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CONTENTS

Sections: Abuse Neglect (AN), Anthropology Archeology (AA), Age Estimation (AE), Bite Marks (BM), Case Report (CR), Dental Damage (DD), Disaster Management (DM), Editorial (ED), Facial Reconstruction (FR), Identification (ID), Jurisprudence/Litigation (JL), Tools and Techniques (TT), Review (RE), Virtopsy (VI).

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VOLUME 30 Number 2 December 2012

SECTION TOOLS AND TECHNIQUE

The Development of a Colorimetric Scale as a Visual Aid for the Bruise Age Determination of Bite Marks and Blunt Trauma

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ABSTRACT
Medical examiners and forensic odontologists are frequently asked to establish the age of a bruise or bitemark on either a living and deceased subjects. The age of bruising has an important medico-legal significance and may be relevant in the investigations related to such crimes as child abuse, domestic violence and homicide. A colorimetric scale for forensic photography based on the colors of the bruise itself, has never been proposed due to the fact that photographic reproduction of color is unreliable and depends on several factors; the camera used, lighting, printer and photo-editing color calibration.
The authors propose two colorimetric scales, both with and without linear measurements, and with 90° angulations, six bruise colors, and three circles with black and white calibrators, which are to be used for the forensic photography of injuries involving the epidermis of Caucasian subjects. The two scales could also be employed on living subjects during different stages of the healing process, or on cadavers in order to provide evidential documentation, image verification and analysis. Such an aid would provide a reliable standard condition and allow for color calibration. The colors represented on the scales would be an aid for the interpretation and objectivity required in estimating the age of the bruise, particularly when the analysis is made directly onto computer images prior to printing. The proposed colorimetric scales do not attempt to give a definitive account of the diverse scientific methods available for the assessment of the age of bruising. The observation of a large sample of blunt trauma and bite mark injuries employing the proposed colorimetric scales would be needed in order to verify and validate the use of these scales. It should be borne in mind that bruise age estimation requires an expert opinion with several degrees of accuracy and variability involved. The age of a bruise cannot be determined by color alone.

KEYWORDS**:** Bruise age estimation, Bite mark analysis, Forensic odontology, Forensic pathology, Forensic science

JFOS. December 2012, Vol.30, No.2 Pag 1-6
ISSN :2219-6749

INTRODUCTION

Forensic medicine and forensic odontology study the application of both medical and dental science related to legal issues and often employs forensic photography: the medico-legal imaging which involves photographing the relevant findings on the cadaver of victims. Medical examiners and forensic odontologists are frequently asked to establish the age of a bruise on either a living or deceased subject. Injuries may be the result of bite marks or non accidental trauma, and have great medico-legal significance in cases involving child abuse and domestic violence. The duration of bruising will be related to the severity of the trauma in terms of strength and how long force was applied for[1.] Color alone is not sufficient in order to determine the age of a bruise[2,] but the variation regarding the color changes may help in age determination and the assessment of blunt trauma injuries and bite marks. Several color changes have been identified in bruises and some, such as yellowing, may indicate that the bruise is over 18 hours old[3-5].

In June of 1996, the U.S. Department of Justice distributed a pamphlet to practitioners involved with investigations concerning child abuse and neglect entitled, "Recognizing When a Child's Injury or Illness is Caused by Abuse"[6]. A specific part of the pamphlet was dedicated to the age determination of bruises and gave an exacting description regarding the color of bruises in direct relation to the age of the bruise itself; red 0-2 days, blue or purple 2-5 days, green 5-7 days, yellow 7-10 days and brown 10-14 days. The exact age of a trauma from photographic evidence alone, remains imprecise and controversial due to the fact that it is difficult to identify the precise color sequences of the healing process in each individual[1-7]. In addition, an individual may receive two bruises at the same time and exhibit different coloration and a different speed of resolution[7]. However, several attempts have been made in order to provide a color chart guideline for a qualitative evaluation of bruise imaging by visual analysis[8-9]. A colorimetric scale for forensic photography based on bruise colors has never been proposed, as photographic color reproduction is unreliable and depends on several factors such as, the camera used, lighting, printer and photo-editing color calibration. Visual assessment remains an unreliable method for the age determination of bruises and the accuracy of aging of bruises is not improved by the degree of forensic experience[10]. This paper proposes two prototype colorimetric scales which may be used for the forensic photography of epidermal injuries of Caucasian subjects, during different stages of the healing process. The RGB color model proved to be a reliable technique with which to assess the color of a bruise[8-10]. The proposed prototype scales are intended to be used by forensic photographers, crime scene police officers and forensic experts. It should be borne in mind that good quality evidence is fundamental for a more objective forensic assessment and a reduction in observer bias[11]. Forensic odontologists may not be on the scene of the crime or present during the period of initial assistance of an abused victim and thus, are not able to directly observe bite mark lesions immediately after they have been inflicted. In these circumstances, bite mark analysis could be requested *a posteriori* by either the coroner or the medical examiner, with assessment being possible exclusively via the photographic evidence of the lesions in question. Recent research has demonstrated that the accuracy and the quality of bite mark

evidence will lead to greater disagreement or agreement in the odontologists'

conclusions[12]. The evaluation of bruise color remains extremely subjective even when photographic evidence is able to accurately convey the original colors of the lesion at the time the photographs were taken.

BACKGROUND

Various methods, such as metric and angular referencing, are available in order to evaluate both the morphological and dimensional properties of a lesion. As there is no standard color reference that is reliable over time, these do not allow for the determination of the age of a bruise. The ruler type metric device, which is the basic model currently employed, is provided with both linear and angular referencing as well as black and white calibration, and was approved in 1988 in accordance with the scientific publication of Hyzer and Krauss[13]. This paper introduces two devices which are easy to use while photographing or video recording traumatic lesions. This enables additional colorimetry based information to be made available for the age determination of lesions. It is both reliable and reproducible after the images have been analyzed, thus improving the efficacy of forensic photography.

COLORIMETRIC SCALES 'NNDV'

The scales have been named according to the initials of the authors' surnames (Nuzzolese, Neri, Di Vella). The patent of the invention belongs to the University of XX (Italy). Both scales consist of an L-shaped ruler provided with double references, both dimensional and colorimetric. Linear references consist of a six centimetre scale per side and three circles, each measuring one inch in diameter. Each circle is divided into four black and white sectors for the black and white levels. Both scales have the same dimensions, but only scale no. 2 has the centimetre reference on both sides (Fig. 1). The colorimetric references consist of six colors pertinent to the various skin color variations of a traumatic bruise lesion; dark red, bluish, purple, greenish, yellow, light brown. The colors, based on the RGB and CMYK color models, have been chosen arbitrarily by reviewing different papers published in related literature[6, 8-10,14,15] regarding the visual assessment of bruise age determination. CMYK and RGB color specifications: dark red (C:40 M:100 Y:100 K:0; R:168 G:25; B: 25), bluish (C:100 M:70 Y:30 K:0; R:0 G:78; B: 126), purple (C:15 M:50 Y:10 K:0; R:215 G:145; B:177), greenish (C:30 M:30 Y:70 K:0; R:194 G:168; B:92), yellow (C:8 M:8 Y:40 K:0; R:241 G:228; B:168), light brown (C:25 M:80 Y:100 K:0; R:195 G:75; B: 14).

CASE STUDY

The case described in this paper concerns a traumatic accidental injury localized on the left forearm of a young adult. The first photographic evidence was recorded eight hours post trauma using a professional digital camera, (Fig. 2). The second recording of photographic evidence was carried out five days post trauma using the same digital camera, lighting and environment, (Fig. 3). Color scale no. 1 was employed in this case.

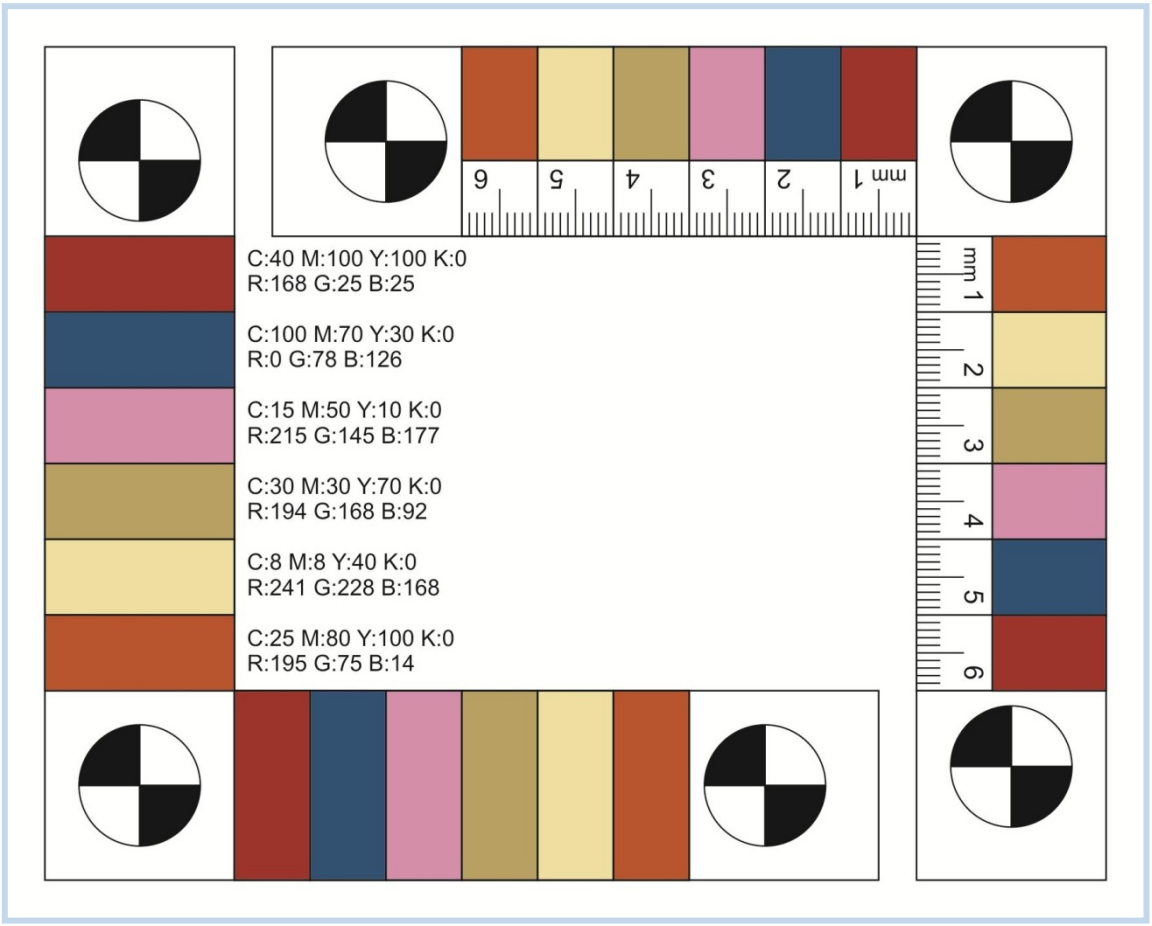


Fig. 1. Colorimetric scales © no. 1 (left) and no. 2 (right) with RGB and CMYK color values.

