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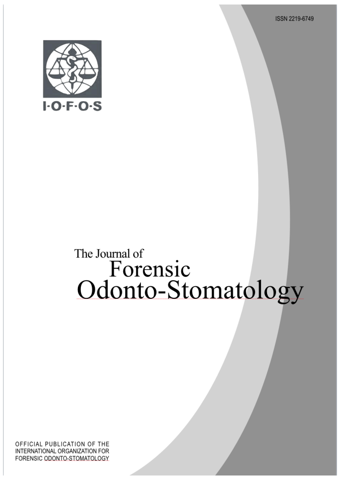

THE JOURNAL OF FORENSIC ODONTO-STOMATOLOGY

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CONTENTS

Intersection of forensic odontology and psychology

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KEYWORDS

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ABSTRACT

Forensic odontologists are expected to deal with challenging demands which can affect their mental health while dealing with forensic activities. This study aimed to explore the psychological impacts of forensic activities on forensic odontologists and students undertaking training. Firstly, it of an integrative review (part I) on the psychological effects of forensic odontology practice. The review was performed on Scopus, Medline and Web of Science. Next, an anonymous online survey using JISC Online Surveys tool (part II) was performed to assess the inherent opinions of forensic odontologists from the the International Organization for Forensic Odonto-Stomatology (IOFOS), and Association of Forensic Odontologists for Human Rights (AFOHR), and Dentify.me. Results were quantitatively evaluated by means of descriptive statistics and qualitatively upon reflection using Microsoft Office Excel (2010). Part I, only one full-text article out of 2235 (Webb et al., 2002) was found eligible indicating a low number of eligible studies. Part II, 75 forensic odontologists and 26 students (49.9% male; 50.5% female) from over 35 countries participated. Results showed that forensic dentists are more psychologically or emotionally affected by child abuse cases and least affected by age estimation cases. Most experienced forensic odontologists reported the lowest scores of discomforts. Males were more comfortable than women in dealing with stress. 80.77% (n= 21) of the students have not experienced any behavioural changes following mortuary sessions but 19.2% (n= 5) witnessed stress. All respondents support the inclusion of a module in Psychology or stress management in training programmes in Forensic Odontology. Suggestions to maintain mental health are considered by the respondents and topics to be taught suggested by a psychologist.

INTRODUCTION

Psychology is defined as *"the study of the nature, functions, and phenomena of behaviour and mental experience"*.[1] In criminal profiling, psychology is used for psychological interviews of serial killers, studies of crimes involving high profile criminals, rapists etc., whose brains need to be understood. Experts working in the field of Forensic Odontology come across a plethora of challenging cases such as human dental identification, dental age estimation of both living and deceased, analysis of bite mark identification and oro-facial injuries, child abuse or neglect and malpractice or fraud accusations in dentistry.[2] Dentists trained in Forensic Odontology should be prepared to work in psychologically challenging environments, such as the mortuary.[3] Forensic Odontologists are eventually accustomed to dealing with the deceased, or they are called to the courtroom as expert witnesses in both criminal and civil cases, but this comes with risk factors such as burnout, compassion fatigue, post-traumatic stress disorder.[3, 4]

Dentistry is a stressful vocation that not only affects the people who are practising but also affects other disciplines of dentistry.[5] Working conditions or circumstances of a forensic odontologist magnify the less desired side of human life which is physically and emotionally challenging. Despite the honour the forensic odontologists have in the society, they are sometimes vulnerable and should reach out for help when in need. It is suggested that forensic dentists must be aware of the potential negative impacts and should have an active check on their mental and physical health at regular intervals.[2]

Overall, forensic odontologists may have to deal with some areas of mental concern such as cognitive bias, post-traumatic stress disorder and death. According to the Cambridge Dictionary, cognitive bias is "*the way a particular person understands events, facts, and other people, which is based on their own particular set of beliefs and experiences and may not be reasonable or accurate*."[6] Our brains work very effectively and in a systematic manner. Every individual recognises and explicates information, distinguishes between right or wrong, thus reaching a decision which is called complex cognitive mechanism. In contradiction, when people become experts in a particular field, their brains become very capable to give an expert opinion but at the same time, they become susceptible to complex cognitive mechanism leading to biases by neglecting other useful facts.[7]

Acute Stress Disorder is the initial trauma response and occurs prior to the chronic post-traumatic stress disorder (PTSD).[8] It is quite temporary in many but can be progressing throughout life in a few, leading to post-traumatic stress disorder.[9] PTSD has been recognized by American psychiatry as the stress-induced mental sickness.[10] It has been debated that the research materials are lacking because it is believed that the forensic professionals are expected to deal with stress and demands as part of their job. However, it has been viewed that the forensic professionals work under pressure and this pressure varies by field, casework, experience, and reporting conclusions.[11] A previous study, 31 dentists who had performed post-mortem identification reported post-traumatic symptoms.[12] 5% to 32% PTSD prevalence is seen in rescue or recovery profession, where the highest has been reported in workers with no prior training in disaster work.[2]

A forensic odontologist or a student under training might experience the death of a close friend or family. This can bring about a temporary deterioration in their physical, social, and psychological well-being as they come across dreadful circumstances during their work and education. In a study conducted in Australia, a chain of destructive repercussions was experienced by the individuals who lost their dear ones. The bereaved men experienced a gradual decline in their mental health when compared to females who experienced the same at a much greater level up to four years. Symptoms such as sadness, depression, irritability, anger are seen in common, and has the possibility to be triggered when working in a mortuary.[13]

Considering the sensitivity of the matter, this study aimed to explore the psychological impacts of forensic activities on forensic odontologists and students undertaking training.

MATERIALS AND METHODS

Ethical approval was granted from `*School of Health Sciences and Dentistry Research Ethics Committee*`, reference number UOD-SHS-SDEN-TPG-2020-027.

Part I – Integrative review

A research question '*What are the psychological effects of the forensic practice in male and female forensic odontologists?*' was based on PICO strategy for integrative review where, Population (P) stands for forensic odontologists; Intervention (I) stands for forensic practice; Comparison (C) stands for sex; Outcome (O) stands for psychological effects.

Studies assessing the psychological or emotional effects on forensic dentists were included. Exclusion criteria consisted of studies not related to topic of interest; literature reviews; studies that did not distinguish males and females; books and book chapters; personal opinions; teaching materials; scientific reports;

abstracts; patents; papers that investigated forensic experts other than forensic odontologists; case reports; letters to the editor and/or editorials; research articles published in languages other than English.
This integrative review was performed on the following electronic databases: Scopus, Medline and Web of Science. The Medical Subject Headings (MeSH) terms were used to create a strategic search string and are given in Table 1. Boolean operators such as "AND", "OR" were used to merge the strategic terms. The articles retrieved were exported to Endnote X9 v9 to search for duplicates. PRISMA checklist (www.prisma-statement.org) was used to screen the articles eligible for the study.[14]

Table 1. Terms used for Integrative Review using four different concepts.

	Concept 1	Concept 2	Concept 3	Concept 4
TERMS	Forensic	Dentist* Odontology* Science* Scientist* Expert*	Psychological Well-being "Mental health" Emotional Posttraumatic Resilience Mindfulness Stress Burnout Pressure	Work-related Job-related Career Job Workplace Casework Mortuary Occupational

This process was carried out in three steps: 1) the titles were assessed by the reviewer and those which matched the research topic were selected; 2) abstract reading and 3) non-eligible articles were excluded with reasons and the eligible ones were used for a full-text reading.
The following data were collected from the paper: identification of study (authorship, year and country of publication); characteristics of sample (sample size, response rate, age range of the sample, distribution of sex and geographic origin of sample). The assessment of studies was based on the following categories: a) Aim; b) Year of publishing; c) Type of paper; d) Included subjects (Total, Males/Females*); e) Age range; f) Geographic location; g) Area of forensic activity; h) Conclusion; i) Possible recommendations.

Part II – Survey
The survey aimed to investigate the opinions on the psychological effects of forensic activities for forensic odontologists and students undertaking forensic odontology training.
The online survey was designed to be implemented using Jisc Online Surveys and comprised of 2 sections (I and II), where the first introductory three questions were common for the professionals and students, and it was preceded by a Participant Information Statement (PIS). Section I consisted of nine questions to be answered by the professionals (8 closed-ended and 1 open-ended) and Section II consisted of eight questions (7 closed-ended and 1 open-ended) to be answered by students as seen in Table 2. The first three questions as well as the last question were common for both the categories of participants. A pilot study was performed prior to the survey, and it was circulated to 35 individuals, which included current and former staff and students at the Centre for Forensic Medicine and Dentistry, University of Dundee, Dundee, Scotland, UK. Out of 35, only 18 responded and necessary modifications were done to the final survey.
Forensic odontologists from professional organizations such as International Organization for Forensic Odonto-Stomatology (IOFOS) and associations named Association of Forensic Odontologists for Human Rights (AFOHR) and Dentify.me received a link to the survey via email to be answered in a period of two weeks. Data were quantitatively evaluated by means of descriptive statistics and qualitatively upon reflection using Microsoft Office Excel (2010).

Table 2. Distribution of survey questions according to categories

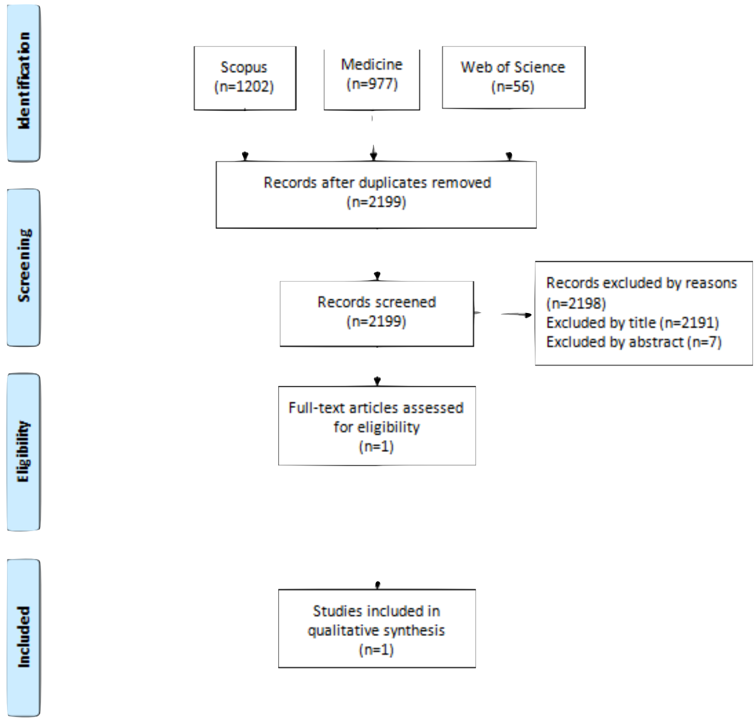
Questions 1-3 for all	**Categories**
1) Please state your gender. 2) Please choose the country you are based in. 3) Please mention the number of years of experience in Forensic Odontology.	General information of the participant.
Questions 4-11 for professionals only	
4) On a range of 0-4, how psychologically/emotionally comfortable are you in dealing with any forensic activity in general? 5) How easy is it to deal with stress in this field most of the time?	Level of comfort (0-Very uncomfortable; 1-Uncomfortable; 2-Neutral 3-Comfortable; 4-Very comfortable)
6) Which aspect of Disaster Victim Identification (DVI) is more difficult to deal with? Please select all that apply. a. Practical work b. Psychological state c. I never worked in DVI but willing to work d. I used to work in DVI in the past e. None of the above f. Other 7) Which aspect of bite mark cases is more difficult to deal with? Please select all that apply. a. Practical work b. Psychological state c. I never carried out bite mark analysis but willing to do d. I used to do bite mark cases in the past e. None of the above f. Other 8) Which area of an investigation do you think is more likely TO NOT affect you psychologically/emotionally? Please select all that apply. a. Dental identification of a traumatised body b. Dental identification of a burnt victim c. Dental identification of a body found in water d. Dental identification of a decomposed body e. Child Abuse- Physical or sexual f. Child abuse- emotional or negligence g. Bite Mark Analysis or other injuries h. Age estimation of a crime suspect i. Age estimation of an asylum seeker j. Disaster Victim Identification- number of bodies or body parts	Case type and possible challenges
9) How would you deal with your colleague who is distressed by Disaster Victim Identification (DVI) operation? Please select all that apply. a. Talk to the colleague personally. b. Talk to the team leader. c. Advise the colleague to consult the psychologist on site. d. I have never worked in DVI e. Other 10) What would you suggest, on a personal level, to your colleague who is facing any psychological/emotional work distress for some stress relief? Please select all that apply. a. Companionship b. Exercise c. Music d. Funny movie e. Games f. Go for a walk g. Take a break/vacation h. A cup of tea/coffee/meal i. Speaking to a friend/ family j. Advice to consult a psychologist k. All the above l. Other	Solutions to the problems.

11) Do you agree or disagree with the following statement: "*There should be an inclusion of a module in psychological/stress management in the Forensic Odontology programmes*"	Inclusion of Psychology module
Questions 12 - 19 for students only	
12) Are you aware that visiting a mortuary for dental identification might be part of a Forensic Dentistry programme? 13) In case you have already attended a mortuary for the very first time, what were your concerns before going to? a. 0- very apprehensive b. 1- slightly apprehensive c. 2-neutral d. 3- slightly excited e. 4-very excited f. I have never been to a mortuary 14) In case you have been to the mortuary, have you witnessed a classmate that has been distressed? 15) Did you ever have behavioral changes in your routine following a forensic case? Please select all that apply. a. Yes - Unable to sleep b. Yes - Unable to concentrate c. Yes - Anger or irritability d. Yes - Avoiding conversations, thoughts, places or people who remind you of it. e. No, never f. Other	Experiences related to mortuary sessions.
16) What would you suggest, on a personal level, to your colleague who is facing any psychological/ emotional work distress during training for some stress relief? Please select all that apply. a. Companionship b. Exercise c. Music d. Funny movie e. Games f. Go for a walk g. Take a break/vacation h. A cup of tea/coffee/meal i. Speaking to a friend/ family j. Advice to consult a psychologist k. All the above l. Other	Solutions to the stress challenges
17) Do you agree or disagree with the following statement: "There should be an inclusion of a module in psychological/stress management in the Forensic Odontology programmes". 18) In your opinion, a psychologist/counsellor should be part of the team delivering Forensic Odontology programmes?	Inclusion of psychology module and a psychologist
Question 19 for all	
19) Do you have any suggestions to support the mental health of Forensic odontologists or forensic odontology students?	Suggestions to improve mental health of forensic odontologists and forensic odontology students.

RESULTS

Part I, a total of 2235 studies were obtained on performing an integrative search. 116 duplicates were found, which gave a total of 2199 studies to be further screened. 2191 results were excluded by title and seven studies were excluded by abstract. Only a single full-text article was found eligible (Figure 1).

Figure 1. Flowchart of integrative literature search and selection criteria by PRISMA (53)



There is a lack of exploration on the psychological obstacles faced during forensic activities by odontologists. The last study on this topic was carried out in 2002, and therefore there is a huge interval resulting into lack of research materials till now. Details can be seen in Table 3. Part II, 101 participants (50 males and 51 females) completed the survey being 75 forensic odontologists and 26 students from over 35 countries as seen in Table 4 and Figure 2.

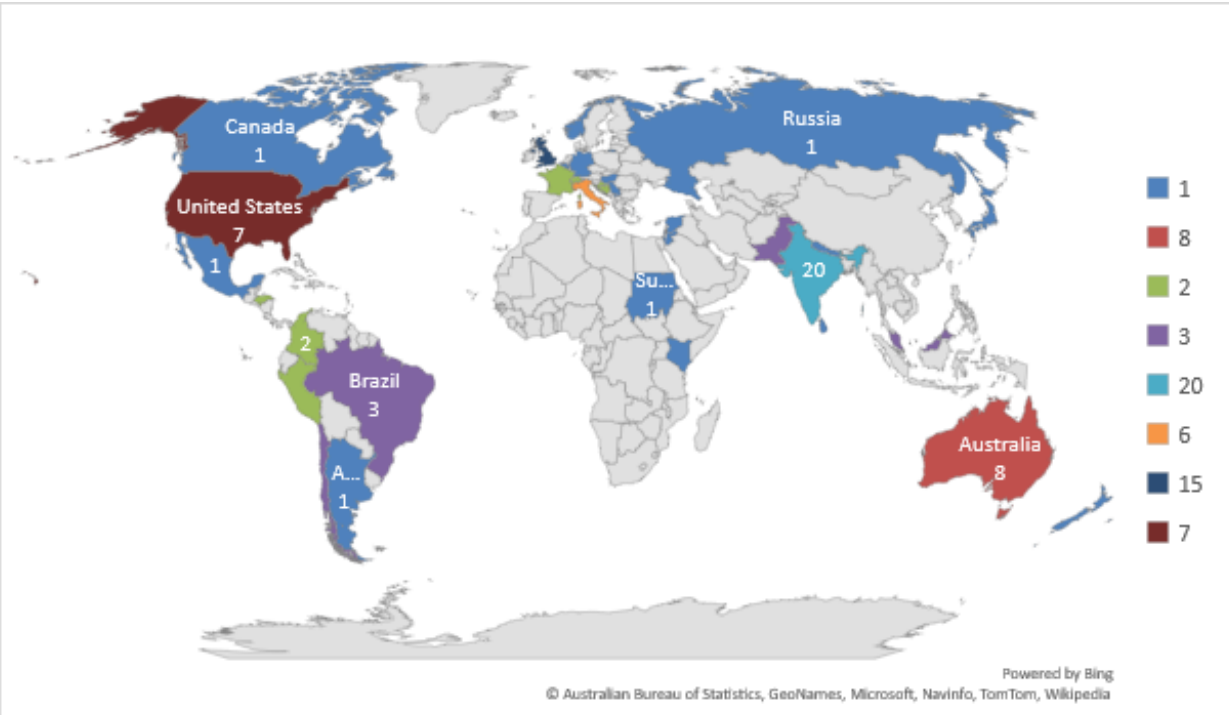
Table 3. Characteristics of eligible study

Title	The Emotional and Psychological Impact of Mass Casualty incidents on forensic odontologists
Year of publishing	2002
Type of paper	Survey based (Quantitative and Qualitative)
Included subjects (Total, M/F*)	Total=38, Masculine=34/Feminine=4 (95% response rate)
Age range	50-64 years (50% respondents)
Geographic location	United States and Canada
Area of forensic activity	Mass fatality incidents (airplane crash, vehicle accidents, natural disasters, fire. (Average of nine incidents attended).
Conclusion	Quantitative findings (one third respondents were upset, distressed or irritable, while the rest of them had a positive experience. Qualitative findings (Positive- mutual trust and friendship and sense of achievement, Negative- victims, politics and working conditions).
Possible recommendations	Preparedness among forensic odontologists have to be systematically studied and its potential psychometric properties must be explored.

Table 4. Breakdown of number of countries and participants

Countries	No. of participants
India	20
United Kingdom	15
Australia	8
United States	7
Italy	6
Brazil, Chile, Malaysia, Pakistan	3 each
Bosnia Herzegovina,	2 each
Colombia, Croatia, France, Honduras, Peru and Switzerland	
Argentina, Belgium, Canada, Germany, Hungary, Israel, Japan, Jordan, Kenya, Kosovo, Mexico, Nepal, New Zealand, Norway, Russian Federation, Serbia, Sri Lanka, Sudan and Syria	1 each

Figure 2. Breakdown of 35 countries participated in the survey



Students with no experience constituted about 20.79% (n= 21), followed by current students with experience 4.95% (n= 5), Professionals were of 0-5 years of professional experience 16.83% (n= 17), 6-10years of experience 15.84% (n= 16) and above 11 years of experience 41.58% (n= 42).

Forensic Odontologist's opinions

In this study, 45.33% (n= 34) were very comfortable in dealing with the forensic activities marking it to be the highest. 18.67% (n= 14) had neutral experience, whereas 30.67% (n= 23) were comfortable. Only 5.33% (n= 4) were uncomfortable and no one mentioned to be very uncomfortable. Table 5 and Figure 3 demonstrates that, on comparing the experience of forensic odontologists with level of psychological and emotional comfort at work, the level of psychological and emotional comfort while dealing with forensic activities increases along with their professional experience. On comparison between sex and level of psychological and emotional comfort at work, Table 6 and Figure 4 show that males are more comfortable than women in dealing with forensic activities.

Table 5. Experience VS Level of psychological comfort at work

Level of comfortability	0-5 years	6-11 years	Above 11 years
0-Very uncomfortable	0.0%	0.0%	0.0%
1-Uncomfortable	11.8%	6.2%	2.3%
2-Neutral	29.4%	18.8%	14.2%
3-Comfortable	35.2%	56.2%	19.3%
4-Very comfortable	23.6%	18.8%	64.2%

Figure 3. Illustration of the graph between experience and level of comfort at work

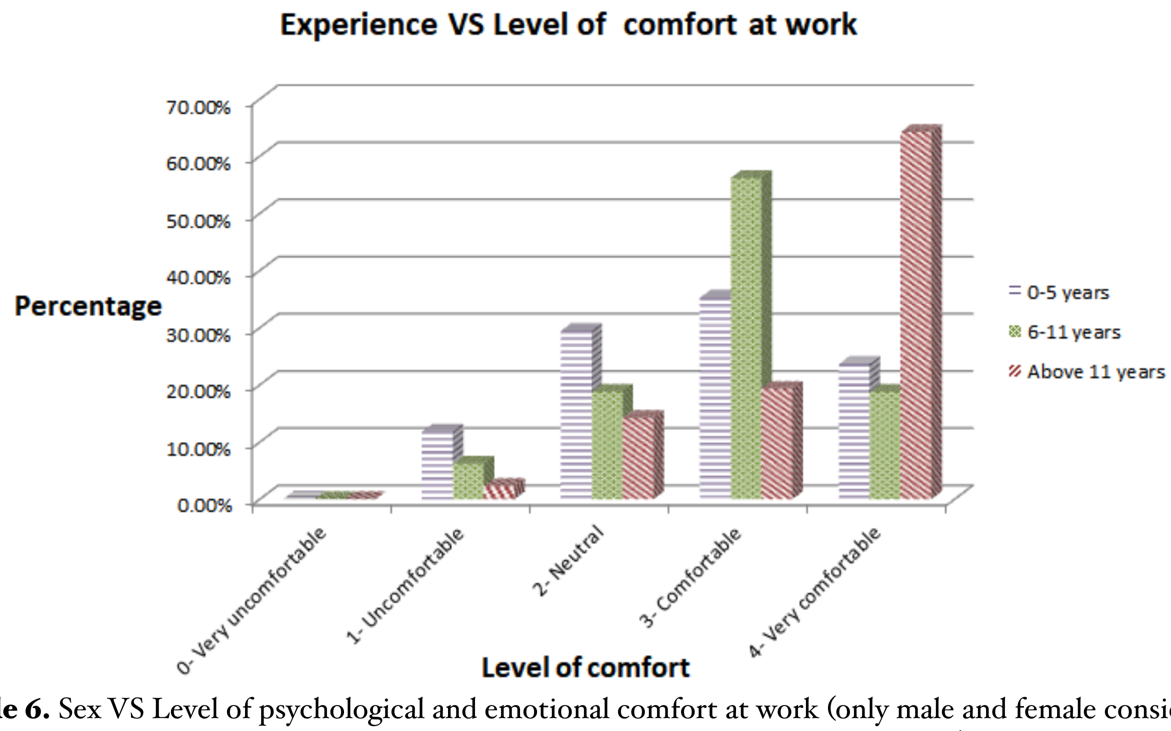
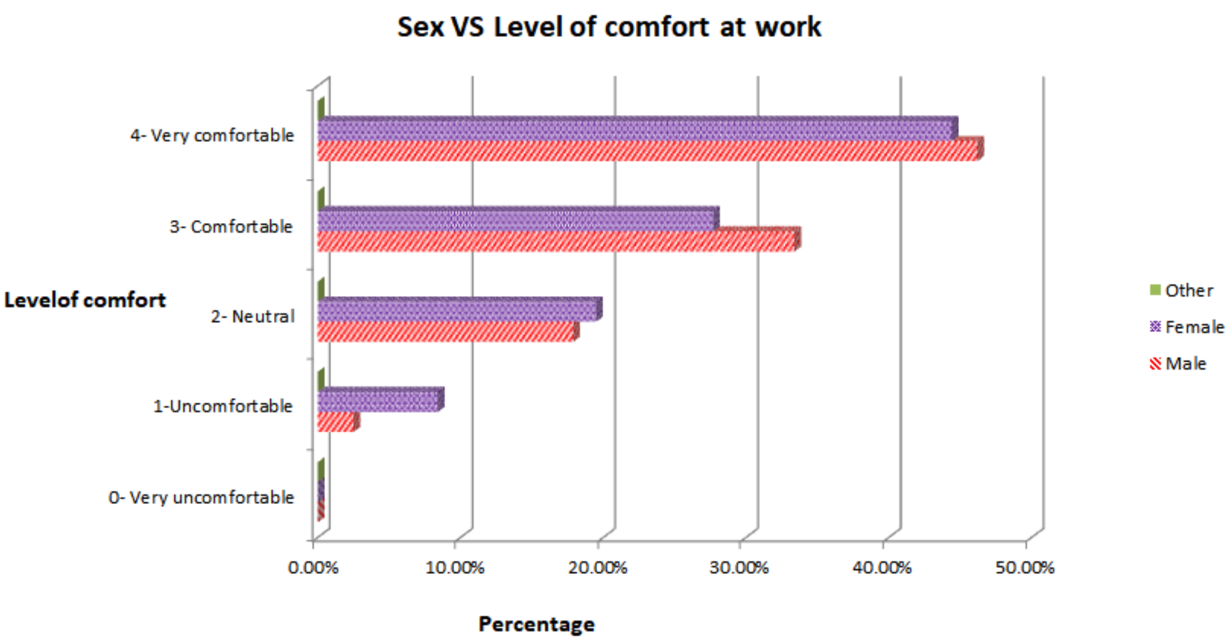


Table 6. Sex VS Level of psychological and emotional comfort at work (only male and female considered because the option 'other' was not chosen by any participant)

Level of comfort	Male	Female
0-Very uncomfortable	0.0%	0.0%
1-Uncomfortable	2.5%	8.4%
2-Neutral	17.9%	19.5%
3-Comfortable	33.4%	27.7%
4-Very comfortable	46.2%	44.4%

Figure 4. Illustration of the comparison between sex and level of comfort dealing with forensic activities.



Majority of the participants i.e., 32% (n= 24) were of the opinion that it is easy and 20% (n= 15) found it very easy to deal with the stress at work. None of the participants considered to be very difficult to deal with the stress level in their profession. 18.67% (n= 14) found the level of stress to be difficult. On the other hand, 29.33% (n= 22) felt that it is neither difficult nor easy. As per Table 7 and Figure 5, on comparing experience and stress level, results show that the level of stress reduces along with an increase in experience.

Table 8 and Figure 6 demonstrates that, on comparing two sexes and stress level, males handle stress more easily when compared to females.

Table 7. Experience VS Stress level at work

Stress level	0-5 years	6- 11 years	Above 11 years
0- Very difficult	0.0%	0.0%	0.0%
1-Difficult	23.5%	25.0%	14.2%
2-Neutral	29.5%	43.7%	23.8%
3-Easy	35.3%	31.3%	31.0%
4- Very easy	11.7%	0.0%	31.0%

Figure 5. Illustration of the comparison between experience and stress level at work

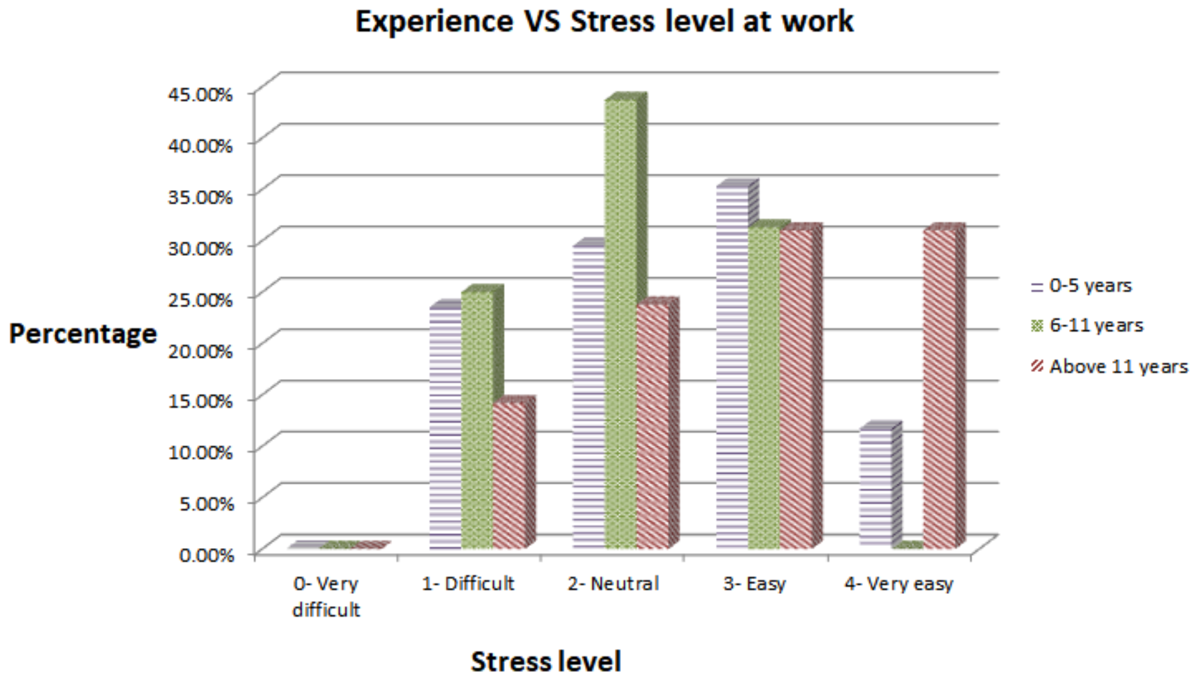
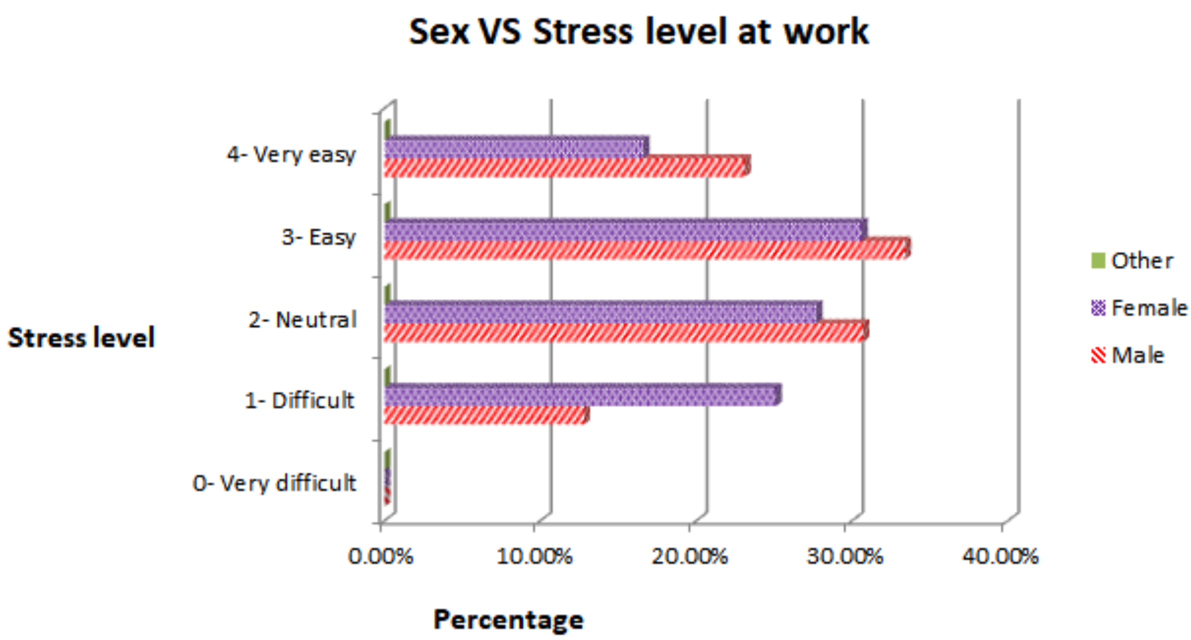