RESULTS

The proposed scales serve as a guide to the qualitative evaluation of bruise imaging by visual analysis. The devices find widespread use in all cases where photography must be employed for clinical, but above all medico-legal and forensic analysis. In live subjects the use of the scales at different moments in time (e.g. at a time interval of 2, 5-7 or 14-15 days) allows for a comparative evaluation of the evolving color stages of one or more traumatic lesions, and thus serves as a visual aid in the chronological evaluation of bruising. In cadavers, the scales fulfill all qualitative criteria for forensic photography with the additional benefit of supplying a standard colorimetric reference based on the CMYK and RGB color models allowing for the black and white, and color calibration of the employed digital cameras and photo-editing software.

Figures 1 and 2 demonstrate two traumatic accidental injuries. The findings confirm the visual color aid of color scale no. 1 and color scale no. 2.

DISCUSSION

This study does not attempt to give a definitive account of the different scientific methods available for the assessment of the age of bruising. Pathologists and odontologists have additional options for an objective assessment, including microscopic examination and closer gross assessment

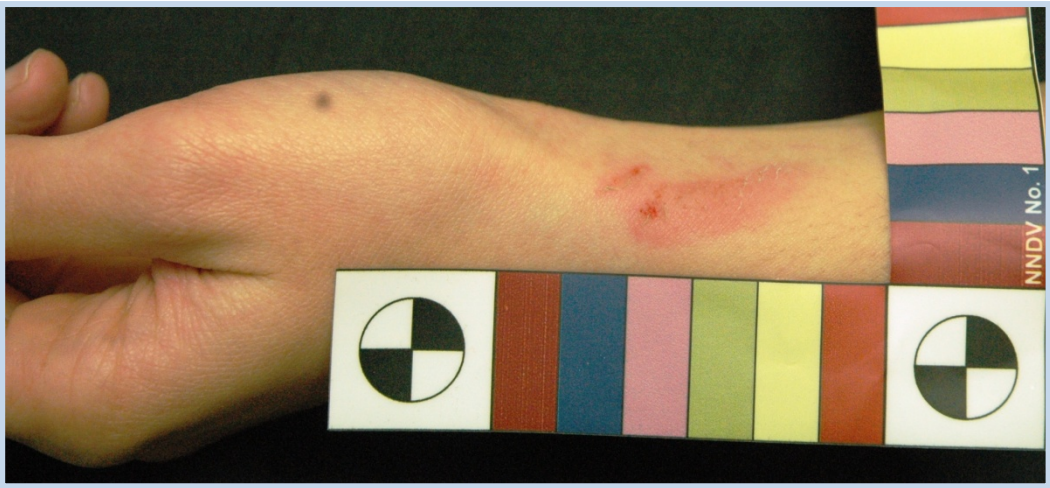
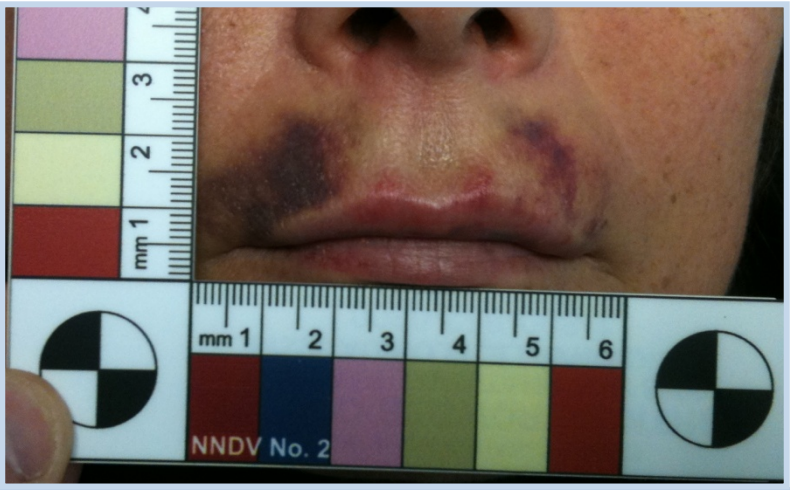


Fig. 2. Left forearm of a young adult: traumatic accidental injury. Photographic evidence was recorded eight hours post trauma, using a professional digital camera and colorimetric scales



© no. 1

Fig. 3. Upper lip of an adult: traumatic accidental injury. Photographic evidence was recorded 1 hour post trauma, using a professional digital camera and colorimetric scales © no. 2.

by dissection. Conventional and specific forensic photography represents one of several aids used to ascertain the age of a bruise[14]. It is the authors' opinion that a color aid could assist with the interpretation and accuracy of the determination of bruise age when analyzing photographic evidence, particularly when the analysis is made directly on computer images prior to printing. Such an aid could create a reliable standard condition and allow for colour calibration and chronological order of the trauma particularly when the bruises pertain to the same individual[15]. In some bite mark cases the estimation of the time of injury may become a relevant issue in legal proceedings. This is particularly true when considered along with other lesions which may either be contemporary or prior to the death of the victim. In a live Caucasian subject the bite mark healing process may indicate a time frame, as the bruise would change color from blue to yellow-green, yellow and finally fade from view[16]. During court testimonials, it is advisable that the forensic odontologist together with the medical examiner, take

into account all available evidence and not just the bite mark itself[12]. Different bruises and bite marks of differing ages may also be an indication of child abuse revealing continual or regular violence[17]. In these cases, the use of forensic photography together with the use of a colorimetric scale may provide strong evidence regarding the bruising and ensures a standard and reliable assessment.
The proposed colorimetric scales need to be validated through the observation of a large sample of blunt trauma and bite mark injuries.

Final Remarks

The applied discipline of forensic science is evolving rapidly, and may be classified as interdisciplinary. Fields such as pathology, odontology and forensic photography develop and expand incorporating new methods allowing for a multi disciplinary involvement and approach. Bruise and bite mark age estimation requires an expert opinion with several degrees of accuracy and variability. The use of color along with all relevant findings and observations pertaining to the investigation, requires experienced and confident observers in order to prevent errors or misjudgment.
Synergy between medical examiners and odontologists is always advisable for an assessment and accurate interpretation in bite mark age evaluation.

Disclosure

The device proposed in this paper is patented "Colorimetric Scale" in the USA with the reference no. 13/225,844. The invention is the property of the University of Bari(Italy); the inventors are: Nuzzolese Emilio, Di Vella Giancarlo, Neri Margherita.

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JOURNAL of FORENSIC ODONTO-STOMATOLOGY
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SECTION JURISPRUDENCE/LITIGATION

Ethical and Legal Issues on HIV Testing, Policy and the Practice of Dentistry

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ABSTRACT

This paper is structured around the following: autonomy and consent, confidentiality, disclosure, knowledge of patient and provider HIV status, the right to choose whom to treat, testing for HIV and the importance of HIV policies in the workplace to guard against discrimination. The emergence of the HIV/AIDS pandemic has challenged traditional ethical values of the health care profession. These include the infectious nature of HIV, the social stigma of the disease and its ethical and legal dilemmas. This paper addresses some of the pertinent questions related to HIV infection and AIDS. The three broad principles of ethics, namely, autonomy, beneficence and justice, provide the basic framework on which this paper is based. Advances in the biotechnology of rapid oral fluid testing particularly in the detection of HIV antibodies from patients in the dental setting have raised additional ethical and legal considerations in the subsequent management of HIV infected patients to include disclosure of test results to the patient and proper referral to physicians or nurse practitioners. The oral health care worker must thus have a solid foundation in the application of bioethical principles. A clinical case scenario related to HIV testing in the dental setting is presented to illustrate how a lack of understanding and the wrongful application of ethical principles may lead to patient harm and legal liability. Given the increasing infection rate of HIV worldwide, polices must be upheld and revised as needed to protect healthcare providers, patients, and society generally against discrimination

JFOS. December 2012, Vol.30, No.2 Pag 7-16
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INTRODUCTION

Dentists can often detect early manifestations of systemic diseases in the oral cavity and may function as gatekeepers in the healthcare system by referring patients to physicians and nurse practitioners for evaluation and treatment. Advances in the biotechnology of oral fluid testing and the direct accessibility of the oral cavity to examination may change the scope of dental care whereby the dentist can promote public health, advocate needed changes in health policy, and align dentistry more closely with medicine and nursing. That alignment was not fully realized generations ago when dentistry was largely isolated from its allied health professions and focused almost exclusively on the restoration of teeth. Advances in oral fluid testing received widespread attention when antibodies to HIV infection could be detected from oral transudate, and forever changed the erstwhile, relatively tranquil landscape of dental practice. Dentistry was catapulted into the center of the HIV/AIDS crisis. Dentists saw a major shift in their role as oral diagnosticians testing for HIV antibodies. That shift also necessitated an in-depth understanding of bioethical principles and their judicious application in clinical management. The principles of biomedical ethics from medical practice have now become even more important and integrated into dental practice.