Table 8. Sex VS Stress level at work (only male and female considered because the option 'other' was not chosen by any participant)

Stress level	Male	Female
0- Very difficult	0.0%	0.0%
1-Difficult	12.8%	25.1%
2-Neutral	30.7%	27.7%
3-Easy	33.4%	30.6%
4- Very easy	23.1%	16.6%

Figure 6. Illustration of the comparison between sex and stress level at work

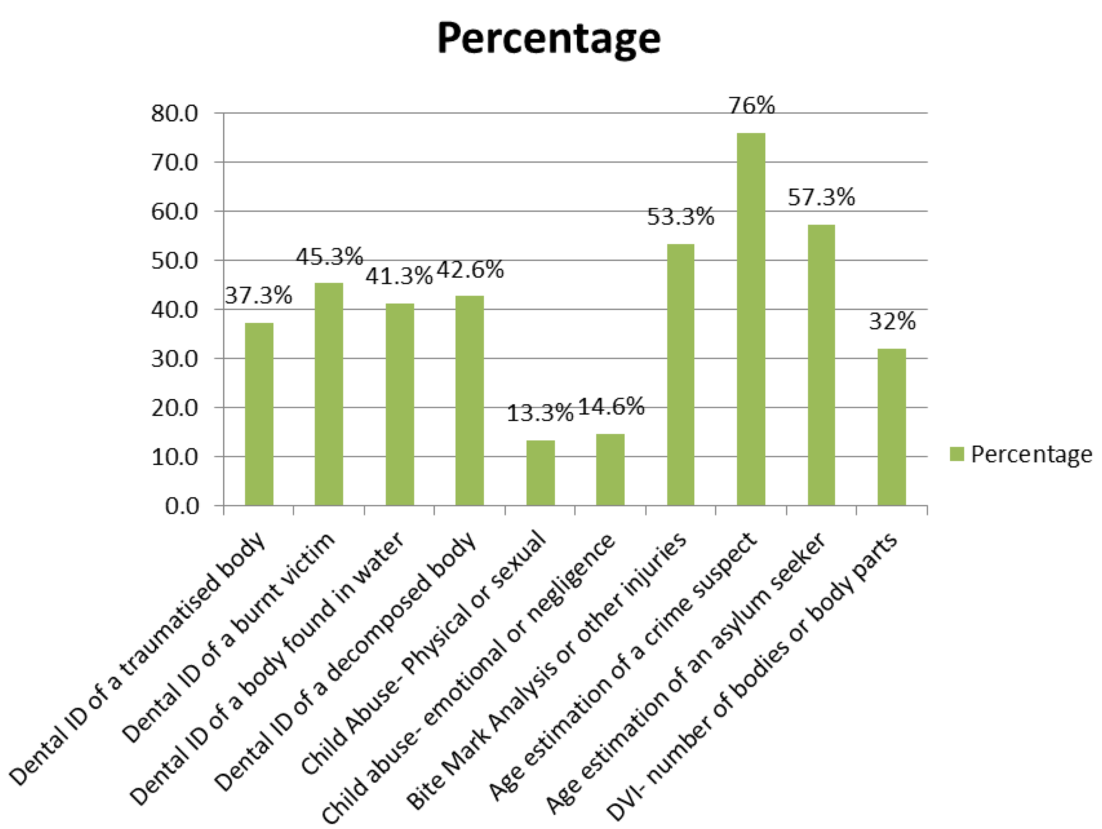


The psychological aspects of DVI were more challenging to the 28% (n= 21) of participants, whereas 17.3% (n= 13) participants felt the logistical aspect of work to be of a concern. 8% (n= 6) of the participants had other concerns such as lack of resources, dealing with child victims in DVI and relatives, also the authorities not following a standardised `*International Criminal Police Organization*` (INTERPOL) protocol and depending on local procedures making it difficult for the forensic odontologists to perform the best of their jobs.

Considering challenges in bite mark analysis, 26.6% (n= 20) considered the practical work more challenging than the psychological state with only 4% (n= 3). 5.3% (n= 4) raised concerns such as 1) adoption of standardized protocols, 2) preparation to be an expert witness in court and 3) interdisciplinary communications.

Most of the professionals admitted that they are not psychologically or emotionally affected in the areas of investigation such as the age estimation of a crime suspect with the highest percentage of 76% (n= 57). The professionals seemed to be most affected by child abuse-emotional or negligence with 14.6% (n= 11), followed by physical or sexual child abuse with 13.3% (n= 10) as seen in Figure 7.

Figure 7. Distribution of areas of investigation least affecting forensic odontologists (X axis= Areas of investigation; Y axis= Percentage of participants affected by respective investigation)



When dealing with work colleagues distressed by DVI operations, forensic odontologists mostly agreed to '*to talk to the colleague personally*', followed by '*to advise the colleague to consult the psychologist*' and '*to talk to the team leader*' as seen in Figure 8. The first two preferred options indicated the tendency to help the colleague directly.

The main suggestions given by professionals for stress relief included: a) advice to consult a psychologist, b) companionship, c) go for a walk, d) speaking to a friend or family, e) taking a break or vacation and f) music. A distribution of suggestions according to popularity is shown in Figure 9.

Figure 8. Suggestions to deal with colleagues on site

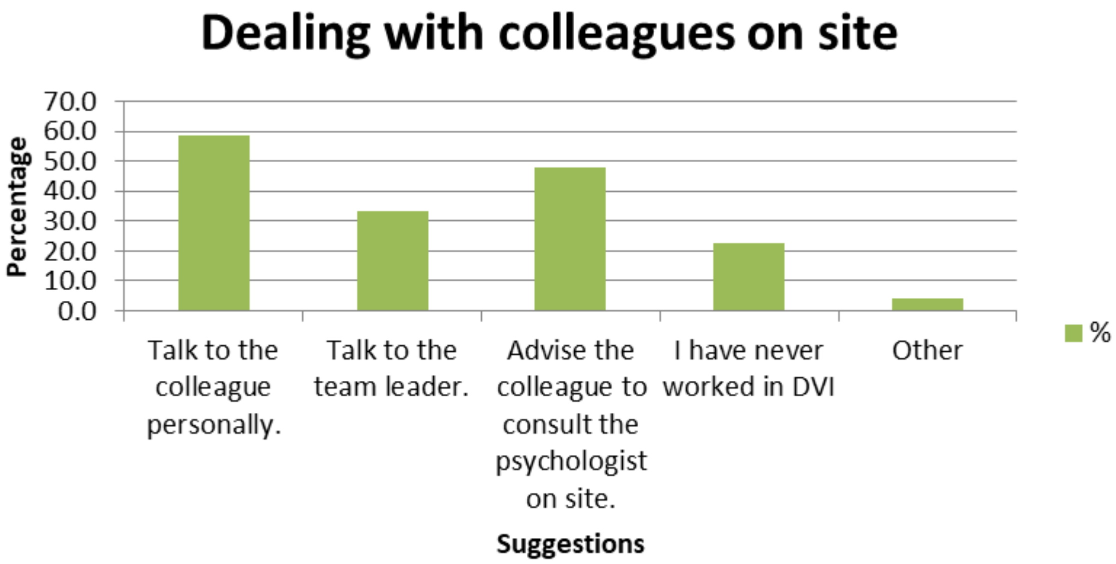
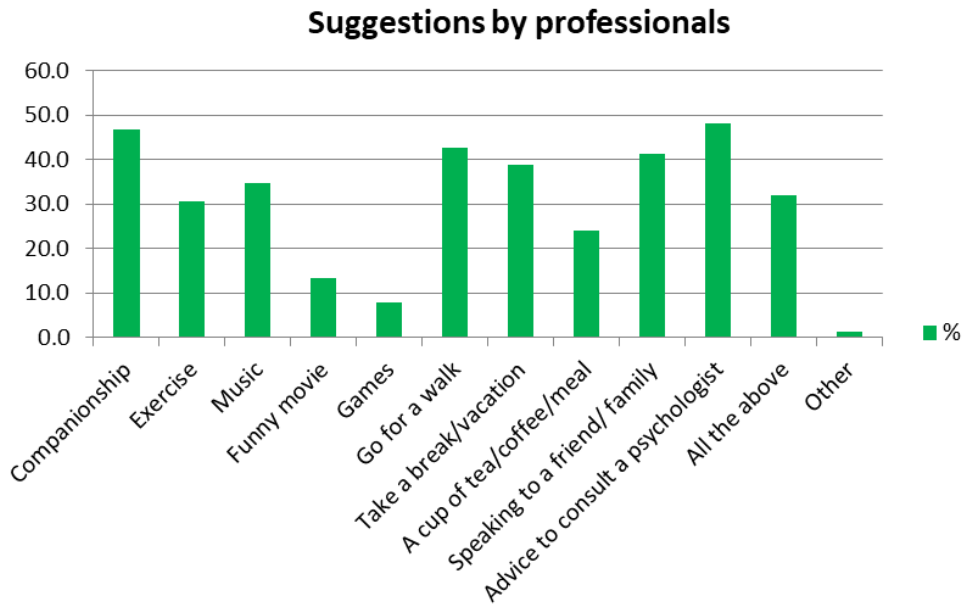


Figure 9. Suggestions by professionals for stress relief.



Students' opinions

All 26 students were aware that a visit to the mortuary could be part of the training; however, 23.08% (n= 6) students have never been to a mortuary before or during the course. About 19.2% (n= 5) of the students were aware of the fact that their classmate had been distressed due to condition or decomposition of the body and feeling dizzy on opening the body bag. 38.4% (n= 10) have never faced any such incidents. 15.3% (n= 4) are unaware of any such incidents if happened, while 26.9% (n= 7) have never been to any mortuary yet.

80.77% (n= 21) never had any behavioural changes but 11.54% (n= 3) ended up avoiding conversations, thoughts, places, or people which triggered them. 7.69% (n= 2) were unable to sleep and 3.85% (n= 1) was unable to concentrate. None of them had any issues such as anger or irritability.

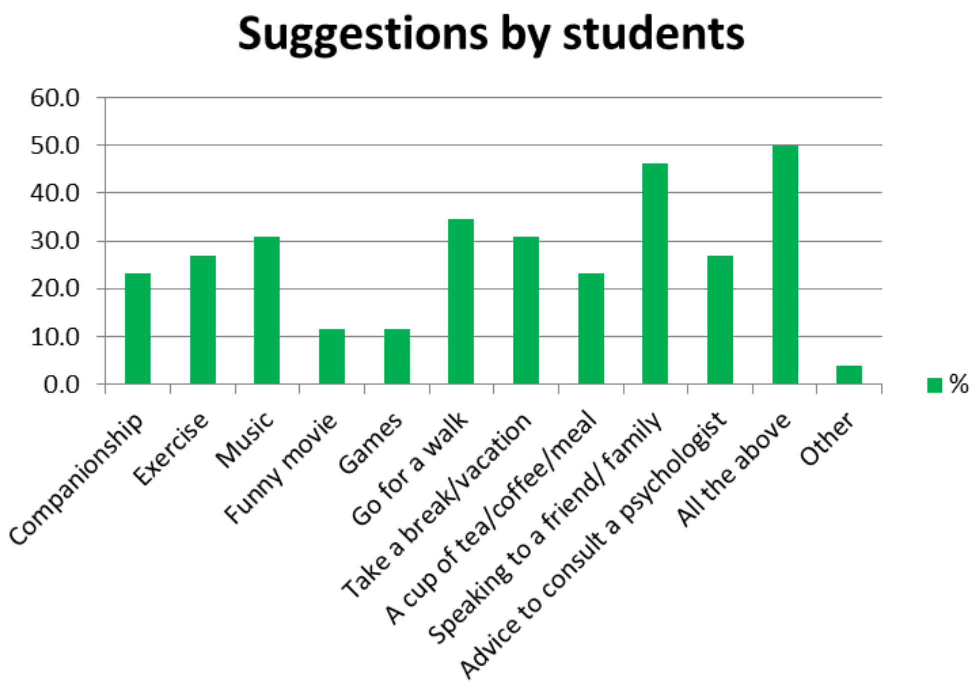
A distribution of suggestions given by students for stress relief according to popularity is shown in Figure 10 , and included: a) speaking to a friend or family, b) go for a walk and c) music.

Opinions of all

None of the professionals and students disagreed to the inclusion of a module in psychological or stress management in forensic odontology.

Majority of the students agreed to a psychologist being part of the Forensic Odontology programme. It has been observed in this study that, 68 participants out of 101, including both professionals and students contributed to making a list of similar suggestions to maintain mental health: yoga, meditation and sleep, training and drills of probable work situations, inclusion of Psychology module in trainings, reflective logs and debriefing following mortuary sessions, mental health assessments 2-3 times a year, tourism or vacations, inculcating hobbies and practicing normal dentistry were mentioned.

Figure 10. Suggestions by students for stress relief



DISCUSSION

Dentists contribute to the society in their most efficient ways and in return it brings them rewards and a chance to help the society. Therefore, people may choose dentistry as a profession because of the reputation and honour the degree offers or the dedication towards health care or serving the society through their profession.[15]

Forensic odontology has been into existence as a specialist discipline for a long time and for people who do not belong to the field of forensics; it is usually remembered as a tool for human identification. However, forensic odontology is broader than this. Forensic odontologists are asked to give opinion on age estimation in living and deceased persons, injuries related to child abuse and neglect, identification and analysis of bite marks (although the forensic value of this evidence is highly challenged) and civil cases which involve malpractice and fraud.

Forensic dentists should have a wide knowledge about dentistry, a systematic and an organised way of approaching cases in a patient and meticulous manner. Personal probity and emotional stability are essential too.[2]

On performing integrative review, it was observed that, there was a vast research gap on psychological impacts faced by professional forensic odontologists and students of Forensic Odontology, as the last paper published was in the year 2002. It is worth pointing out that, there has been a scarcity of research that addresses psychological issues in the field of Forensic Odontology. In the recent times, extreme efforts have been taken to strengthen mental health during the pandemic. Interventions like emergency psychological crisis treatment, 24-hour hotline assistance, online counselling services, mental health courses are given more attention.[16]

According to William Nicholson- "*Experience: that most brutal of teachers. But you learn, my God do you learn*."[17] The results of this study reveal that, the experience of the forensic odontologists increases the level of comfort dealing with forensic activities. It is presumed that experts are trained aptly, and they then progressively gather knowledge over extensive periods through their experience. However, qualifications and experience are not good indicators of performance because their worth is dependent on the features of the task setting in which they are attained.[18] Experienced forensic odontologists may face their first case of child abuse, for instance, later in their career; or may not have training experience with this subtopic of forensic odontology in their early education. It should be further investigated that experience and qualifications are necessary but does the features of a particular task and the working environment influence one`s ability to work, need to be explored. A study published in 2019, 38.9% of the forensic odontologists who work as lecturers have 2 and 5 years of experience in teaching.[3] It is important that the academic staff must have a good scientific background and a good amount of practical experience. More importantly, the teaching experience should be challenged with the experience as a forensic odontologist. This is to say that a forensic odontologist that is experiencing psychological issues in practice may reflect the negative impact of these issues on studies, while working as lecturer.

This study revealed that, males are more comfortable than women in dealing with forensic activities. In an illustrative study,[19] it was mentioned that emotional experience faced by men and women are alike to an extent, but women are more expressive with emotions such as fear, love, and sadness than men, whereas males express anger more frequently than females. It was found that, stereotypes based on adults' gender-emotion were based on a deficit model, which believed that men do not express what they feel.[19-21] Also, in the educational field of Forensic Odontology, male lecturers are predominant as compared to female lecturers.[3] But on the contrary, Forensic Science has managed to attract many females to the field.)[22] In a Brazilian dental website, it is noticed that the females working in Forensic Odontology accounted for 474 and as opposed to 341 males.[23] In a 2019 study, all 10 females who participated in the survey were postgraduates in the field of Forensic Odontology[3] This was assumed that they were not encouraged in this field, and they expressed their interests in the evolving course options in Forensic Odontology.

According to this study, males found it easier in handling stress than females while dealing with forensic activities. On assessing males and females with stress responsiveness, the actions of the Hypothalamic-Pituitary-Adrenal (HPA) axis such as cortisol and sympathetic nervous system (heart rate, blood pressure) plays an enormous role.[24] The HPA axis is a centrally controlled and regulated system that connects the central nervous system (CNS) with the hormonal system.[25] However, female sex organs shrink the effect of HPA response. Therefore, the cortisol feedback on the brain is decelerated and leads to less or late stress response. Depression is more likely to be developed in females because of the compromised reaction of cortisol.[24] Between puberty and menopause, HPA-axis and autonomic responses is lower in females than males of the same age. Also, sex differences before puberty, after menopause, use of oral contraceptives and pregnancy influences the physiology of the HPA axis too.[26] Some women are stressed because of the lack of motivation and encouragement from co-workers and superiors. However, the number of women working in forensics is large because of their maternal nature and maturity.[27] According to (psycho) sociological literature, "sex" defines the physiological and genetic differences between men and women, whereas "gender" refers to the experiences being a man or a woman. Women who work have more problems related to physical and mental health when contrasted to women who do not work. In different cultures, women are expected to take the role of a caregiver,[28] particularly to family members and to toddlers which sometimes take a negative toll on bodily and mental health. As women are considered caregivers when compared to men, the risk of exposure to such pressure is higher influencing their health.[29] It needs to be investigated if a man is the only caregiver of the house in the absence of a woman in the family, will it change the perceptive of men towards work or will it influence his mental and physical health. It has been argued that, when men and women work under similar work conditions, there is no difference in the release of the stress hormones, stress experience or heart rate.[30] On accounting age, education and marital status, the work-related stress in women disappears when compared to men.[31]

It is observed in the results of this study that 80.77% students never had any behavioural changes, but a few manifested minor behavioural changes following mortuary sessions. Not only can the experience in the mortuary but also the graphic images shown during lectures or practical activities have adverse effects. In previous surveys, examinations or assignments and grades expected to become perfectionist, increased stress levels in students with limited time for extra-curricular activities. Also, students living away from home were more stressed that the ones living with parents and vice versa. Lack of entertainment facilities within the accommodation developed more stress among males. Personal factors such as financial or family problems, time constraints, less time for relaxation, reduced holidays and language barrier can also play a vital role.[32, 33] Lack of confidence can lead to many unwanted errors which is highly unacceptable especially dealing with people who are not trained in forensics. Stress responses are also influenced by one's approach of beliefs, culture and attitude, another area that encourages further investigation.[32]

A full-time psychologist should be hired in the forensic department, for the students to be provided with counselling to help them manage any source of stress and its effect. An emotional intelligence test [34] and personality inventories [35] can be suggested for student selection for the admission of this course.[32] Stress management in education system is the key to avoid a substantial number of issues for future forensic odontologists. Stress for odontologists could be because of the gradual exposure or followed by any critical incident. Counselling facilities along with a Critical Incident Stress Management (CISM) program should be made available to the students by the odontology coordinator. Debriefing sessions such as Critical Incident Stress Debriefing (CISD) should be encouraged and combined with services supporting crisis such as crisis management for individuals, family support services, referring to professional care and also education programs after incidents. This could be well conjugated with the help of police and other emergency services.[2]

Tools to maintain mental well-being should be discussed. In a study related to Cochrane systematic reviews, exercising or yoga can improve mental well-being for both medically compromised patients and healthy individuals.[36] Listening to music, talking to your best friend, watching movies, playing video games and controlled caffeine intake maybe contribute to improving one`s mental health.[37-41]

Future studies in the upcoming years are encouraged to obtain unbiased answers, as it is a sensitive topic and mental well-being is still a taboo in the society. Fear of discussing the topic, survey fatigue, time constraint due to busy schedule or issues with internet connections could have affected the wiliness to answer to the survey and quality of the responses. Questions on sensitive topic such as death must be explored.

Finally, the results of this study encouraged the addition of sessions on Psychology in the MSc Forensic dentistry and MFOdont Forensic Odontology programmes of Centre for Forensic Medicine and Dentistry, University of Dundee, Dundee, Scotland, UK. The sessions cover: a) Psychology and death; b) Cognitive bias; c) Psychological aspects of giving evidence in court; and d) Mental health disorders and stress relief in Forensic Odontology; Dr Giselle Mânica delivers the lectures and simulated case scenarios for group discussions since the academic year 2021-22. The feedback from the students was extremely positive and the experience could be replicated by other courses.

CONCLUSIONS

It is believed that professionals working in forensics are trained to work under pressure and are resilient under a stress situation but, sometimes, pressure and stress take a toll on professionals and students who invest their time, money and sometimes resettle to another location across countries. Consulting a psychologist or counsellor for these reasons or having a personal conversation with someone on mental health is still taboo in society. A concern of being judged by the community still harbours in the minds of everyone; however, a psychologist can help with improving the decision-making of the people in need, or stress management and behaviour based on understanding the past behaviour which can help in predicting the future behaviour. It is recommended that we must also help people who face psychological issues, treat mental health-related problems, improve the education system, behaviour in the workplace and relationships.

The importance of psychology should be understood and inculcated during the training period itself. This could be done by appointing a psychologist for the department during the training period. Many forensic dentists might have struggled and suffered silently in the past. The mental health of students away from academic work is vital and it must function in a balanced manner. Yoga, exercise or other leisure activities must be encouraged at an individual level. Encouragement should be given to talk about the problems more than the solutions, interact with people from all age groups to understand human nature, watch psychological interviews, read more regarding the positivity towards life, express gratitude and feel grateful for the little things in life. As the mental well-being of forensic odontologists is dependent on the successful management of professional strains, this topic deserves more attention than it has received so far, especially in training programmes in Forensic Odontology.

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Accuracy of age estimation using root dentin translucency in Peruvian adults. A pilot study

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KEYWORDS

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ABSTRACT

Introduction: Age estimation is an important forensic resource in human identification. Amongst the different methods of dental age estimation, root dentin transparency (RDT) is considered a reliable parameter, as well as an indicator of chronological age at time of death in human adult remains. The aim of this study was to estimate the age of individuals using the Bang and Ramm method and to derive a new formula suitable for age estimation in the Peruvian population by assessing the length and percentage length of RDT.

Materials and methods: The sample consisted of 248 teeth collected from 124 deceased individuals, between the ages of 30 and 70 years. RDT length was digitally measured from sectioned and photographed teeth. Linear and quadratic regressions were made to derive Peruvian formulae and the new formulae were applied to another group of samples (n=30).

Results: Data showed a significant correlation ($p<0.01$) between chronological age and translucency length (Pearson´s correlation=0.775) and percentage length (Pearson´s correlation=0.778). Linear and quadratic regressions for obtaining Peruvian formulae showed that quadratic equations expressed greater determination coefficients. Comparisons between estimated age using Peruvian formulae showed that dental age from percentage of length of RDT a higher percentage of estimates with errors <± 05 and <± 10 years. The accuracy of the new Peruvian formula using the percentage of length of RDT (MAE=7.83) can be considered acceptable.

Conclusion: As shown in the results, age estimation using the Peruvian formula derived from the percentage of length of RDT has proven to be more accurate than estimates using the Bang and Ramm method. Thus, it could be used in age estimation for Peruvian individuals, as it is the most accurate methods and provides a larger number of acceptable estimates.

INTRODUCTION

Age is a relevant detail in dental and anthropological forensic cases of body identification, especially in cases of massive disasters, genocides and court cases.[1] The importance of age estimation as a forensic resource has been increasing in the recent past, due to the rise of unidentified bodies and human remains. It is even used in cases of living people who cannot prove their real date of birth.[2] There are diverse techniques which have been described and published to estimate chronological age, including skeletal growth and teeth development. Nevertheless, dental tissue is one of the most resistant parts of the body, remaining intact even in post-mortem stages.[3] Teeth are not affected by the environment or pathological factors and they remain well preserved in cases where the body of the deceased person is decomposed, skeletonized or burnt.[4,5]

Many methods have been used to estimate dental age in deceased adults, through direct assessment of the degree of dental attrition;[6] the extraction of teeth to analyze translucency of dentin, [7] secondary dentin deposits[8] and cementum annulations; [9] or the use of chemical substances.[10]

Amongst the different dental parameters used in age estimation, root dentin transparency (RDT) is considered a stable parameter and an indicator of chronological age at death in adult human remains.[11] Gustafson was the pioneer in using RDT as one of the six criteria for age estimation, reporting an increase of the translucency when related to increasing age.[12] Since then, several studies have reported using translucency in age estimation.[13,14] In 1970, Bang and Ramm presented a more detailed method to measure RDT, which was developed through the assessment of 926 teeth from the Norwegian population, establishing different formulae for each specific intact or sectioned tooth.[15]

The aim of this study was to estimate the age of Peruvian individuals using the original Bang and Ramm method and to derive a formula suitable for age estimation in the Peruvian population by assessing the length and percentage length of RDT.

MATERIALS AND METHODS

The sample consisted of 248 teeth. Only lateral and central lower incisors (left and right) were collected from 124 deceased individuals between the ages of 30 and 70 years during a period of 3 months in the Peruvian Institute of Forensic Thanatology (Lima, Peru). Teeth with radicular resorption, radicular cavities, root pathologies and fractures of the crown were excluded. Permission was obtained from the Institute of Legal Medicine. The real age was provided by the National Registry of Identification and Civil Status (RENIEC in Spanish) and treated as an accurate indicator of chronological age at death. Teeth extracted were cleaned with distilled water and soft tissues around the root were removed. Root length of all the sample were measured; then, teeth were sectioned by using a micromotor and placed next to an ABFO scale on a flat-bed scanner for photography. Translucency length was digitally measured from the apex to the coronal extent of the translucency using Adobe Photoshop software. After all procedures, teeth were replaced into their alveolus. According to Bang and Ramm's method, specific equations for each tooth were used to estimate the real age.

SPSS 26 software was used for statistical analysis. Pearson's correlation coefficient was used to calculate the correlation between chronological age and length/percentage length of RDT. Linear and quadratic regression functions for chronological age and length/percentage length of RDT were performed.

To aid in assessing it, as well as to compare accuracy of the new formula as compared to the Bang and Ramm method, it was applied to a new group of samples (n=30). Paired sample T-tests were run to determine the difference between chronological age and estimated age derived from Bang and Ramm formula and Peruvian formulae, using both length and percentage length of RDT.

To evaluate the accuracy of dental age estimation, the Mean Absolute Error (MAE) was calculated using MS Office Excel Spreadsheet. MAE is the average of the absolute values of the difference between chronological and estimated age.

RESULTS

From 248 teeth collected, 76 incisor teeth were from females and 172 were from males (Table 1). The minimum age was 30 years and the maximum was 70 years (mean age=46.99).

Although results showed a strong correlation between chronological age and length of RDT (0.775), a stronger correlation was found between chronological age and percentage length of RDT (0.778) (Table 2).

Linear and quadratic regressions for obtaining Peruvian formulae showed the relationship between real age and length and percentage length of RDT (Figure 1 and 2). Determination coefficients from quadratic functions were greater than those from linear regressions (Table 3).