AUTONOMY AND CONSENT

The oral health care worker (OHCW) can only examine or treat patients who have given their consent. Such consent is based on the patient's voluntary authorization of a dental procedure on his or her understanding of the relevant information provided by an OHCW.[1] Consent rests on the principle of respect for autonomy which acknowledges the ability of persons to comprehend knowledge, weigh alternatives and form judgments. Cultural differences also need to be taken into account as well as a right to respect autonomy. The doctrinal principle of informed consent and confidentiality both flow from autonomy. One important purpose of the doctrinal principle of informed consent is to protect people not only from unnecessary treatment but also all forms of unwanted treatments even if they are deemed medically necessary. Each individual who is competent has the fundamental right to control who can touch his or her body. While autonomy is a hugely important value, , the ability of teh health worker to provide care must also be respected. This leads to the crux of moral issues involved in setting limits to individual autonomy in health care.[2]

CONFIDENTIALITY

The obligation of confidentiality is virtually universal in professional codes of ethics, particularly with respect to HIV/AIDS. There is an inherent conflict between a patient's interest in confidentiality and the public's interest in protection from infectious diseases. The presumption is that only patients themselves can know which disclosures to third parties will have consequences in their private, public and professional lives.[3] A person with HIV/AIDS has a right to privacy, especially with regard to the doctor-patient relationship. Deliberate breach of this right by disclosing confidential information to another, constitutes an unlawful act. Courts have awarded damages in an instance whereby a medical doctor disclosed his patients' HIV status to a colleague on the golf course without the patients' consent as in *Jansen van Vuuren v Kruger*.[4]

From one standpoint, confidentiality is a branch or subset of informational privacy, it prevents re-disclosure of information that was originally disclosed within a confidential relationship.[5] An infringement of a person's right to confidentiality occurs only if the person (or institution) to whom the information was disclosed in confidence fails to protect the information or deliberately discloses it to someone without first party consent. By contrast, a person who without authorization enters a hospital record room or computer data bank violates rights of privacy rather than rights of confidentiality. Only the person or institution that receives the information in a confidential relationship can be charged

DO CERTAIN CIRCUMSTANCES WARRANT BREACHING PATIENT CONFIDENTIALITY?

Confidentiality is not absolute and clinical information must sometimes be shared by other health professionals.[6] In fact, no moral norm is absolute. The ethical duty of the OHCW extends not only to patients but also to other individuals whose life and safety may be affected by non-disclosure of information. The autonomy of that patient ought to then be limited accordingly. Consequential arguments thus support disclosure of confidential information such as HIV status as ethically permissible in the context of a "duty to warn", as in *Tarasoff v. Regents of the University of California*, if and only if the probability and magnitude of harm are major to a third party such as a patient's spouse, partner or other third party.[5, 7, 8] For the dental practitioner, however, risk is minimal to the practitioner who uses proper barrier techniques and protocol. In the absence of major risk of harm from HIV infection, disclosure is no more compelling than for any other chronic illness, e.g., diabetes mellitus.

There may however be another circumstance in which it is ethically permissible to disclose a patient's HIV status without the patient's previous permission and informed consent. Let us say that while the patient was competent, he had made every effort to keep his positive HIV status strictly confidential. However, his HIV infection ultimately progressed to AIDS and disclosure of the patient's HIV status is likely to be critical in his end of life care to avoid harm or needless suffering. The advanced HIV infection may thus become the proximate cause of the patient's death. In that specific regard, Vernillo, Wolpe, and Halpern[9] proposed that disclosure of an incompetent patient's HIV status may usefully inform surrogate decision-making as much as terminal cancer may. In contrast, if a competent patient after appropriate counseling refuses to have other health care workers informed of his/her HIV status, then the patient should be told that the OHCW is duty bound to divulge this information to other health care workers who are also involved in the management of the patient. However, counseling of the patient is an absolute prerequisite to disclosure of the patient's HIV status. It is ethically unjustified to disclose the HIV status of a patient to a referring physician whether it is necessary or not without informing the patient about such disclosure. Furthermore, the disclosure is warranted only in as much as such information will primarily lessen the risk of clinical complications for the patient and not necessarily the potential for HIV exposure to the healthcare professional.

Doctors have an ethical duty to disclose the patient's positive HIV status but those who wish to make such disclosures should always first have a comprehensive

discussion with the patient because the patient may also refuse consent to disclose his or her HIV status. A doctor is legally bound to such a decision and the same would apply to oral health care workers. As discussed in the next section, an HIV test result particularly one confirmed by a physician or nurse practitioner from a dentist is no longer preliminary but definitive and thus represents a diagnostic finding of infection. The patient may then decide whether or not to accept medical treatment if treatment is recommended.

HIV TESTING IN DENTAL PRACTICE

The ethical and legal issues pertaining to HIV testing in the dental setting have been discussed previously.[7, 10] A recent qualitative study in an urban university dental clinic showed fairly consistent results and indicated relatively high levels of acceptability among dental students and faculty for implementing rapid oral fluid HIV testing.[11] Dental practice sites present unique opportunities for implementing the Center for Disease Control (CDC) recommendations for routine HIV testing.[12] The availability of rapid diagnostic test kits that detect HIV-1 and HIV-2 antibodies in oral fluid has greatly facilitated the acceptability and potential for widespread HIV testing in dental sites and elsewhere.[7, 13, 14] On July 3, 2012, the US Food and Drug Administration approved the OraQuick In-Home HIV Test, the first over-the-counter home-use rapid HIV test to detect the presence of antibodies to HIV.[15] The dentist can also play an important role in explaining the interpretation of test results to patients, given the emergence of in-home testing. However, this technological advance raises additional ethical considerations. The patient who asks his or her dentist about an in-home test must understand that a positive result does not mean that the individual is definitely infected with HIV but rather that additional testing should be done in a medical setting to confirm the oral fluid test result. Similarly, the patient must understand that a negative test result does not mean that an individual is definitely not infected. Counseling patients about test results also help ensure that patients know how to use an in-home kit properly. It is equally important for the dental patient to know that rapid oral fluid tests for HIV antibodies have had a history of persistent false positive test results[16] reinforcing the need for patients to follow-up with a medical healthcare professional. Nonetheless, advances in rapid oral fluid diagnostic technology generally may not only promote the public good but also reshape the scope of dental health care delivery and informed consent.[17] Obtaining fully informed and voluntary consent for HIV testing is absolutely critical and nowhere more so than in the dental setting. With advances in rapid oral fluid testing, the dental practitioner must have a sound working knowledge of bioethical principles to include respect for patient autonomy, confidentiality, and the subsequent management of a preliminary test result.[7]

Let us consider the following case scenario in a dental practice which offers an additional ethical analysis. A dentist informs a patient that he or she will do a rapid test for HIV infection from the patient's oral fluid. The patient is fully informed about the purpose of the test and any risks and benefits as part of pre-test counseling. The patient is also told that he or she can refuse to have the HIV test (opt-out consent) without any prejudice to receiving dental care. Refusal however must be made before the oral fluid sample is obtained and tested for HIV antibodies. If the patient chooses to get tested and a

sample is obtained, then the patient cannot later refuse the test result. About 10 to 15 minutes into the dental procedure, the patient says, "Doctor, I understood what you told me before but I really do not want the test result. I am frightened. You have not yet seen the test result nor have I. So, why not discard the HIV test result?" The dentist wrongfully interprets this patient's request as an exercise of his or her autonomous right and willfully discards the test result. The patient visits a physician about a year later with symptoms of fatigue and weight loss. The physician obtains a positive HIV test result from the patient who comments that a dentist had performed a rapid HIV test but agreed with the patient to discard the test result without any knowledge of the result.

In the above clinical scenario, the dentist is likely to be legally liable for negligence whereby failure to use a reasonable amount of care (i.e., providing the preliminary HIV test result to the patient) may have led to harm. When the patient had requested that his or her HIV test result be discarded, the dentist should have understood the limits of patient autonomy and thereby not acquiesced to that request. If the patient had known his or her HIV test result sooner, then a physician could have treated him or her before the infection progressed to symptomatic disease. Discarding an oral fluid sample is no different than discarding a biopsy specimen. If diagnostic information is obtainable (even if preliminary), then it must be evaluated further by a physician or nurse care practitioner. The result must also be reported to the patient. Failure of the OHCW to report the HIV test result to the patient by discarding it undermines the patient's autonomy to make future decisions regarding his or her healthcare. Such an action may also represent a violation of that patient's civil rights. How then is a patient to act when he or she cannot possibly know the harm that might befall him or her?

SHOULD THE PATIENT'S SEXUAL PARTNER/S BE INFORMED OF THE HIV STATUS OF THE PATIENT?

The answer in general depends upon the magnitude and the probability of harm as previously discussed. In all likelihood, the physician or nurse practitioner will assess the risk of HIV exposure through post-counseling with that patient. One study concluded that it is very ineffective to leave partner-notification to patients.[18] Perhaps, the only responsible strategy is the one proposed by the American Medical Association (AMA) Council on Ethical and Judicial Affairs: a physician who knows that a seropositive individual is endangering a third party should (1) attempt to persuade the infected patient to cease endangering a third party. If the infected patient recants on the clinician's recommendation and infects his partner, then that patient assumes the legal liability. However, the OHCW must have complete documentation in the clinical chart to show that the infected patient was persuaded to cease the practice of unprotected sex with his partner (2) if persuasion fails, notify authorities, e.g., public health officials, law enforcement; and (3) if the authorities take no action, notify the endangered parties.[19] Failure of the individual to disclose his or her HIV status to a sexual partner results in willful exposure of that third party to HIV infection and legally constitutes an act of assault. From a legal point of view, a person may act in what is termed "private defense" of another, when it appears that such person's physical integrity may be threatened by another's unlawful action. Negligently or deliberately infecting another person with HIV could give rise to civil claims as was the case in *Venter v Nel*

in 1997.[4] Oral health care workers should always first discuss the issue of notifying patients or face possible civil claims for damages.

Questions of conflicting obligations may understandably occur when an OHCW is faced with the decision of whether the status of an HIV positive individual should be disclosed to a third party without the consent of the patient. The above AMA tiered strategy[19] offers some guidance but physicians or nurse practitioners should assume the role of notifying third parties when previous steps have failed. There are grounds for such a disclosure only where there is a serious and identifiable risk to the specific individual(s) who, if not so informed, would be exposed to infection. The patient's autonomous right to confidentiality is thus trumped. Justice to a third party deems it appropriate to inform the third party who can now obtain HIV testing and medical treatment, if needed. Therefore, when a person is found to be infected, the OHCW is obliged to discuss the matter with the patient's physician who can take the lead in a post-counseling session.

KNOWLEDGE OF PATIENT'S HIV STATUS

Some OHCW feel strongly that they should know the HIV status of high-risk patients because of fear of possible infection. Testing for HIV should only be suggested if the degree of security it affords the OHCW is substantially more than the potential harm it may cause the patient. However, as stated previously, dentists who properly use barrier technique are generally at a low risk of contracting HIV infection from patients regardless of the patient's risk status. It is the duty of the practitioner to *suggest* that tests be carried out. Should patients refuse to undergo testing, they should be advised to seek a second opinion. If such a test is deemed essential to the management of the patient, pre-test counseling needs to be performed by a professionally trained counselor. A doctor or dentist, whose diagnostic ability is compromised by the persistent refusal of a patient to undergo a simple investigation, is free to terminate the professional relationship. Such decisions would be taken in a spirit of compassion and understanding and every effort should be made by members of the profession to avoid such situations.[20] A caveat does exist: : if dentists insist that patients get HIV tested primarily out of concern for the practitioner's own well-being, then patients may equally insist that their dentists undergo HIV testing.

CAN AN OHCW REFUSE TO TREAT A PATIENT?

Although there is no legal obligation of an OHCW to treat a patient, the issue is a complex one, because health professionals have taken the Hippocratic Oath which affirms the ethical obligation to treat and there is no need to modify dental care for HIV infected patients. Furthermore, no personal characteristics, such as race, colour, creed, sexual identity, and culture should impinge on treatment planning.[21] Williams[22]also argues that dental ethics codes make no exception to the dentist's duty to treat all patients equally to include patients with infectious diseases such as HIV/AIDS. Up to 70% of patients with HIV/AIDS have oral manifestations of the infection. OHCW are often the first to diagnose this and need to discuss their findings with their patients. Under such circumstances, an OHCW must also acknowledge the rights of patients[23] and need to take into account the ethical principles of beneficence and justice.[24]

Beneficence encompasses the following: not to inflict evil or harm, to prevent evil or harm, to remove evil or harm and to do or promote good.[25] Justice has been described in terms of fairness and "what is deserved".[24] Doyal[20] argues that in the ethical management of patients with HIV, the virtues of courage, prudence, charity and hope need to be part of the approach to treatment. There are however, limits to the exercise of these virtues in the course of work. It is accepted that the courage, which is expected within professional practice must be mediated by the additional virtue of prudence.

CAN YOU ASK AN EMPLOYEE TO HAVE A PRE-EMPLOYMENT HIV TEST?

In South Africa, unless it can be shown that a job applicant's medical status could affect an inherent job requirement, pre- and post- employment testing for *any* medical condition is specifically prohibited by the Basic Conditions of Employment and Employment Equity Acts as being unfair and discriminatory. Unlike in previous years, the definition of "employees" includes job applicants, in as much as every person must be treated equally when applying for a job (except in as much as affirmative action policies justify discrimination). Section 6(1) of the Employment Equity Act (EEA) states that "*No person may unfairly discriminate, directly or indirectly, against an employee, in any employment policy or practice, on one or more grounds, including ... HIV status ...*" This section applies to all employers and employees. Notably, the omission of the word 'positive' from the phrase 'HIV status' means that discrimination on the grounds of an employee's perceived HIV status is also prohibited. Therefore it is not a prerequisite that an employee be HIV positive before he can succeed with a claim of unfair discrimination on the grounds of his or her 'HIV status'. Section 7(2) of the EEA states that *'testing of an employee to determine that employee's HIV status is prohibited unless such testing is determined to be justifiable by the Labour Court'*.[4]

CAN AN OHCW WHO IS HIV POSITIVE CONTINUE TO TREAT PATIENTS?

The labor law clearly states that as long as a person is not physically ill, there is no reason for an employer to discontinue, reduce or alter the employee's duties whatsoever. The complication which clearly demarcates this from any other situation is the possibility of infecting a patient. Under normal circumstances there is no, or negligible risk. When procedures require the use of sharp instruments, then the risk increases. It will depend on the kind of action the OHCW intends to take with the patient, which should delineate various forms of treatment that may expose the patient to high or low risk. There are no compelling data to show transmission of HIV infection from a dentist to a patient when the dentist is using barrier technique to include gloves, mask, and a clinical gown.[26] Some patients in a dental office, however, may still remain suspicious of a dental health care provider and the risk of HIV transmission from that provider. The Centers for Disease Control and Prevention (CDC) and the Florida Health and Rehabilitative Service Department (HRS) initially dismissed the case in which an HIV-positive dentist, David Acer, had transmitted HIV infection to at least six of his dental patients. In the light of withheld behavioral evidence from medical records, legal testimonies, and personal interviews obtained during the investigation, the

information strongly supported the conclusion that these six HIV transmissions were most likely intended by the dentist to execute a political and social agenda.[27] Nonetheless, the OHCW should seek specialist advice on the extent to which they should limit their professional practice in order to protect patients. They must act upon that advice, which in some circumstances would include a requirement not to practice or to limit their practice in certain ways. No OHCW should continue in clinical practice merely on the basis of their own assessment of the risk to patients. The OHCW must also keep in mind that he or she is prone to opportunistic infections that may be contracted from an ill patient. It is unethical for OHCW who know or believe themselves to be infected with HIV to put patients at risk by failing to seek appropriate counseling, or to act upon it when given. The doctor, who has counseled a colleague who is infected with HIV to modify his or her professional practice in order to safeguard patients, and is aware that his advice is not being followed, has a duty to inform an appropriate body that the fitness to practice of the OHCW may be seriously impaired.

THE IMPORTANCE OF HIV/AIDS POLICIES

The Health Professions Council of South Africa (HPCSA) [28] provides a set of ethical guidelines fully cognizant of the increasing infection rate of HIV. However, differences in adherence to acts, policies, and/or ethical guidelines may exist in other countries. Universality in terms of the ethical principles of beneficence and non-maleficence as it guides the provision of healthcare does indeed exist.Polices must be upheld and revised as needed to protect healthcare providers, patients, and society generally against discrimination. Given the increasing infection rate of HIV worldwide, the financial cost of the impact of HIV/AIDS is huge and will escalate dramatically in the work force. Aside from the possible future cost of absenteeism, employers should take every precautionary step against possible labour problems, arising from HIV in the workplace. This requires the education of employees about AIDS and ensuring that the working environment is safe. Methods of education may include publishing articles in employee newsletters and on notice-boards about AIDS transmission and prevention; providing AIDS 'hotline' numbers as part of the employee assistance program; conducting meetings with medical and legal specialists on AIDS to inform employees of the syndrome and to inform management and supervisors of the rights of AIDS sufferers and other employees.[4]

CONCLUSION

The multifaceted challenge of the HIV/AIDS pandemic has had a profound effect in healthcare practice necessitating a re-examination and application of the concepts of ethics, responsibility, autonomy and justice. There have been sweeping changes in social attitudes, policy and regulatory frameworks. On a global scale it took activism to new heights and raised moral concerns of social justice with regard to access to health care, basic human rights, the government's responsibility to care for its citizens, and the duty of beneficence of the developed towards the developing world. On a national level it has re-opened debates on issues of distributive justice and fairness. HIV has forced society to look at innovative ways of collectively managing the pandemic in a responsible, sustained and equitable manner. Healthcare providers including dentists, physicians,

and nurses play a decisive role in the referral of patients and in the detection and treatment of HIV infection. The understanding of bioethical principles and their judicious application should ensure that patients with HIV infection are justly treated commensurate with any patient who suffers from a chronic illness.

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SECTION AGE ESTIMATION

Comparison of the applicability of four odontological methods for age estimation of the 14 years legal threshold in a sample of Italian adolescents.

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ABSTRACT

The 14-years age threshold is especially important in Italy for criminal, civil and administrative laws. Several methods relying on dental calcification of the teeth, up to the second molar, are used for the evaluation of age in childhood.

The objective of the research was to compare the inter-rater agreement and accuracy of four common methods for the dental age estimation – Demirjian (D), Willems (W), Cameriere (C) and Haavikko (H) – in a sample of Italian adolescents between 11 and 16 years. The sensitivity and specificity, and the different level of probability, according to the peculiarities of Italian criminal and civil law, were compared for the methods examined, considering the threshold of 14 years.

The sample was composed of 501 digital OPGs of Italian children (257 females and 244 males), aged from 11 years and 0 days to 15 years and 364 days.

The maturation stage of the teeth was evaluated according to D, W, H and C methods by three independent examiners. Mixed statistical models were applied to compare the accuracy and the errors of each method.

The inter-rater agreement was high for the four methods and the intraclass correlation coefficients were all ≥ 0.81. Methods H and C showed a general tendency to underestimate the age in the considered sample while the methods D and W tended to overestimate the child's age. In females, D and W were more accurate than C, which is more accurate than H. In the males, W is the most accurate method even though it over-estimated age. Considering the 14-years threshold, the sensitivity of D and W methods is quite high (range 0.80; 0.95) and specificity is low (range 0.61; 0.86).

The principal findings of the research are: the W and D methods are much more accurate than C and H, but they tend to overestimate the age. The C method largely underestimates the age (by ~1 year) for both genders and for all operators. H is unsuitable for dental age estimation in the Italian population, while W and D yielded high sensitivity but low specificity, thus producing high rates of false positive results. The choice of method to estimate if an Italian child has reached the 14-years legal threshold should mainly be chosen according to the different legal milieu (if civil or criminal) and the gender of the examined individual. The age assessment in criminal case must be prudently managed.

Keywords: dental age estimation; forensic odontology; legal threshold of age; Demirjian method.
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INTRODUCTION

There is now full accordance in the literature that the methods of dental age estimation (DAE) relying on the evaluation of the mineralisation and growth stage of the teeth seem to be scarcely affected by local and systemic factors (1-3) but are dependent on the genetics of the populations as they show an ethnic variability (4-5). The estimation of age through the study of the calcification of the permanent teeth has been demonstrated to provide reliable and accurate methods and results. The dentition up to the second molar is useful in the procedures in which subjects <16 years are involved, and the remaining third molars are evaluated after this age threshold. Most studies on DAE, and especially those concerning the dentition up to the second molar and the threshold of 14 years, have focused on the methods or the comparison of methods with little consideration of the accuracy of the estimation in cohorts around this age and in classifying individuals in respect to the threshold. In contrast, judges, institutions and agencies generally ask not simply an age estimation but more strictly an age assessment with respect to the legal threshold of age. The age evaluation procedure of the living minor is usually required to determine if the subject is accountable for his actions in criminal law. According to Italian law, the lowest age threshold for criminal accountability is 14 years; the actual accountability between 14 and 18 must be established case by case mainly relying on a psychological maturity assessment basis. In another context - mostly of administrative or civil law significance - the age evaluation of the minor is necessary to determine if the subject shall undergo specific obligations (educational, for instance) or should receive specific aides or other providences from the state administration (immigration, adoption, motorcycle driver license, passport release - to cite only the most common cases). Criminal law requires that the age over the threshold has to be assessed "beyond any reasonable doubt" (according to the Latin aphorism "in dubio pro reo") and, if doubts persist, the lower age has to be assigned by the judge/court. The reasonable doubt is logically connected to scientific evidence provided by experts, who are aware that only evidence endowed with very high probability, at least > 90%, may turn useful in criminal proceedings and meet the standard of proof. In criminalproceedings the percentage of false positives deserves special consideration, because the overestimation ofthe age threshold is the less desirable error both from the legal and the ethical point of view.