Table 1. Distribution of sample according to age groups, sex, and tooth type

Age group (years)	Sex		Tooth				Total
	M	W	LRCI	LLCI	LRLI	LLLI	
30-40	54	22	22	16	24	14	76
41-50	52	34	10	33	14	29	86
51-60	32	12	11	11	9	13	44
61-70	34	8	15	6	13	8	42
TOTAL	172	76	58	66	60	64	248

LRCI: Lower Right Central Incisor; LLCI: Lower Left Central Incisor; LRLI: Lower Right Lateral Incisor; LLLI: Lower Left Lateral Incisor

Table 2. Correlation between age and length and percentage length of translucency

Variables	Pearson's coefficient
Chronological age / Length of RDT	0.775
Chronological age / Percentage length of RDT	0.778

$p<0.05$

Figure 1. Scatter plot showing the association between age and translucency length

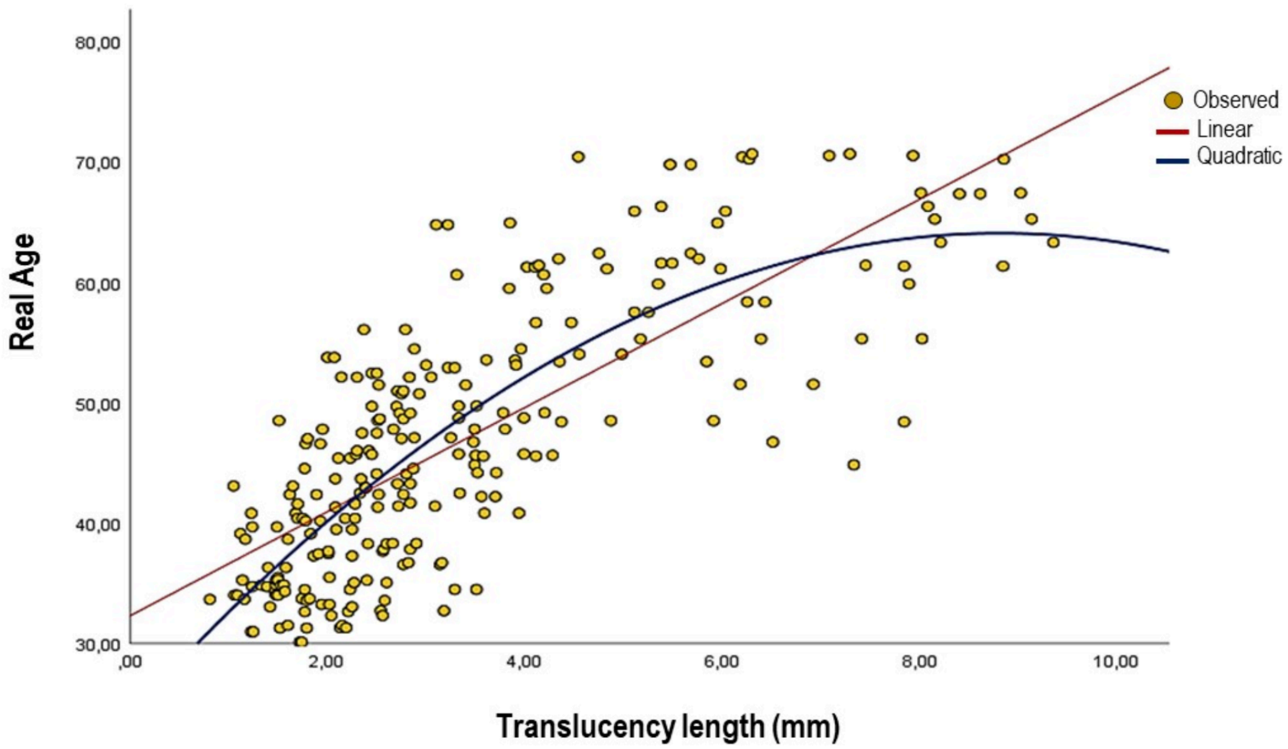

Figure 2. Scatter plot showing the association between age and percentage of translucency length

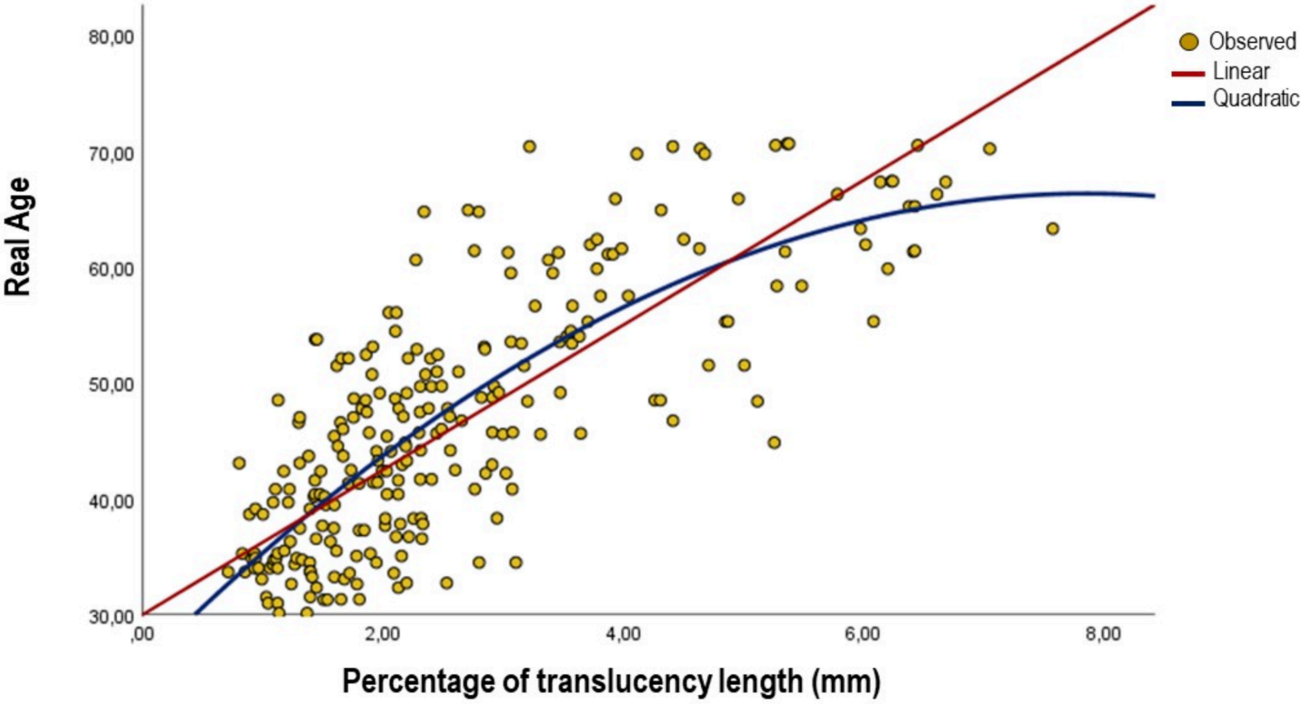

Table 3. Regression analysis, coefficients and formulae derived for length and percentage length of translucency

Variables	Regression analysis	R	R^2	Regression equation/formula
Chronological age / Length of RDT	Linear regression	0.775	0.600	Age= 32.294 + 4.323×T
	Quadratic regression	0.800	0.641	Age= 24.028 + (9.090×T)+(-0.516×T^2)
Chronological age / Percentage length of RDT	Linear regression	0.778	0.605	Age= 31.913 + 0.569×P
	Quadratic regression	0.793	0.629	Age= 25.623 + (1.037×P)+ (-0.007×P^2)

SE: Standard Error T: Transparency length P: Transparency length/Root length×100

The mean of differences between real and estimated age from Bang and Ramm formula (-6.701) pointed out an overestimation of chronological age. Nevertheless, the mean of differences from Peruvian formula (percentage length of RDT) showed better results (-5.999), which also represented an overestimation of chronological age. In relation to age groups, differences from all three formulae do have significant variations in the ranges of 30-40 and 41-50 years old ($p<0.05$) (Table 4).

The assessment of errors among chronological and estimated age revealed that quadratic functions derived from both Peruvian formulae produce smaller MAEs in comparison to estimates using Bang and Ramm formula (MAE=8.97). Thus, percentage length of RDT presented more errors with regards to age estimates at <±5 years and <±10 years (Table 5).

Table 4. Paired t test evaluating the mean of differences between real and estimated age across age groups

Paired samples	Age group	N	r	Mean	95% CI		t	sig
					Inferior	Superior		
Chronological age / Bang and Ramm formula	30-40	10	0.621	-8.508	-14.890	-2.125	-3.015	0.015
	41-50	9	0.583	-10.054	-15.610	-4.499	-4.174	0.003
	51-60	9	0.403	-3.168	-11.219	4.884	-0.907	0.391
	61-70	2	1.000	1.519	-66.370	69.408	0.284	0.824
	Total	30	0.606	-6.701	-10.134	-3.269	-3.993	0.000
Chronological age / Peruvian formula (T)*	30-40	10	0.546	-9.986	-14.937	-5.034	-4.562	0.001
	41-50	9	0.568	-9.228	-14.641	-3.815	-3.931	0.004
	51-60	9	0.328	-1.003	-7.250	5.245	-0.370	0.721
	61-70	2	1.000	3.352	-57.651	64.354	0.698	0.612
	Total	30	0.564	-6.174	-9.317	-3.032	-4.018	0.000
Chronological age / Peruvian formula (P)**	30-40	10	0.555	-9.466	-13.334	-5.597	-5.535	0.000
	41-50	9	0.491	-8.548	-14.246	-2.851	-3.460	0.009
	51-60	9	0.273	-1.395	-7.372	4.583	-0.538	0.605
	61-70	2	1.000	2.088	-22.188	26.365	1.093	0.472
	Total	30	0.627	-5.999	-8.853	-3.145	-4.299	0.000

*using Transparency length
**usingTransparency length/Root length×100

Table 5. Accuracy of the original method of Bang ad Ramm and Peruvian formulae

Formula	MAE	Error <± 05 years	Error <± 10 years
Original Bang and Ramm formula	8.97	36.7% (11/30)	63.4% (19/30)
Peruvian Formula (T)*	8.40	33.3% (10/30)	63.3% (19/30)
Peruvian Formula (P)**	7.83	46.7% (14/30)	70% (21/30)

*using Transparency length
*using Transparency length/Root length×100

DISCUSSION

Root dentin transparency (RDT) is described as the appearance of translucency on the external tooth root surface when the tooth is observed through a source of light.[16]

Since Gustafson first employed root dentin transparency as one of six parameters for age estimation in adults,[12] several studies using RDT have been published.[13,14] Nevertheless, Bang and Ramm were the ones who reported the largest sample of teeth, assessed, and made different formulae for sectioned and/or unsectioned teeth.[15] Although the first attempts at measuring the length of RDT were made manually,[17] current studies report digital approaches for more reliable measurements than those obtained by using calipers.[18,19] Additionally, digital images allow for easier storage and thus allows the samples to be used again in the future if required.[20] In this study, digital copies of tooth images were made by uploading the images to Adobe Photoshop, in concordance with previous articles which used the same software program.[20,21]

Although intact teeth can be used for the macroscopic assessment of the transparency of root dentin, better details are provided by sectioned teeth.[15] This is the reason why the current study only used sectioned samples, because better evidence of the RDT is obtained and its measurement is made faster in comparison to the entire tooth. Furthermore, Soomer et al. reported greater accuracy and precision in sectioned as opposed to intact teeth using Bang and Ramm's method.[22]

Regarding the quantification of RDT, it is important to point out that reports have calculated it by measurements of length,[13] area;[23] length expressed as percentage of total root length[24] and area expressed as percentage of total root area.[25] However, most studies have prefered to use the length of RTD as a variable, showing a varied range of coefficients' correlation with real age, from r values less than 0.15[26] to a coefficient higher than 0.90.[27]

In this study we decided to employ the length and the percentage length of RDT. Pearson's coefficients for correlation between real age and length and percentage length of RDT shows strong correlations ($r=0.775$ and $r=0.778$ respectively). Even though Thomas et al. conducted a study using the same variables involved in our study (i.e. length ($r=0.59$) and percentage ($r=0.583$) of RDT),[24] we found similar results with the research of Whittaker et al. ($r=0.797$) in Caucasian individuals.[28] In addition, Whittaker et al. reported that higher correlation coefficients were obtained using percentage length of RDT compared with percentage area of RDT.[28] Furthermore, Sengupta et al. quantified and assessed four variables which included the length and area of root dentin translucency; the percentage of length and percentage of area of RDT, showing that correlation coefficients were improved when length and area of RDT were expressed as percentages.[29]

Considering the population used, the sample consisted of 248 teeth, which is the largest Peruvian sample which has been reported to date. Although a previous study using RDT height for age estimation in Peruvian population showed a correlation with real age of $r=0.69$,[30] a better coefficient of correlation between real age and length of RDT was found in this study ($r=0.775$).

Though a universal formula for dental age estimation was proposed due to the ease of use in forensic cases,[19] Ubelaker et al. have pointed out that a maximum accuracy for estimating age in adults is obtained with population specific formulae.[31] Additionally, Lucy et al. reported the need to fit the original Bang and Ramm method and obtain new suitable formulae for different populations.[32] Concerning the above, we completely agree with the mentioned statements. Thus, linear and quadratic regressions have been made following Bang and Ramm's methodology to find appropriate coefficients and determine a more accurate formula for the Peruvian population.

Similarly, Acharya et al. performed both linear ($r^2=0.55$) and quadratic ($r^2=0.60$) regressions demonstrating better results in terms of larger coefficient of determination for quadratic equations.[11] In our study, regression analysis expresses an improvement in coefficients of quadratic regressions, using both length and percentage length of RDT (Table 3). Results about r squared mean that the proportion of data to predict real age in function of the length of RDT is 64.1% and 62.9% in function of the percentage of length of RDT.

Bang and Ramm noted a bias for transparency length to slow down principally after 60 years.[15] Furthermore, Acharya et al.,[11] in concordance with our study (Fig. 1 and 2), found that length of RDT begins to decrease approximately after 60 years. This fact, added to larger coefficients for quadratic equations, suggests the presence of a curvilinear relationship between real age and length of RDT. Consequently, in this study we decided to focus on quadratic rather than linear regressions and compare those determinants.

Regarding the mean of differences between chronological and estimated age, results involving the percentage of length of RDT express the lowest mean of difference and tend to overestimate in 5.999 years (Table 4). According to Shruthi et al., estimated age using translucent dentine had a mean difference of -5.6 years (overestimation) with its lowest value in the 6675 year-old group.[33] As for the Peruvian population, a previous study showed the lowest mean errors of age estimation in the group of 30–39 years old.[31] However, in this study differences between real

and dental age from all three formulae do present a significant variation ($p<0.05$) and show their lowest value for the 30-40 and 41-50 age group.
As a measure of accuracy of age estimation methods, the present study used the MAE. Results show that the Peruvian formula (percentage of length of RDT) is more accurate than the Bang and Ramm method because of lower MAE (7.83), with more percentage of estimates with errors <±5 years (46.7%) and <±10 years (70%). The Indian formula used by Acharya et al. produced larger MAE (>8 years) and less age estimates with errors <±10 years than ours.[11] A further study in the Indian population showed a larger percentage of estimates with errors <5 years (61.4 %) and less result for <10 years (12.9%) in comparison to ours.[34] As for the Peruvian population, Ubelaker et al. obtained a lower mean error of 6.29 years.[31] Solheim et al. consider that errors <± 3 years are excellent, while errors <± 10 years are acceptable in dental age estimation.[35] Therefore, MAEs from the Peruvian formula using percentage of length of RDT can be considered acceptable.
It is important to point out that the sample in this study was collected from the region of Lima. In order to prove the suitability of the Peruvian formula, further studies should include teeth from other regions.

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Performance of different dental age estimation methods on Saudi children

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ABSTRACT

Aim: To evaluate and compare the performance of six dental age estimation methods (Moorrees, Fanning and Hunt, Demirjian, Gleiser and Hunt, Nolla, Chaillet et al., and Nicodemo et al.) on a sample of Saudi children.

Method: This cross-sectional study was based on the evaluation of a sample of 400 archived digital panoramic radiographs of healthy Saudi children (200 each from boys and girls) aged 6 to 15.99 years. Panoramic radiographs acquired during 2018–2021 were obtained from the information technology department of the dental clinics at King Saud University, Riyadh, Saudi Arabia. Dental age was evaluated using the six dental age estimation methods on the developing permanent dentition in both jaws of the left side. The accuracy of each method was assessed in relation to chronological age, and a comparison between these methods was made.

Result: For all the tested methods, significant differences were found between chronological and dental age (P<0.001). The mean difference between dental and chronological age was (-2.19 years) for Chaillet et al. method, (0.15 years) for the Demirjian method, (-1.01 years) for the Moorrees, Fanning and Hunt method, (-1.72 years) for Nicodemo et al. method, (-1.29 years) for Nolla method, and (-1.00 years) for Gleiser and Hunt method.