On the contrary, in civil proceedings the general rule is just "more probable than not", so that a percentage of probability just above the 50% may suffice.

Furthermore, the different kinds of error in assessing the age have to be differently regarded with respect to the criminal or civil law context. The false positive is the worst and the least desirable error for criminal law, given the heavy legal consequences that it implies. In civil proceedings the false positive and false negative tend to have the same meaning: they are both errors, but the first is not necessarily worse than the second. In fact, when the age is over the threshold the subject may be entitled to some civil rights.

To achieve the best accuracy, both in DAE and in assessing if a child has attained the legal threshold, it is therefore important to test the different dental methods of age estimation on a specific ethnic group. Few

papers have been published about the comparison of the accuracy, sensitivity and specificity of the different methods for DAE in a sample of Italian children at the 14-years age threshold. Moreover, many studies have reported that the accuracy of the method is dependent on the age of the subject and that the uncertainty of the predictions grows in the older cohorts (6). Therefore, it is meaningful to evaluate the methods comparing the sensitivity, the specificity and the accuracy for age cohorts close to the threshold. To address this issue, we selected ~50 Italian subjects per gender, and an age cohort around 14 years (age span 11-16), and compared the results obtained through four of the most known and adopted methods for DAE: the Demirjian's, the Willems', the Cameriere's and Haavikko's methods. The comparative analysis evaluated the inter-rater agreement, the mean error of estimations, the sensitivity and specificity of the selected methods as indexes that allow the evaluation of the accuracy of the method to predict the attainment of the 14-yrs threshold (5110 days of age).

MATERIALS AND METHODS

A total of 501 digital orthopantomographs (OPGs) of Italian children of Caucasian origins were taken from three selected clinical radiology offices in northern, central and southern Italy. As shown in table 1, the sample consisted of 244 males and 257 females, almost equally divided in age cohorts from the age of 11 years (4015 days) to 15 years and 364 days (5839 days) (Table1)

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Table 1: Frequency and percentage of the subjects for age and gender.

Age	Females (%)	Males (%)
11	56 (22)	46 (19)
12	51 (20)	46 (19)
13	47 (18)	43 (18)
14	50 (19)	56 (23)
15	53 (21)	53 (22)
All	257 (100)	244 (100)

Table 2: Intraclass correlation coefficients (R) for the 3 examiners on 501 radiographs. R: Intraclass correlation coefficient. CI95%: Confidence Interval at 95%

Method	R	Lower limit CI95%	Upper limit CI95%
Cameriere	0.84	0.80	0.87
Demirjian	0.81	0.76	0.85
Haavikko 50	0.83	0.78	0.86
Willems 50	0.88	0.86	0.89

The chronological age from the OPGs has been recorded as number of days. All the individuals of the sample were healthy and the OPGs had been taken for clinical control purposes. Exclusion criteria were the following: systemic diseases, premature birth, congenital anomalies, tooth agenesia, endodontic treatments, large carious lesions involving the dental pulp, gross mandibular pathologies, poor quality X-rays. 103 subjects (40%) in the female group, and 109 (45%) in the male group, were 14-years old or younger.

The methods utilised to analyse the OPGs and estimate the age were:

The Demirjian's method for seven teeth (D). In the conversion table we chose the estimated age (EA) as the age at the 50th centile (6,7);

The Willems' method (W), specifically the conversion score elaborated with the polynomial regression system for the Belgian sample (8-10). The EA is calculated at the 50^{th} percentile;

The Cameriere's (C) method using the European formula (11-16), as it is presented in the AgEstimation Project website (http://agestimation.unimc.it);

The Haavikko's (H) method at the 50th centile (17).

Three expert forensic odontologists, all of them blind to the chronological age of the subjects, analysed all the OPGs.

A descriptive analysis of the sample was performed and the chronological age of the 501 subjects was distributed in age and gender.

An inter-rater agreement test was calculated on the estimations of the age provided by the three experts for the whole sample. For this analysis, the two-way intraclass correlation coefficient (R) was used. To evaluate the results of the agreement, the standards of Fleiss were used (18). Values of R below 0.4 may be taken to represent poor agreement, value above 0.75 may be taken to represent excellent agreement and values between 0.4 and 0.75 may be taken to represent fair to good agreement (18).

The difference between estimated and chronological age was calculated to evaluate the accuracy of each method (EA-CA). A positive figure indicates an over-estimation and a negative figure indicates an under-estimation of the age. The mean and standard deviation of each method and gender were calculated for each examiner.

To test the difference in accuracy between the four methods adopted, two linear mixed models were applied. The two mixed models were performed for males and females.

For each mixed model, the subject represented the random effect, while the fixed effects were represented by the examiner, the method and the interaction between the examiner and the method. The outcome variable was the difference between the estimated age and the chronological age. Post hoc differences between examiners and methods were tested with the Tukey Honestly Significant Difference test.

To test the discrimination accuracy, considering the threshold of 14 years, the subjects were dichotomised into two cohorts at the cut-off threshold of 5110 days of age. Sensitivity and specificity were calculated for the Demirjian (D) and Willems (W) methods and for each examiner and gender. Sensitivity in this context is defined as the probability of correctly estimating a subject who is 14-years old or above. Specificity in this context is defined as the probability of correctly estimating a subject who is <14-years old. Sensitivity and specificity for the Haavikko (H) and Cameriere (C) methods have not been analysed because they allow an estimation of 14 years as maximum and both of them resulted to underestimate largely the age of the sample so that we would obtain in every case a sensitivity and a specificity respectively equal to 0 and 1. Comparison of sensitivity and specificity were tested for Demirjian and Willems methods with the McNemar test for each examiner and gender.

MedCalc ® version 12.3.0.0 and JMP ® version 9.0 were used for the statistical analysis. Significance was set at 0.05.

RESULTS

The intraclass correlation coefficients (R) and their confidence intervals for each method are shown in Table 2 (Table2). The estimates of R are quite high and all the lower limits of the confidence intervals are above 0.75, representing an excellent agreement among the examiners for all the methods (18).

The mean and standard deviation of the difference between estimated age and chronological age are shown for each method, examiner and gender in Table 3 (Table3).

Table 3: Mean (standard deviation) of the difference between estimated age and chronological age. A positive number indicates an over-estimation of the age and a negative number indicates an under-estimation of the age.

	Cameriere	Demirjian	Haavikko	Willems
Females N=257				
Examiner 1	-0.96 (0.99)	-0.04 (1.10)	-1.47 (1.15)	0.51 (1.33)
Examiner 2	-0.81 (1.05)	0.82 (1.21)	-1.33 (1.16)	0.47 (1.32)
Examiner 3	-1.11 (0.98)	0.45 (1.22)	-1.69 (1.09)	0.34 (1.19)
Males N=244				
Examiner 1	-1.05 (0.94)	0.65 (1.30)	-1.12 (1.03)	0.20 (1.32)
Examiner 2	-0.90 (1.03)	0.77 (1.27)	-0.86 (1.01)	0.40 (1.28)
Examiner 3	-1.26 (0.98)	0.62 (1.15)	-1.09 (0.97)	0.23 (1.10)

Table 4: Tukey HSD for females. The outcome variable is the difference between estimated age and chronological age. Levels not connected by the same letter are significantly different

Method	Examiner							Least Sq Mean
Demirijan	2	A						0.82
Willems	1		B					0.51
Willems	2		B					0.47
Demirijan	3		B					0.45
Willems	3		B					0.34
Demirijan	1			C				-0.04
Cameriere	2				D			-0.81
Cameriere	1				D	E		-0.96
Cameriere	3					E		-1.11
Haavikko	2						F	-1.33
Haavikko	1						F	-1.47
Haavikko	3							G -1.69

It emerges that C and H tend to under-estimate remarkably the age, while D and W are prone to an overestimation.

In the mixed models, the factors represented by the examiner, the method and the interaction between the examiner and the method are all significant. The p-values were <0.0001 for all factors in the model for females.

The estimated difference between the estimated age and the chronological age

and the results of the Tukey HSD test are reported for females in Table 4 (Table4). In females, it emerges that D and W over-estimate the age, but they are more accurate than C and H; C is more accurate than H.

The p-values were <0.0001 for examiner and method and 0.0161 for the interaction term in the model for males. The estimated difference between the estimated age and the chronological age and the results of the Tukey HSD test are shown for males in Table 5 (Table5). The THSD test basically shows that W is the most accurate method for males even if it leads to an over-estimation.

Sensitivity, specificity and the McNemar test values for D and W methods among the three examiners are shown in Table 6 (Table6).

Table 5: Tukey HSD for males. The outcome variable is the difference between estimated age and chronological age.

Method	Examiner								LeastSqMean
Demirijan	2	A							0.77
Demirijan	1	A							0.65
Demirijan	3	A							0.62
Willems	2		B						0.40
Willems	3		B	C					0.23
Willems	1			C					0.20
Haavikko	2				D				-0.86
Cameriere	2				D	E			-0.90
Cameriere	1					E	F		-1.05
Haavikko	3						F	G	-1.09
Haavikko	1						F	G	-1.12
Cameriere	3							G	-1.26

Table 6: Sensitivity, specificity and McNemar test for Demirijan and Willems method among the three examiners. Sensitivity: proportion of subject classified ≥ 14 years old when they are ≥ 14 years old.Specificity: proportion of subject classified < 14 years old when they are < 14 years old. * McNemar test; F: Females; M: Males.

Threshold 14 years	Examiner	Gender	Demirijan	Willems	p-value*
Sensitivity	1	F	0.80	0.95	<0.0001
Sensitivity	2	F	0.94	0.94	1.0000
Sensitivity	3	F	0.81	0.89	0.0027
Sensitivity	1	M	0.85	0.84	0.3173
Sensitivity	2	M	0.94	0.92	0.1573
Sensitivity	3	M	0.89	0.89	1.0000
Specificity	1	F	0.86	0.62	<0.0001
Specificity	2	F	0.62	0.64	0.1573
Specificity	3	F	0.80	0.72	0.0073
Specificity	1	M	0.61	0.65	0.0143
Specificity	2	M	0.61	0.61	0.3173
Specificity	3	M	0.73	0.73	1.0000

The sensitivity values of the methods are quite high, but the specificity values are

low, especially for males. For females, examiners #1 and #3 yielded the higher sensitivity with W and the best specificity with D. For the male sample, the three examiners gained similar sensitivity and specificity values for W and D, with the only exception of a slightly better specificity value obtained by examiner #1 with the W method.

DISCUSSION

Many studies have demonstrated that dental calcification evaluated on OPG provides reliable evidence to estimate the age of children and youths. The scientific literature is committed to providing ever more accurate methods for the age assessment and to classify individuals with respect to their age given a legal threshold of interest (i.e. 14, 16, 18 years). Many variables potentially can affect the estimation of age and explain some differences in findings among studies even if applying the same method of DAE. The appropriateness of the statistical approach, the influence of the operator (19-21) and the real role of the ethnicity or environmental factors assume a crucial importance. On the other hand, especially criminal law requires that the age assessment or classification are provided with very high probability and a special attention to false positive rates.

In this study, we compared four well-known methods for DAE in an Italian sample distributed around the threshold of 14 years (11-16 years).

The inter-operator agreement was tested for the three experts and on the whole sample and our high values are consistent with those reported by other studies (22) and demonstrates the high repeatability of all the applied methods.

The Demirjian's and Willems's methods turn out to be the most accurate but they

were demonstrated to be gender-sensitive and partially influenced by the operator performances. Consistently with numerous studies for the D method (23-27) and with a few for the W method (28-29) as the principal finding, it emerges that the Willems' and Demirjian's methods lead to an age overestimation in both genders. Recently Butti et al. (30) concluded that dental maturation standards as described by Haavikko do not appear suitable for Italian children. In our research, the large underestimation of age for both female and male individuals in every cohort of age supports the aforementioned conclusion.

In 2011 Galic et al. (22) reported that the C method is the most accurate for both genders followed by Haavikko and Willems, which was the least accurate. El-Bakary (28) reported an average underestimation by 0.26 years for girls and 0.49 years for boys in the Egyptian population and similar results were observed by Cameriere in a European population (12-16). Our findings contrast with these results given the consistent age underestimation (~1 year) that the C method produces for both genders and in every cohort of age. Few papers have been published about the comparison of the accuracy, specificity and sensitivity of the DAE methods for the 14-years age threshold. Given the different relevance of false attribution in age assessment in civil and criminal proceedings, we have focused our attention on sensitivity (false negative) and specificity (false positive). The specific features of H and C methods and the large underestimation rates they have shown, renders the evaluation of their sensitivity and specificity values meaningless, hence the use of the Cameriere and Haavikko methods cannot

be considered reliable for the assessment of the attainment of the 14-years threshold. The Haavikko method is not suitable for

the evaluation above the 12-years cohort because it leads to a substantial and progressive inaccuracy (increasing with age of the subjects); the Cameriere European formula, on the other hand, does not allow estimations above the age of 14,06 years in the male individuals and even above the age of 13,68 years (13, if we use a completed year figure) in the female individuals.

The higher sensitivity values obtained with the use of the W and D methods, and therefore the higher probability of identifying the subjects who really are above 14-years old, allow us to say that in a civil law context, in which the evaluation methods that lead to an overestimation are essentially acceptable, the W and D are the methods of choice (and among them the W method when applied to female individuals). In contrast, the age overestimation and the low specificity values obtained by these methods impose prudent application in criminal case, possibly being advisable to apply two or more methods of DAE and/or to compare conclusions with those obtained through other methods of age estimation (e.g. maturation of wrist-hand bones). The forensic application of W or D in criminal cases always requires an accurate and prudent review of the results before the final conclusion is expressed and the odontologists should, at least, provide to the judge or the institution the percentages of false positive results expected with the adoption of these methods.

CONCLUSION

The authors, examining a sample of Italian children aged between 11 and 16 years, verified that:

- The Willems and Demirjian methods are more accurate than Cameriere and Haavikko, but they tend to overestimate the age.
- The Cameriere method largely underestimates the age (~1 year) for both genders and with all the operators.
- The Haavikko method is not suitable for dental age estimation in the Italian population.
- The Willems and Demirjian methods yielded high sensitivity but low specificity, thus producing consistent rates of false positive cases.
- As for the specificity rate, the Demirjian method is more suitable for females, while, for the sensitivity rate, the Willems's method is the most indicated.

The results from our research make it difficult to identify a method that can be considered universally valid and the best to estimate the age of an Italian sample of children at the 14-years threshold, either for civil or criminal proceedings. Given the different legal requirements imposed by civil and criminal law, and especially for the latter, the examiner should therefore apply at least two methods of DAE for a sound comparative examination and provide estimations that report the false positive rates registered for the applied methods.

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I-O-F-O-S

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SECTION IDENTIFICATION

Sexual Dimorphism in Brazilian Human Skulls: Discriminant Function Analysis

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The authors declare that they have no conflict of interest.

ABSTRACT

Many anthropological studies have shown that sex can be determined using the human skeleton, especially by examining the pelvis and skull. The aim of this study was to verify the presence of sexual dimorphism in the Brazilian population by craniometric analysis; to identify the most reliable measurements and to propose a discriminant function for sex determination. The selected sample was composed of 100 adult skulls, 50 male and 50 female, from Cuiabá city, Mato Grosso State, Brazil. Of all the measurements taken, only the difference between the bi-euryon distances has proven insignificant, while themost dimorphic measure was the bi-zygomatic diameter. A discriminant function was obtained by applying the bi-zygomatic and the basion-lambda measurements, with a confidence level of 72%. The authors concluded that most of the traits analyzed are sexually dimorphic and the discriminant function elaborated is reliable for sex determination in human identification for forensic purposes.

KEYWORDS: *Forensic anthropology, Sexual dimorphism, Craniometry, Forensic Dentistry.*

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INTRODUCTION

Human identification is one of the major and most important tasks of Forensic Medicine and Dentistry. The identification of a deceased individual holds social, economic and legal repercussions. Soft tissues are commonly no longer present, due to carbonization, trauma or advanced decomposition. In those cases, forensic anthropology serves an important role in human identification.[1,2]

One of the main features considered in anthropological analysis for identification purposes is the sex. The most reliable bone structures for sex determination are the pelvis and the skull.[1,3,4] Studies based on metric features of teh pelvis and skull have been considered most reliable, given their objectivity, reproducibility and statistical value.[5, 6]

Anthropological features vary from one population to another. Therefore, human identification methods within this science must be validated before they can be used in each geographical group[5]. Sex determination by metric traits of cranial structures has been tested by many authors around the world.[1-3, 6-13]

The aim of this study was to evaluate the sexual dimorphism in metric relations between anatomical points of the skull, as well as to compile an effective mathematical function for sex determination in a Brazilian population, located in Central-Western region of the country.

MATERIAL AND METHODS

A sample composed of 100 human skulls with known sex and age at death, with equal number of each of the sexes. After cleaning the material, each skull received a code number to minimize operator bias. The damaged skulls were excluded, as well as those with signs of gross asymmetry, trauma, or that belonged to individuals under the age of 22 years at death.

Craniometric landmarks were located according to standard textbooks of anthropology.[14-5] described on Table 1, shown on Figure 1

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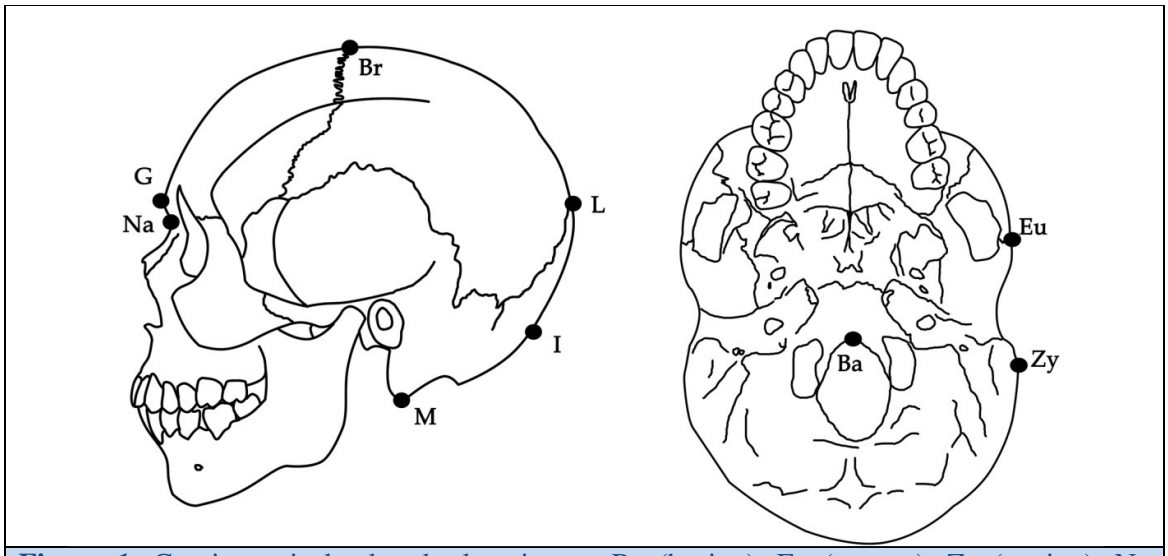

Figure 1: Craniometric landmarks locations – Ba (basion), Eu (euryon), Zy (zygion), Na (nasion), G (glabella), Br (bregma), L (lambda), I (inion), M (mastoid).[14-15]

Table 1: Definition of craniometric landmarks.[14-5]

Basion	The most inferior point on the anterior border of the foramen magnum
Bregma	The point where the sagittal suture ends anteriorly at the coronal suture in the sagittal plane
Euryon	The lateral point on the parietal bone that marks the greater transverse diameter of the skull
Glabella	The most anterior point on the frontal midline, between the supraorbital tori
Inion	The most prominent projection of the occipital bone at the most superior point of the external occipital protuberance
Lambda	The midline point where the sagittal suture meets the lambdoid suture
Mastoid	The most proeminent and inferior point of the mastoid process
Nasion	The point where the internasal suture meets the nasofrontal suture in the midsagittal plane
Zygion	The lateral point on the zygomatic bone that marks the greatest bizygomatic diameter

Table 2: Mean, standard deviation, Student´s t-test for paired samples, and Dahlberg´s test for random and casual error.