Conclusion: Among the tested methods, the accuracy in Saudi subjects was the highest for Demirjian's method, followed by the Moorrees, Fanning and Hunt method. The methods proposed by Nicodemo et al., and Chaillet et al., were the least accurate.

INTRODUCTION

Age is determined by a person's date of birth and the amount of time or years elapsed from that date to any point in time and is termed chronological age (CA)[1]. The CA can be estimated by determining the physiological age [1]. Physiological age, also known as biological age, is based on the degree of maturation of different tissue systems.[2] Several biological ages have been developed, including skeletal, morphological, secondary sexual, and dental age (DA).[2]

DA is of particular interest to many scientific and clinical fields of application, including orthodontists and pediatric dentistry in choosing a timing and treatment plan, and in forensic dentistry, and pediatric endocrinology studies.[2] DA estimation is more reliable and genetically controlled than age estimation using skeletal indicators such as cervical vertebrae and hand-wrist bones.[3]

Additionally, DA can be determined by assessing tooth emergence or eruption in the oral cavity and observing the mineralization of developing teeth on radiographs.[4] Tooth mineralization is a more reliable indicator of dental maturity than emergence because it is not affected by factors such as ankylosis, early or delayed extraction of primary teeth, impaction, or crowding of permanent teeth.[2, 4]

Among all the methods used to estimate DA, such as visual, radiological, morphological, biochemical, and histological methods, the radiological method is a less invasive, simple, and reproducible and can be employed on both living and unknown dead.[5] Several radiological methods have been developed and studied to analyze dental mineralization as age indicator. The Demirjian method is the most widely used radiological method. Demirjian *et al*.[2] developed an eight-stage system in 1973 based on an analysis of French Canadian children. Chaillet et al.[6] obtained high accuracy in estimating DA in a Belgian population after adapting the Demirjian scores and using Belgian weighted scores. In 2005, Chaillet *et al.*[7] published international maturity curves for age estimation based on the evaluation of samples from eight different populations to overcome variations among different populations and use them when the ethnic origin of individuals is unknown. Nolla[8] created a DA system with 11 developmental stages, including tooth crypt staging, before the initial calcification.

Additionally, Gleiser and Hunt[9] devised a thirteen-stage system in 1955. Moorrees, Fanning, and Hunt (MFH)[4] evaluated dental development in 14 stages of mineralization, ranging from "cusp formation" to "root apex closure," for the development of single and multirooted permanent teeth. In 1991, Smith[10] used MFH charts to develop tables showing the age at which each tooth reached each stage and a formula for age estimation, which made the MFH method easier to use. Nicodemo et al., in 1974[11], provided a representative chart of the mineralization of all permanent teeth using eight developmental stages, with four stages each for the crown and the root.

Most DA estimation studies in the Saudi population have focused on the Demirjian method alone, and few studies have used and compared more than one method. Therefore, this study aimed to evaluate and compare the performance of six DA estimation methods that utilize the development of permanent teeth (MFH, Demirjian, Gleiser and Hunt, Nolla, Chaillet et al., and Nicodemo et al.) in a sample of Saudi children.

MATERIALS AND METHODS

Ethical Approval

The Institutional Review Board (E-21-6175) approved this study, followed by the approval of the College of Dentistry Research Center (PR 0124) at King Saud University.

Sample Selection And Size

This was a retrospective cross-sectional study involving children aged 6-15.99 years. Each chronological year was assigned to an individual group. A list of all Saudi patients aged (6–15.99 years) who had a panoramic radiograph acquired between 2018 and 2021 was obtained from the Information Technology department of the dental clinics at King Saud University. The inclusion and exclusion criteria were applied to the radiographs in reverse chronological order (from newest to oldest) until 400 cases were included. If a patient had multiple radiographs in the file, the oldest and most recent one that reflected the selection criteria were included.

The sample size was calculated for an effect size of 0.188 based on the Cohen equation and previous studies[12], at a level of significance of 0.05 and statistical power of 0.9. An analysis of variance (ANOVA) was performed for repeated measurements for ten age groups and the six methods, using GPower software.[13] The sample size in each age group was determined to be 40, which was subdivided into 20 boys and 20 girls; therefore, 400 digital panoramic radiographs (200 each from boys and girls) were used. The radiographs were initially assessed for the presence of radiographically visible exclusion criteria. The Salud file was then checked for other exclusion criteria in patients with acceptable radiographs. The radiographs were selected by ascending file number until each age group was completed.

Inclusion Criteria

The participants were selected following three main inclusion criteria: (1) Saudi patients, (2) children aged 6 to 15.99 years, and (3) presence of a panoramic radiograph in the Romexis server of the KSU College of Dentistry.

Exclusion Criteria

The exclusion criteria were as follows: (1) poor quality radiographs: the overlap of structures and presence of artifacts at the region of interest, (2) non-Saudi patients, (3) the presence of any systemic diseases or developmental conditions, (4) abnormal dental development including amelogenesis/dentinogenesis imperfecta, taurodontism, hypodontia, and hyperdontia, (5) presence of gross pathology related to the left side of the jaw or teeth, (6) presence of gross caries and periapical pathosis on the left side of the jaw, (7) presence of large restorations or crowns on the left side of the jaw, (8) early tooth extraction on the left side of the jaw, and (9) known previous orthodontic treatment.

Data Collection

Digital radiographs were analyzed with the naked eye for DA estimation. Planmeca Romexis 3.6.0.R software available at KSU was used. Each participant's CA was calculated by subtracting the date of birth registered in the file from the date the radiograph was obtained and converted into a decimal system using the Eveleth and Tanner's method.[14] The observer blinded the CA and entered them into a different spreadsheet until all 400 panoramic radiographs were assessed.

Each permanent tooth on the left side was evaluated to determine its developmental stage using the following methods: MFH[4], Nolla[8], Demirjian [2], Chaillet et al.[6], Gleiser and Hunt[15] and Nicodemo et al.[11]

Radiograph viewing conditions were standardized as follows:(1) if image adjustments had been made on the panoramic radiograph before data collection, all adjustments were undone; (2) viewing was done in a dimly-lit room; (3) the zoom level was standardized between methods; and (4) all age estimation methods were applied using the same contrast and density settings.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows version 28. The primary observer was trained and calibrated by a field expert. The main observer performed all measurements. To calculate the intra- and inter-examiner values, a 10% random sample of the digital radiographs was selected using random allocation software and re-evaluated after 2 weeks. Cohen's kappa test was used to verify intra- and inter-observer agreements for all methods.[16]

The accuracy of each method was determined by the mean difference between DA and CA (bias). The DA was compared with the CA for each participant. The CA was subtracted from the DA, and a positive result indicated an overestimation, whereas a negative result indicated an underestimation. This difference and the absolute mean difference for each radiograph were tabulated. The absolute mean difference was used to assess the accuracy range by removing the canceling effect of equal, over, and underestimation. The standard deviation for each CA–year interval was also calculated. One-sample and paired t-tests were used to assess the accuracy of each method in each year interval for the entire sample.

An independent samples t-test was used to compare mean differences in CA and DA between the sexes. Repeated-measures ANOVA and post hoc analysis were used to compare DA and CA among five methods (excluding the Gleiser and Hunt method). The Bland–Altman plot was used to assess the agreement between each method and CA. Statistical significance was set at $P<0.05$.

RESULTS

Reliability Test

Intra-examiner Kappa values were 0.88 (MFH), 0.96 (Demirjian), 1.00 (Gleiser and Hunt), 0.97 (Nolla), 0.96 (Chaillet et al.), and 0.97 (Nicodemo et al.). For inter-examiner agreement, the Kappa values were 0.80 (MFH), 0.81 (Demirjian), 0.87 (Gleiser and Hunt), 0.78 (Nolla), 0.81 (Chaillet et al.), and 0.73 (Nicodemo et al.). These values are "substantial" or "almost perfect".[16]

Description of Sample

A total of 400 digital radiographs were analyzed (200 each from boys and girls). All participants were divided into ten groups based on their CA and sex, with each group having an equal number of boys and girls (Table 1).

Table 1. Distribution of the study sample by chronological age (CA) and gender

Group	Chronological Age (CA)/years	Males No.	Females No.	Total
1	6.00 – 6.99	20	20	40
2	7.00 – 7.99	20	20	40
3	8.00 – 8.99	20	20	40
4	9.00 – 9.99	20	20	40
5	10.00 – 10.99	20	20	40
6	11.00 – 11.99	20	20	40
7	12.00 – 12.99	20	20	40
8	13.00 – 13.99	20	20	40
9	14.00 – 14.99	20	20	40
10	15.00 – 15.99	20	20	40
Total		**200**	**200**	**400**

Accuracy of Each Method

The Chaillet et al. method underestimated CA by -2.03 years for boys and -2.35 years for girls (average, -2.19 years; $P<0.001$) (Tables 2 and 3) (Figs. 1,2, and 3). The Chaillet et al. method underestimated the age of both sexes in all age groups (Table 4) (Fig. 4). A significant difference was found between boys and girls in the age groups of 12,13,14 and 15 years, where the mean difference was lower in boys than in girls ($P=0.035$, $P=0.006$, $P<0.001$, $P<0.001$, respectively) (Table 4).

Table 2. The accuracy of all methods for the entire sample expressed by Bias (the mean difference between dental age (DA) and chronological age (CA) in years) and the absolute mean difference between estimated and Real Age in years) using a one sample t-test

Method	Measure of Accuracy	Mean	SD	p-value	95% C.I	
					Lower	Upper
Chaillet et al	Bias	-2.19	0.98	**<0.001**	-2.28	-2.09
	Absolute difference	2.22	0.89	**<0.001**	2.14	2.31
Demirjian	Bias	0.15	0.63	**<0.001**	0.08	0.21
	Absolute difference	0.49	0.43	**<0.001**	0.44	0.53
Gleiser and Hunt	Bias	-1.00	1.20	**<0.001**	-1.15	-0.85
	Absolute difference	1.22	0.98	**<0.001**	1.20	1.34
MFH	Bias	-1.01	0.82	**<0.001**	-1.09	-0.93
	Absolute difference	1.06	0.75	**<0.001**	0.99	1.14
Nicodemo et al.	Bias	-1.72	1.86	**<0.001**	-1.91	-1.54
	Absolute difference	1.93	1.64	**<0.001**	1.76	2.09
Nolla	Bias	-1.29	0.83	**<0.001**	-1.37	-1.20
	Absolute difference	1.31	0.78	**<0.001**	1.24	1.39

Table 3. The Accuracy for Males and Females

Method	Measure of Accuracy	Gender	Mean	SD	P-value
Chaillet *et al.*	**Bias**	Female	-2.35	1.16	**0.002**
		Male	-2.03	0.74	
	Absolute difference	Female	2.42	0.98	**<0.001**
		Male	2.02	0.74	
Demirjian	**Bias**	Female	0.11	0.65	0.219
		Male	0.18	0.61	
	Absolute difference	Female	0.49	0.44	0.838
		Male	0.48	0.42	
Gleiser and Hunt	**Bias**	Female	-1.16	1.19	**0.042**
		Male	-0.84	1.20	
	Absolute difference	Female	1.31	0.02	0.162
		Male	1.13	0.93	
MFH	**Bias**	Female	-1.14	0.85	**0.001**
		Male	-0.88	0.76	
	Absolute difference	Female	1.18	0.79	**0.001**
		Male	0.94	0.68	
Nicodemo *et al.*	**Bias**	Female	-1.65	1.87	0.401
		Male	-1.80	1.85	
	Absolute difference	Female	1.88	1.63	0.579
		Male	1.97	1.66	
Nolla	**Bias**	Female	-1.50	0.89	**<0.001**
		Male	-1.07	0.70	
	Absolute difference	Female	1.53	0.84	**<0.001**
		Male	1.10	0.66	

Figure 1. Box plot for the bias observed for each method

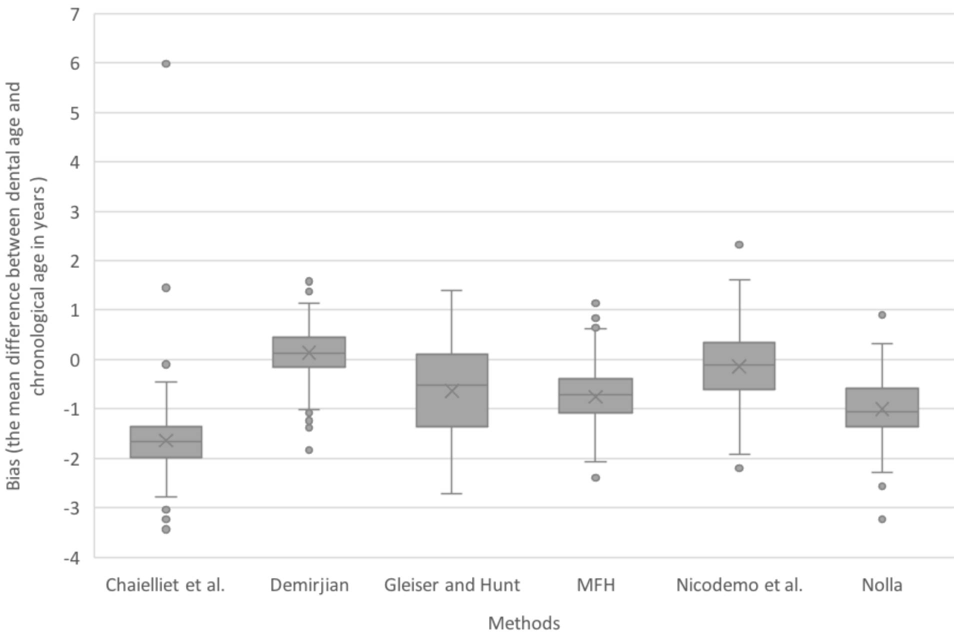


Figure 2. Box plot for the bias observed for each method stratified by sex

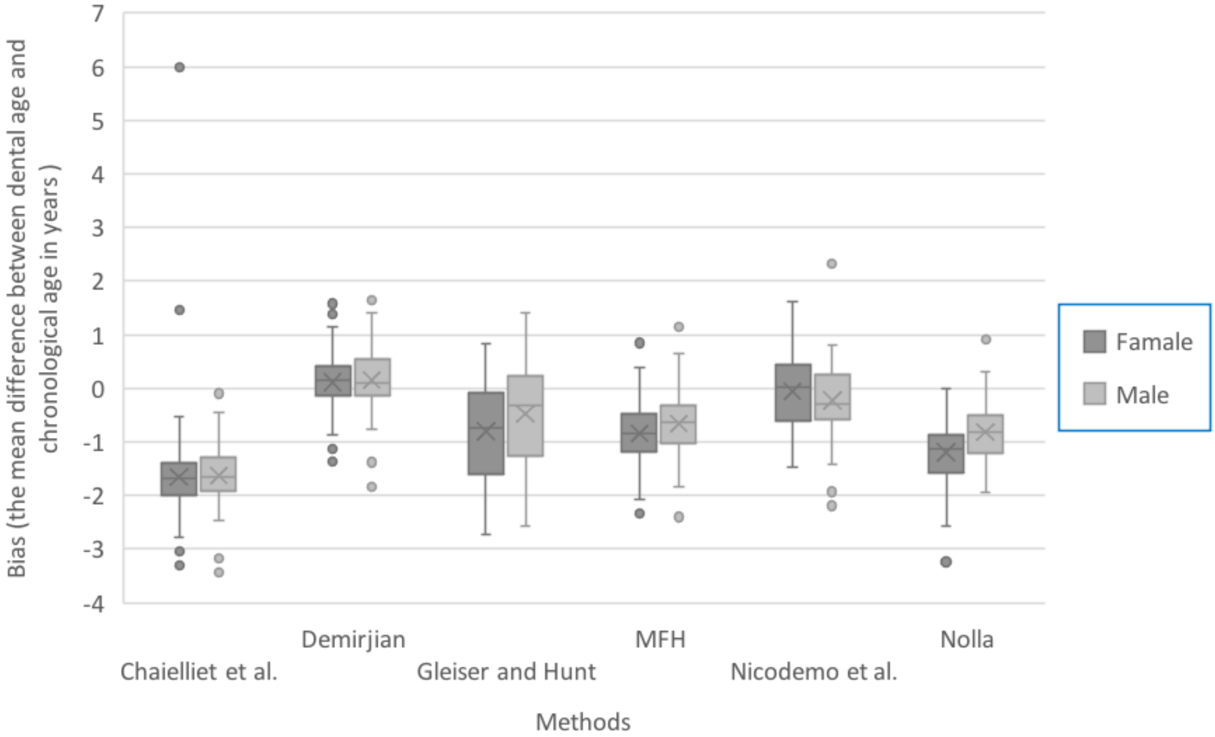


Figure 3. Bland-Altman plot the real age and estimated age for Chaillet et al. method