Measure	1st exam		2nd exam		t	*p*	Error
	mean	SD	mean	SD			
Na-L	171.64	7.09	171.48	7.15	1.398	0.175	0.41
Na-Ba	97.54	5.28	97.02	5.16	2.865	0.009*	0.73
G-L	171.84	7.48	171.70	7.59	1.319	0.200	0.38
G-I	172.60	10.57	172.10	10.54	2.182	0.039*	0.87
G-Br	102.82	6.19	102.34	5.88	1.949	0.063	0.91
Ba-L	111.46	6.19	110.98	6.20	1.193	0.244	1.43
Ba-Br	127.90	8.91	127.96	8.73	0.398	0.694	0.52
Zy-Zy	108.09	8.53	107.04	8.51	3.244	0.003*	1.34
Eu-Eu	140.20	8.74	140.46	8.47	1.162	0.257	0.80
M-M	106.06	6.27	105.82	6.17	2.073	0.049*	0.45

The measurements were taken with a digital caliper (Digimess™, Sao Paulo, Brazil), and outside spring caliper for regions where the digital caliper could not be used. All the exams were performed by a single observer. The measures taken were the following: Nasion-Lambda (Na-L); Nasion-Basion (Na-Ba); Glabella-Lambda (G-L); Glabella-Inion (G-I); Glabella-Bregma (G-Br); Basion-Lambda (Ba-L); Basion-Bregma (Ba-Br); Zygion-Zygion (Zy-Zy); Euryon-Euryon (Eu-Eu); Mastoid-Mastoid (M-M).

Twenty-five skulls were re-examined after a two weeks interval, to evaluate the intra-examiner reliability, by Student´s t-test for paired samples. The random error was assessed by the formula proposed by Dahlberg: error = $\sqrt{\Sigma d^2/2n}$, where d = difference between 1st and 2nd measurements, and n = number of repetitions.

Discriminant analysis stepwise model was used to verify the best mathematical model to discriminate sexes and determine which measure is more relevant. Results with p <0.05 were considered statiscally significant. All statistical procedures were performed using Statistical Package for the Social Sciences – SPSS™, version 13.

RESULTS

The data for the systematic and casual error are presented in Table 2, and four of nine measures of paired samples showed statistically significant results.

The Kolmogorov-Smirnov test was used to check the data distribution, by which one can note that the variations within the measurements were statistically insignificant, as seen in Table 3.

Table 3: Kolmogorov-Smirnov test.

Sex	Measure	Mean	SD	*P*
Female	Na-L	169.47	7.24	0.216
	Na-Ba	96.03	4.83	0.903
	G-L	169.62	7.45	0.335
	G-I	169.07	8.28	0.878
	G-Br	102.36	6.14	0.890
	Ba-L	110.29	7.68	0.943
	Ba-Br	127.28	7.24	0.472
	Zy-Zy	103.29	5.99	0.745
	Eu-Eu	139.42	7.05	0.202
	M-M	103.75	5.05	0.853
Male	Na-L	174.15	5.53	0.915
	Na-Ba	99.72	4.97	0.545
	G-L	175.16	6.34	0.195
	G-I	176.45	7.30	0.620
	G-Br	106.06	5.96	0.934
	Ba-L	114.28	6.27	0.948
	Ba-Br	131.24	8.34	0.824
	Zy-Zy	110.88	7.06	0.746
	Eu-Eu	140.49	7.68	0.859
	M-M	107.05	5.95	0.353

Table 4: Variation of means between sexes analyzed by Student's t-test.

Measure	Female		Male		*p*
	Mean	SD	Mean	SD	
Na-L	169.47	7.24	174.15	5.53	<0.001 *
Na-Ba	96.03	4.83	99.72	4.97	<0.001 *
G-L	169.62	7.45	175.16	6.34	<0.001 *
G-I	169.07	8.28	176.45	7.30	<0.001 *
G-Br	102.36	6.14	106.06	5.96	0.003 *
Ba-L	110.29	7.68	114.28	6.27	0.005 *
Ba-Br	127.28	7.24	131.24	8.34	0.013 *
Zy-Zy	103.29	5.99	110.88	7.06	<0.001 *
Eu-Eu	139.42	7.05	140.49	7.68	0.470
M-M	103.75	5.05	107.05	5.95	0.004 *

With respect to differences between sexes, according to Student´s t-test, all the measures had statistically significant results, with exception of the bieuryon width, as shown in Table 4.

Discriminant analysis stepwise model, considering the nine variables studied Table 5, showed that the best variable to separate the groups is the bizygomatic width ($p < 0.001$) followed by the basion-lambda length ($p<0.001$). Applying other variables did not improve the discrimination obtained with these two variables.

The function obtained by discriminant analysis was: sex= (0.1373 x Zy-Zy)+ (0.0639 x [Ba-L-21.8876]) with a zero result pointing to females, and any greater result pointing to males, with success rates presented in Table 6.

DISCUSSION

Forensic anthropology is a branch of the Forensic Sciences concerned with the application of general anthropological knowledge and methods to the process of law.[16] The results obtained in this study show that for all the measures taken male subjects presented an average higher than for females, indicating that sex determination may be made by cranial measurements, as described by Günay &

Altinkök[7] and Kemkes & Gobel.[5] The accuracy of sex determination methods in highly interbred populations is usually lower than in areas with ethnic predominance of a single group, like in Central Europe.[5,13-4] In Brazil, the population is very miscegenated, due to the mixture between European, African and Asiatic immigrants and the indigenous population who already lived in the country. The population of the analyzed region was made up by the miscegenation of indians, whites and blacks, and the majority is comprised by *caboclos*, result of the miscegenation between whites and Indians, increasing the chances of great part of the sample belongs to this ethnical group.[17]

Table 5: Section points that optimize the success rates for the nine variables that show statistically significant difference between sexes.

Measure	Section point	Success rate		
		Total (%)	Female (%)	Male (%)
Na-L	166.50	65	36	94
Na-Ba	99.50	68	80	56
G-L	166.25	65	34	96
G-I	167.50	67	42	92
G-Br	101.28	63	46	80
Ba-L	114.50	64	76	52
Ba-Br	131.50	65	78	52
Zy-Zy	106.86	75	80	70
M-M	105.59	63	70	56

When determining the sex of a human skull, the randomly guessing would present an average accuracy of 50%, since theoretically the guess would be right in half of the cases.[18] And according to Sweet,[18] every human being has an identity in life and there is a basic societal need for this identity to be recognized after death, both for family consolation and for juridical purposes.

The sex estimation by analysis of the skull can be made by two methods available: the qualitative or quantitative method. The qualitative variables, used glabella features, thick bones and superciliary arches, form of treatment, bone surface appearance due to the action of the muscles, mastoid processes, the parietal eminences, alveolar arch, and coronoid fronto-nasal articulation.[19] The quantitative variables use measurements between pre-set points for sex identification.[20]

Table 6: Success rate of the discriminant function analysis with the measures Zy-Zy, and Ba-L

		Sex	Result		Total
			F	M	
Sample	n	F	38	12	50
		M	16	34	50
	%	F	76	24	100
		M	32	68	100
Total success rate					72

Suazo et al.[21] examined 284 adult skulls from São Paulo State, of which 187 were male and 97 female, and verified through quantitative methods that the best indicators were found in traits whose formation is related to the insertion and action of major muscle groups, such as mastoid process, zygomatic bone, mandible, and roughness of the occipital bone.

According to Vanrell,[22] the differential diagnosis of the sex by the analysis of the morphological characteristics of the skull and mandible has 77% success rate, while the analysis on metric traits of the skull in distinguishing the sex exceed 90% accuracy. In this, research the authors chose to take measurements of the skull with subsequent statistical analysis by discriminant function, which represents an objective method for sex determination, as suggested by Steyn & Iscan[6] and Patil & Mody.[1] According to these authors, a discriminant analysis overcomes subjective methods, providing a more reliable result, reason why it has been increasingly used in sex determination.

Amongst all the measurements performed in this study it was observed, according to discriminant analysis, that the bizygomatic distance presented the greatestsexual dimorphism, corroborating the results of other researchers, such as Krogman & Iscan,[23] Steyn & Iscan,[6] Monticelli & Graw,[8] Kranioti *et al.*[10] and Naikmasur *et al.*[12]

The bieuryon width showed no significant dimorphism in this sample, unlike the data presented by Franklin et al, [24] who analyzed eight measures in skulls of South African indians, and the measures with greatest dimorphism were bizygomatic distance, length and height of the skull.

Skulls from the sample were not distinguished by ethnic traits, and the fact that the regional population is marked for its high level of miscegenation, may have influenced the results obtained. It seems fair to conclude that, as defended by Iscan,[25] cranial patterns are population-specific features, affected by environmental factors such as diet, climate, and culture. Yet, it should be noted that the bizygomatic width represents an important feature in evaluating the sexual dimorphism in several populations, and should always be

considered in sex determination for human identification.

CONCLUSION

From the analysis of the results, the authors conclude that all cranial measurements performed showed significant values for sex determination, except for the cranial width (bieuryon width). Among all the measurements taken in this research, the greatest sexual dimorphism was shown by the bizygomatic width. Through discriminant analysis, a mathematical model for sex determination was developed applying the bizygomatic width and the basion-lambda length, with a cofidence level of 72%.

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SECTION AGE ESTIMATION

Dental Age Estimation in Japanese Individuals Combining Permanent Teeth and Third molars.

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ABSTRACT

The study aim was, firstly, to verify the Willems et al. model on a Japanese reference sample. Secondly to develop a Japanese reference model based on the Willems et al. method and to verify it. Thirdly to analyze the age prediction performance adding tooth development information of third molars to permanent teeth.

Retrospectively 1877 panoramic radiographs were selected in the age range between 1 and 23 years (1248 children, 629 sub-adults). Dental development was registered applying Demirjian 's stages of the mandibular left permanent teeth in children and Köhler stages on the third molars. The children's data were, firstly, used to validate the Willems et al. model (developed a Belgian reference sample), secondly, split ino a training and a test sample. On the training sample a Japanese reference model was developed based on the Willems method. The developed model and the Willems et al; model were verified on the test sample. Regression analysis was used to detect the age prediction performance adding third molar scores to permanent tooth scores.