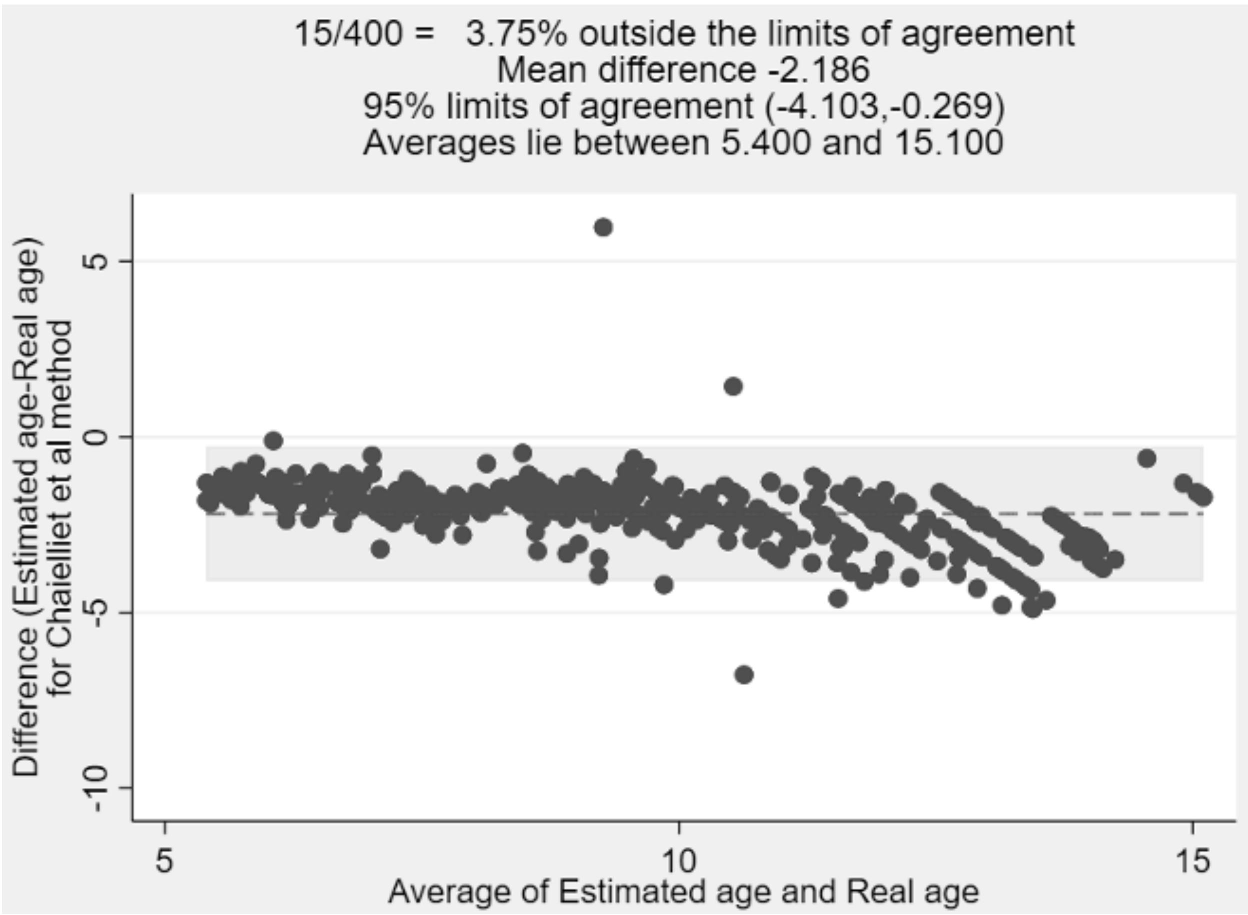


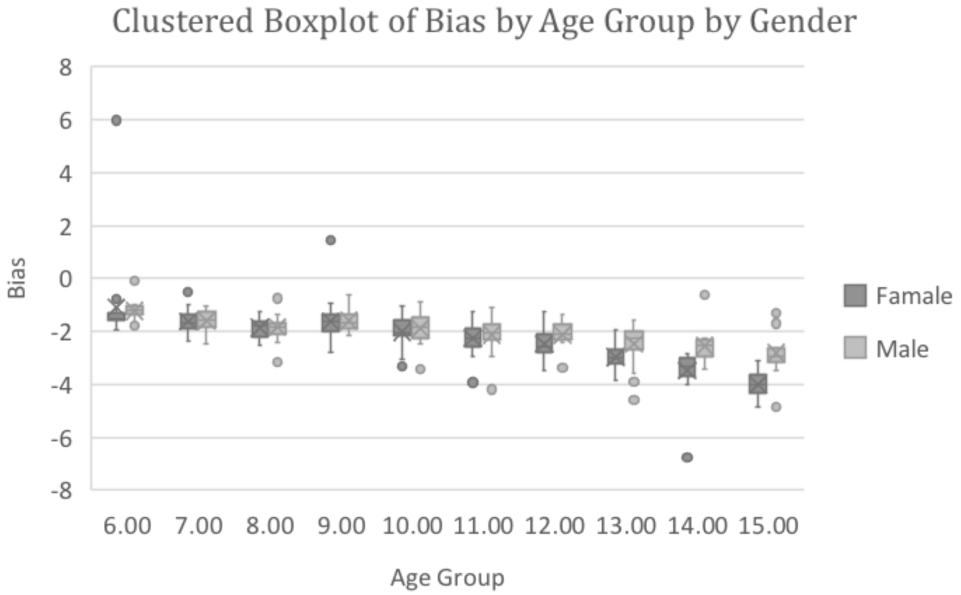
Table 4. The accuracy of Age Intervals of all methods for Males and females

Categorical age midpoint	Method	Gender	N	Mean	SD	P-value	95% C.I	
							Lower	Upper
6.5	**Chaillet et al.**	Female	20	-1.10	1.69	0.764	-0.66	0.90
		Male	20	-1.22	0.36			
	Demirjian	Female	20	0.43	0.26	0.178	-0.33	0.06
		Male	20	0.56	0.35			
	Gleiser and Hunt	Female	20	0.08	0.38	**0.010**	-0.69	-0.10
		Male	20	0.48	0.53			
	MFH	Female	20	-0.57	0.36	0.165	-0.45	0.08
		Male	20	-0.38	0.46			
	Nicodemo et al.	Female	20	0.55	0.40	0.672	-0.25	0.38
		Male	20	0.48	0.57			
	Nolla	Female	20	-0.90	0.42	**0.001**	-0.76	-0.20
		Male	20	-0.42	0.45			
7.5	**Chaillet et al.**	Female	20	-1.62	0.41	0.857	-0.28	0.23
		Male	20	-1.60	0.39			
	Demirjian	Female	20	0.14	0.35	0.856	-0.20	0.24
		Male	20	0.12	0.32			
	Gleiser and Hunt	Female	20	-0.02	0.64	0.311	-0.53	0.17
		Male	20	0.16	0.43			
	MFH	Female	20	-0.68	0.58	0.687	-0.41	0.27
		Male	20	-0.61	0.49			
	Nicodemo et al.	Female	20	0.39	0.43	**0.048**	0.00	0.55
		Male	20	0.11	0.43			
	Nolla	Female	20	-1.08	0.47	0.071	-0.60	0.03
		Male	20	-0.80	0.50			
8.5	**Chaillet et al.**	Female	20	-1.87	0.33	0.751	-0.34	0.25
		Male	20	-1.83	0.57			
	Demirjian	Female	20	-0.19	0.41	0.290	-0.45	0.14
		Male	20	-0.03	0.50			
	Gleiser and Hunt	Female	20	-0.65	0.32	**<0.001**	-0.72	-0.24
		Male	20	-0.16	0.43			
	MFH	Female	20	-0.89	0.39	0.095	-0.58	0.05
		Male	20	-0.62	0.57			
	Nicodemo et al.	Female	20	-0.01	0.42	0.311	-0.12	0.36
		Male	20	-0.13	0.33			
	Nolla	Female	20	-1.44	0.40	**<0.001**	-0.84	-0.23
		Male	20	-0.90	0.53			
	Chaillet et al.	Female	20	-1.63	0.90	0.870	-0.48	0.41
		Male	20	-1.60	0.38			
	Demirjian	Female	20	0.03	0.62	0.808	-0.40	0.32
		Male	20	0.08	0.49			

9.5	**Gleiser and Hunt**	Female	20	-1.36	0.55	**0.016**	-0.63	-0.07
		Male	20	-1.01	0.26			
	MFH	Female	20	-0.96	0.52	0.239	-0.50	0.13
		Male	20	-0.78	0.46			
	Nicodemo et al.	Female	20	-0.34	0.40	0.088	-0.03	0.41
		Male	20	-0.53	0.25			
	Nolla	Female	20	-1.08	0.58	0.176	-0.57	0.08
		Male	20	-0.84	0.41			
10.5	**Chaillet et al.**	Female	20	-2.03	0.63	0.477	-0.52	0.25
		Male	20	-1.90	0.56			
	Demirjian	Female	20	0.15	0.83	0.634	-0.39	0.64
		Male	20	0.03	0.79			
	Gleiser and Hunt	Female	20	-2.05	0.40	0.155	-0.43	0.07
		Male	20	-1.87	0.38			
	MFH	Female	20	-1.14	0.73	0.321	-0.70	0.24
		Male	20	-0.91	0.73			
	Nicodemo et al.	Female	20	-0.89	0.38	0.094	-0.04	0.48
		Male	20	-1.11	0.43			
	Nolla	Female	20	-1.47	0.70	0.083	-0.74	0.05
		Male	20	-1.12	0.53			
11.5	**Chaillet et al.**	Female	20	-2.27	0.57	0.469	-0.55	0.26
		Male	20	-2.12	0.68			
	Demirjian	Female	20	0.36	0.96	0.422	-0.37	0.85
		Male	20	0.12	0.95			
	Gleiser and Hunt	Female	20	-2.96	0.31	**0.002**	-0.50	-0.11
		Male	20	-2.66	0.30			
	MFH	Female	20	-1.13	0.81	0.342	-0.77	0.27
		Male	20	-0.88	0.82			
	Nicodemo et al.	Female	20	-1.62	0.29	0.151	-0.06	0.37
		Male	20	-1.77	0.37			
	Nolla	Female	20	-2.02	0.88	**0.012**	-1.18	-0.16
		Male	20	-1.35	0.71			
12.5	**Chaillet et al.**	Female	20	-2.47	0.51	**0.035**	-0.67	-0.03
		Male	20	-2.12	0.50			
	Demirjian	Female	20	0.52	0.63	0.315	-0.19	0.57
		Male	20	0.33	0.54			
	MFH	Female	20	-1.32	0.65	0.054	-0.90	0.01
		Male	20	-0.87	0.77			
	Nicodemo et al.	Female	20	-2.37	0.34	0.113	-0.04	0.36
		Male	20	-2.53	0.29			
	Nolla	Female	20	-1.90	0.96	0.079	-0.95	0.05
		Male	20	-1.45	0.57			
	Chaillet et al.	Female	20	-3.04	0.51	**0.006**	-0.93	-0.09
		Male	20	-2.48	0.77			

13.5	**Demirjian**	Female	20	0.09	0.39	0.772	-0.48	0.36
		Male	20	0.15	0.85			
	MFH	Female	20	-1.67	0.61	0.121	-0.95	0.12
		Male	20	-1.26	0.99			
	Nicodemo et al.	Female	20	-3.21	0.29	0.147	-0.05	0.34
		Male	20	-3.35	0.32			
	Nolla	Female	20	-2.00	0.78	0.209	-0.96	0.22
		Male	20	-1.63	1.04			
14.5	**Chaillet et al.**	Female	20	-3.48	0.84	**<0.001**	-1.38	-0.46
		Male	20	-2.56	0.58			
	Demirjian	Female	20	0.04	0.47	0.080	-0.60	0.04
		Male	20	0.32	0.52			
	MFH	Female	20	-1.57	1.00	0.115	-0.95	0.11
		Male	20	-1.14	0.60			
	Nicodemo et al.	Female	20	-3.99	0.27	0.084	-0.02	0.34
		Male	20	-4.15	0.30			
	Nolla	Female	20	-1.68	0.89	**0.009**	-1.07	-0.17
		Male	20	-1.06	0.46			
15.5	**Chaillet et al.**	Female	20	-4.00	0.49	**<0.001**	-1.60	-0.76
		Male	20	-2.82	0.80			
	Demirjian	Female	20	-0.50	0.67	**<0.001**	-1.02	-0.33
		Male	20	0.18	0.37			
	MFH	Female	20	-1.50	1.52	0.719	-0.99	0.69
		Male	20	-1.35	1.04			
	Nicodemo et al.	Female	20	-4.97	0.26	0.406	-0.09	0.22
		Male	20	-5.04	0.22			
	Nolla	Female	20	-1.44	1.48	0.409	-1.09	0.46
		Male	20	-1.12	0.82			

Figure 4. Box plot for the bias of Chaillet et al method stratified by sex and age



The Demirjian method overestimated CA by 0.18 years for boys and 0.11 years for girls (average, 0.15 years; P<0.001) (Tables 2 and 3) (Figs.1,2 and 5). Overestimations were significant for the age groups of 6, 7, 12, and 14 years (P<0.001, P=0.034, P<0.001, and P=0.018, respectively). The Demirjian method overestimated CA for both sexes in all age groups, except for boys aged 8 years and girls aged 8 and 15 years, for whom an underestimation was observed (Table 4) (Fig.6). A significant difference was only found between boys and girls in the age group of 15 years, where the mean difference was lower in boys than in girls (P<0.001) (Table 4).