The validated Willems et al. model provided a mean absolute error of 0.85 and 0.75 years in females and males, respectively. The mean absolute error in the verified Willems et al. and the developed Japanese reference model was 0.85, 0.77 and 0.79, 0.75 years in females and males, respectively. On average a negligible change in root mean square error values was detected adding third molar scores to permanent teeth scores.

The Belgian sample could be used as a reference model to estimate the age of the Japanese individuals. Combining information from the third molars and permanent teeth was not providing clinically significant improvement of age predictions based on permanent teeth information alone.

KEYWORDS: *Forensic science, Forensic Odontology, Dental Age Estimation, Willems method, Third molars.*

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INTRODUCTION

Errors in age estimation can be calculated subtracting the predicted age from the chronological age of an individual. Dental age estimations provide the smallest errors using age related tooth developmental variables, especially in children. The main reason is that in children developmental variables can be observed in multiple tooth types and positions and that they can be combined [1]. In sub-adults only third molar(s) development can be considered [2,3]. The development of the seven lower left permanent teeth is used by diverse authors to estimate the age of children until 16 year[4, 5]. In these children the technique described by Willems et al. provides most accurate age predictions[1]. Since third molars start developing in children at the age of 7, they can be considered as an additional age predictor in children[2,6]. Another possible aspect influencing dental age prediction outcomes is discrepancies between the origin of the individuals in the used reference sample and the individual whose age has to be predicted. Verifying an age prediction model developed on a reference population similar in origin as the verification sample and a model developed on a non corresponding reference sample, can reveal possible disagreements[7,8]. Therefore the population considered in the current study was Japanese, allowing to check the performances of the Willems et al. model developed on a reference sample of 2116 Belgium children.

In Japan specific ages are of forensic interest. The national age of sexual consent is 13 years, as specified by the Japanese Penal Code Articles 176 and 177. 'Art. 177 Penal Code ' stipulates that a person who has sexual intercourse (through violence or intimidation) with a female person of not less than 13 years, commits the crime of rape and shall be punished with imprisonment at forced labor for a limited term of not less than two years. Art. 176 Penal Code states that a person who, through violence or intimidation, commits an indecent act upon a male or female person of less than 13 years shall be punished with imprisonment at forced labor for not less than six months no more than seven years. The same shall apply to a person who commits an indecent act upon a male or female person less than 13 years. The Japanese age of majority is 20 years and at the age of 18 year a license to drive can be obtained.

The aim of this study was, to verify the Willems et al. model on a Japanese sample, to construct a Japanese reference model based on the Willems et al. method, and to verify this reference model, and to analyze the age prediction performance adding tooth development information from third molars to permanent teeth.

Materials and Methods

Digitally collected panoramic radiographs of 1877 Japanese individuals (n=1877) with verified chronological age between 1 and 23 year were gender specific selected (Table1). In each age category of 1 year, starting at 5 years, around 50 male and female individuals were selected. Individuals with observed dental pathology influencing tooth development were excluded. The collected sample was divided into children (n=1051) with age between 5 and 15.99 year and sub-adults (n=826) between 16 and 23year. Care was taken that in the sub-adult group at least one third molar was present. In the children dental maturity of the lower left permanent teeth (except the third molar) was registered, using the 8 point staging technique proposed by Demirjian et al.[4]. The maturity of all present third molars was registered the 10 point staging

technique proposed by Gleiser et al.[9], modified by Kohler et al.[10]. In case different developmental stages were observed on the diverse roots of a third molar, the lowest stage was registered. The staging was facilitated using the image ameliorating tools of Adobe®Photoshop® CS2 version 9.0 photo editing software. In particular the image magnification, contrast and brightness were optimized.

The children sample was used to verify the Willems et al.[5] model.

Table1: Age and sex distribution of Japanese sample

SEX	N	MEAN	STD	MEDIAN	MIN	MAX	Q1	Q3
F+M	1877	13.9	5.0	14.2	0.5	23.8	9.7	17.7
F	974	14.2	5.2	14.5	0.5	23.8	9.9	18.2
M	903	13.6	4.8	14.0	5.0	22.9	9.4	17.3

F= female, M=male, N= number, STD= standard deviation, MIN= minimum age, Max= maximum age, Q1= first quartile, Q3= third quartile. All values (except N) are expressed in years.

Randomly, but stratified on age and gender, the children sample was split into a training (n=532) and a test (n=519) sample. On the training sample a Japanese reference model, based on the Willems et al. method, was developed. The test sample was used to verify the new developed Japanese reference model and the Willems et al. model.

The error in age predictions was calculated subtracting the estimated age from the chronological age. Calibrations of the error were obtained calculating mean error (ME), quantifying the direction of the error (positive ME= underestimation); mean absolute error (MAE), quantifying the magnitude of the error and the root mean square error (RMSE), enabling to quantify the variability in errors (giving more weight to large errors).

On all subjects with registered Demirjian stages on the mandibular left permanent teeth (PM) and Gleiser et al. stages, modified by Köhler on third molars, regression models were developed: a model with only PM, a model with only TM and a model with PM and TM as predictor(s) and age as response. The RMSE were calculated from the models to evaluate the magnitude of the error in age prediction.

All statistical analyses were done using the SAS software, version 9.2 of the SAS system for windows (SAS

Results

Verification of the Willems et al. model on the Japanese children sample provided overestimated age predictions (ME=-0.02 year, MAE= 0.80 year, RMSE= 1.10 year). Comparing the predictions from the new constructed Japanese reference model and the Willems et al. model resulted in comparable calibration measures, except for the difference in ME in females (0.14 year).(Table 2).

In the Japanese children the smallest RMSE values were obtained from the model with only PM compared to the model with only TM and the model with PM and TM combined. The RMSE values were not constant over the different age categories (Table 3)

Discussion

The differences in ME, MAE and RMSE between the prediction from the Willems et al. model and the prediction from the new constructed Japanese reference model reflect the usefulness of the Belgian population as reference. A factor

Table 2: Mean error, mean absolute error and root mean squared error in females and males verifying the Willems et al. and the Japanese reference model on the test sample**.**

	FEMALE							MALE						
Model	**n**	**ME**	**SD**	**MAE**	**SD**	**RMSE**	**95% CI**	**n**	**ME**	**SD**	**MAE**	**SD**	**RMSE**	**95% CI**
Willems et al.	257	0.08	1.10	0.85	0.70	1.10	1.01;1.20	262	-0.06	0.97	0.77	0.59	0.97	0.89;1.06
Japanese reference	257	-0.06	1.04	0.79	0.67	1.04	0.95;1.13	262	-0.09	0.95	0.75	0.58	0.95	0.87;1.03

n= number; ME= mean error; SD: standard deviation; MAE: mean absolute error; RMSE: root mean square error; CI: confidence interval

Table3: Root mean square errors from the regression model with only permanent teeth, only third molar and the combined tooth development information as predictor(s) and age as response.

	Female			Male		
Age category	**RMSE PT**	**RMSE TM**	**RMSE PT+TM**	**RMSE PT**	**RMSE TM**	**RMSE PT+TM**
<8 years	0.80 (0.67;0.94)	1.97 (1.65;2.30)	0.76 (0.63;0.88)	0.77 (0.64;0.89)	2.31 (1.91;2.68)	0.75 (0.63;0.88)
8-10 years	1.03 (0.81;1.25)	0.81 (0.64;0.98)	0.91 (0.72;1.10)	1.01 (0.80;1.22)	0.58 (0.46;0.70)	0.94 (0.75;1.14)
10-12 years	1.43 (1.11;1.75)	1.83 (1.41;2.24)	1.45 (1.13;1.78)	1.18 (0.93;1.44)	1.80 (1.41;2.20)	1.19 (0.93;1.45)
12-14 years	0.79 (0.60;0.97)	2.62 (2.00;3.24)	0.890 (0.69;1.11)	0.92 (0.71;1.13)	2.82 (2.18;3.47)	0.90 (0.70;1.11)
14-16 years	1.26 (0.98;1.54)	2.89 (2.24;3.54)	1.27 (0.98;1.56)	0.98 (0.75;1.21)	2.71 (2.06;3.36)	1.03 (0.78;1.28)
8-16 year	1.06 (0.96;1.16)	2.09 (1.90;2.29)	1.05 (0.95;1.14)	0.96 (0.87;1.05)	2.16 (1.96;2.36)	0.95 (0.86;1.04)

RMSE: root mean square error; PT permanent teeth; TM: third molars

influencing these comparable results was the difference in size of the current Japanese training set (n= 539) and the set of subjects used by Willems et al. to develop their prediction model (n= 2116). In the Willems et al. model no linearity assumption was made with respect to the

Demirjian scores. Therefore, the same approach was being used in the current analysis. Allowing non-linearity by using the score as a categorical predictor in the regression model, increases the risk of overfitting. This phenomenon will be stronger, the smaller the training data set

and is therefore another factor possibly influencing the study results. The fact that the results of the verification of the Willems et al. model on the Japanese children sample, provided similar or better age prediction results than the verified new

developed Japanese reference model, additionally justifies the use of the Willems et al.[5] model for age estimations in Japanese children.

Liversidge et al.[11] concluded that significant differences in Demirjian's dental maturity method in different groups are incorrectly interpreted as population differences. This conclusion is confirmed in the current study. Indeed the used Willems et al. model was derived from the Demirjian's dental maturity method and the obtained results of the verified new developed Japanese reference model and the Willems et al. Belgium reference model did reveal similar results in age predictions. The fact that the test sample used for this verification was from Japanese origin had no influence on the error in age prediction.

In the Japanese children an overall decrease in RMSE values (age range 8-16 year) was detected adding TM information to PM information. This decrease was not big enough to provide better RMSE values than obtained with PM information alone. Moreover added TM information to PM information decrease the RMSE values obtained from PM information alone. These finding proofs that only the combined age related information of the mandibular left 7 permanent teeth (PM) should be considered for optimal age predictions in (Japanese) children.

An age of forensic interest in Japan is the age of 13 years because the punishment of a crime committed to on a child above or under this age will be different. It was found that in this age category adding third molar development information to the permanent teeth development information was not decreasing the obtained RMSE values. Therefore in a Japanese population the ages in this particular age category are at best estimated based on the permanent teeth information alone.

Conclusion

The Belgian population used as a reference sample to develop the Willems et al. age prediction model was proven useful in Japanese children. In Japanese children age related information collected from third molars and permanent teeth provided no or clinically insignificant improvement in age prediction accuracy.

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