Figure 5. Bland-Altman plot the real age and estimated age for Demirjian method

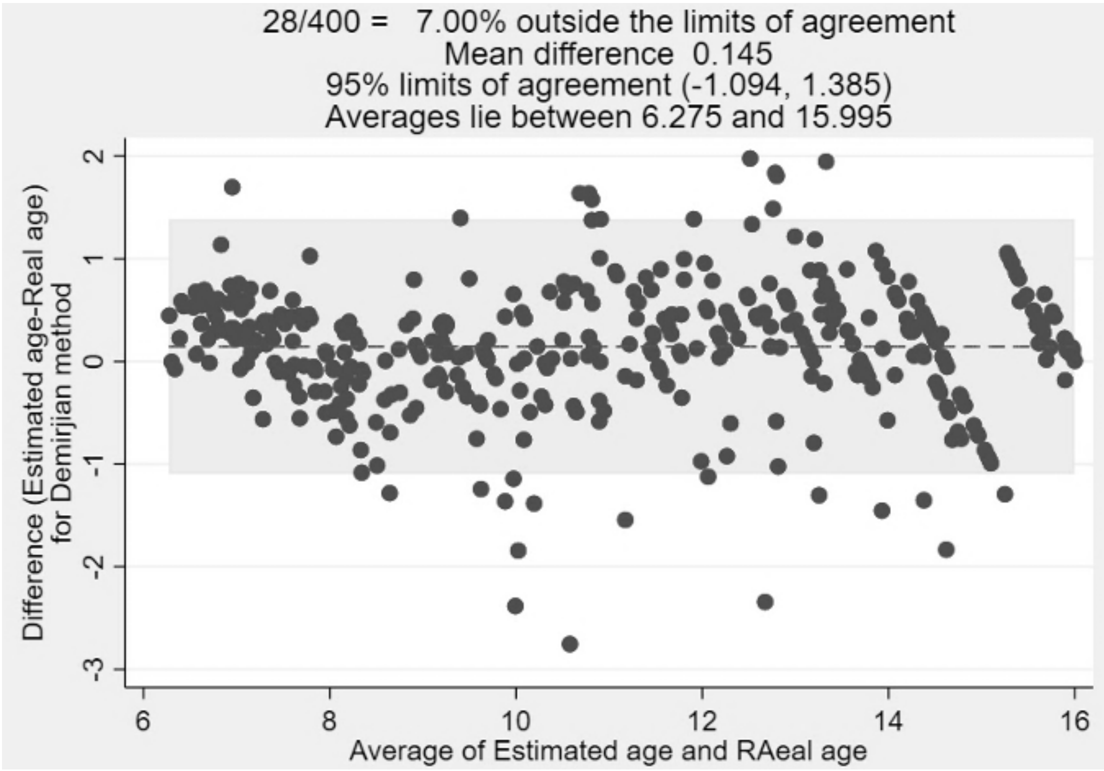
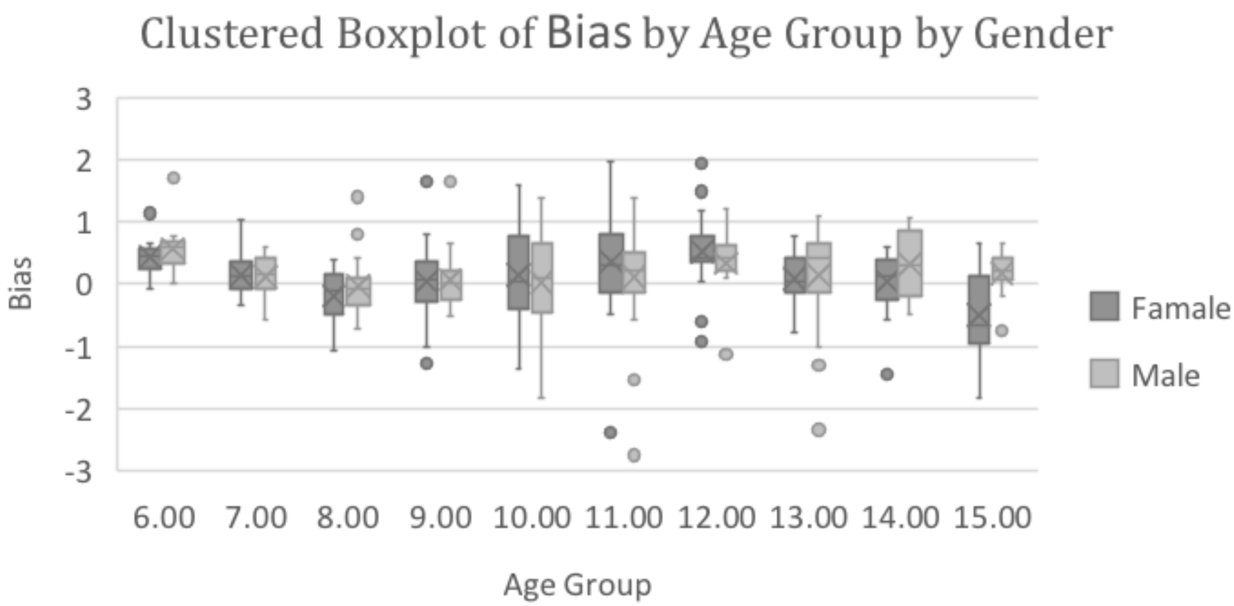


Figure 6. Box plot for the bias of Demirjian method stratified by sex and age



The MFH method also underestimated age by -0.88 years for boys and -1.14 years for girls (average, -1.01 years; P<0.001) (Tables 2 and 3) (Figs. 1,2 and 7). Underestimations were observed for both sexes in all age groups (Table 4) (Fig.8).
The Nicodemo et al. method underestimated CA by -1.80 years for boys and -1.65 years for girls (average, -1.72 years; P<0.001) (Tables 2 and 3) (Figs.1,2, and 9). Underestimations were significant for the age groups of 9, 10, 11, 12, 13, 14, and 15 years (P<0.001). However, age was significantly overestimated in the age groups of 6 and 7 years (P≤0.001, P=0.001,respectively). A significant difference was found between boys and girls in the age group of 7 years, where the mean difference was lower in boys than in girls (P=0.048) (Table 4) (Fig.10).

Figure 7. Bland-Altman plot the real age and estimated age for MFH method

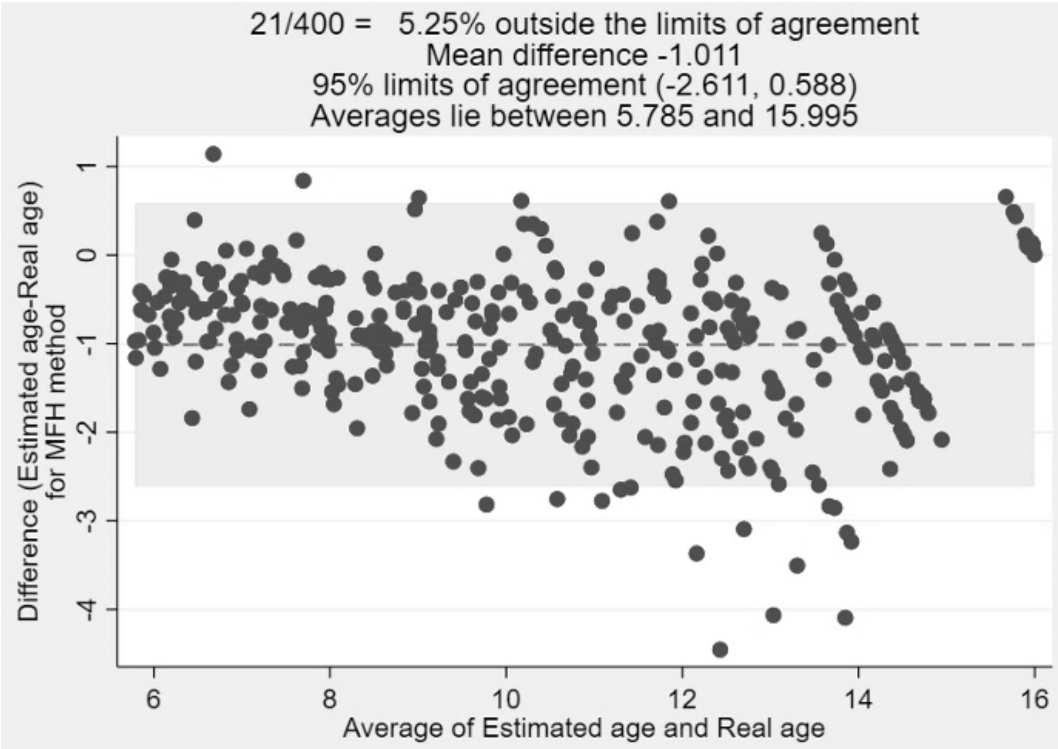


Figure 8. Box plot for the bias of MFH method stratified by sex and age

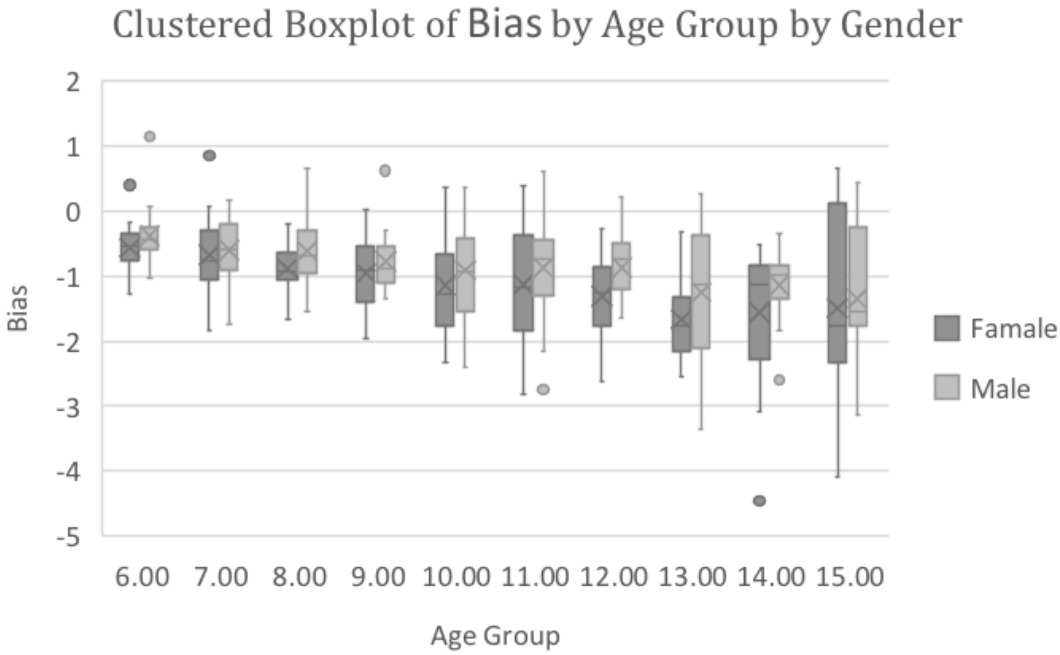


Figure 9. Bland-Altman plot the real age and estimated age for Nicodemo et al method

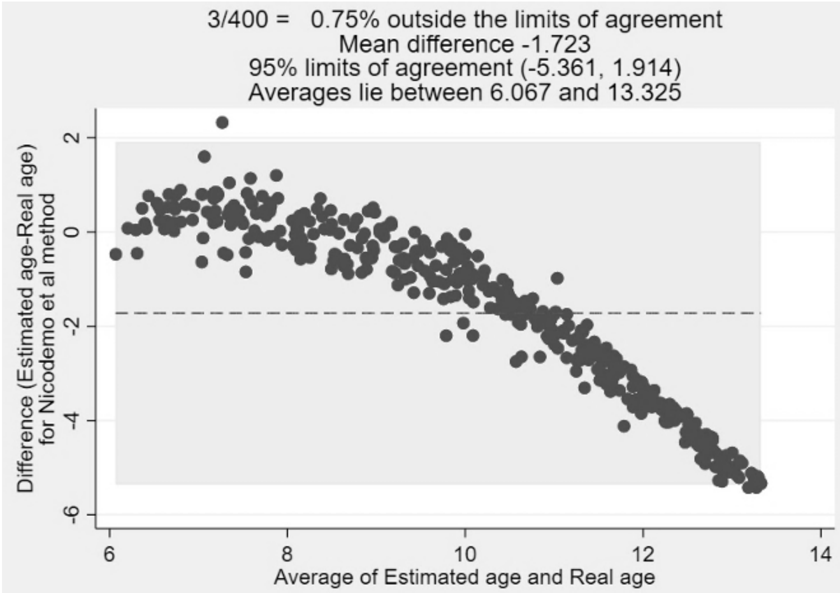
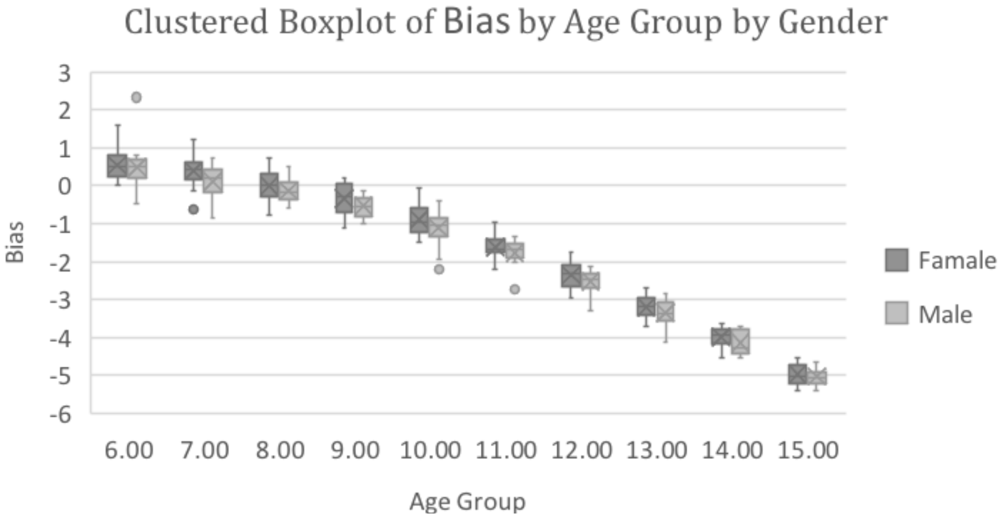


Figure 10. Box plot for the bias of Nicodemo et al. method stratified by sex and age



The Nolla method underestimated CA by -1.07 years for boys and -1.50 years for girls (average, -1.29 years; P<0.001) (Tables 2 and 3) (Figs. 1,2 and 11). The Nolla method underestimated CA for both sexes in all age groups (Table 4) (Fig.12). A significant difference was found between boys and girls of age groups 6, 8, 11, and 14 years, where the mean difference was lower in boys than in girls (P=0.001, P<0.001, P=0.012, respectively). P=0.009, respectively) (Table 4).

The Gleiser and Hunt method underestimated CA by -0.84 years for boys and -1.16 years for girls (average, -1.00 years; P<0.001) (Tables 2 and 3) (Figs. 1,2,and 13). The Gleiser and Hunt method underestimated CA for both sexes in all age groups, except for boys aged 6 and 7 years and girls aged 6 years, for whom an overestimation was found (Table 4) (Fig. 14). A significant difference was found between boys and girls of age groups 6, 8, and 9 years, where the mean difference was lower in girls aged 6 years and lower in boys aged 8 and 9 years (P=0.010, P<0.001, P=0.016, respectively) (Table 4).

Comparison of Bias Between Different Methods

Significant differences in bias were found among the different methods. Post-hoc comparisons showed that there was a statistically significant difference in bias between all methods (P<0.001) (Table 5).

Figure 11. Bland-Altman plot the real age and estimated age for Nolla method

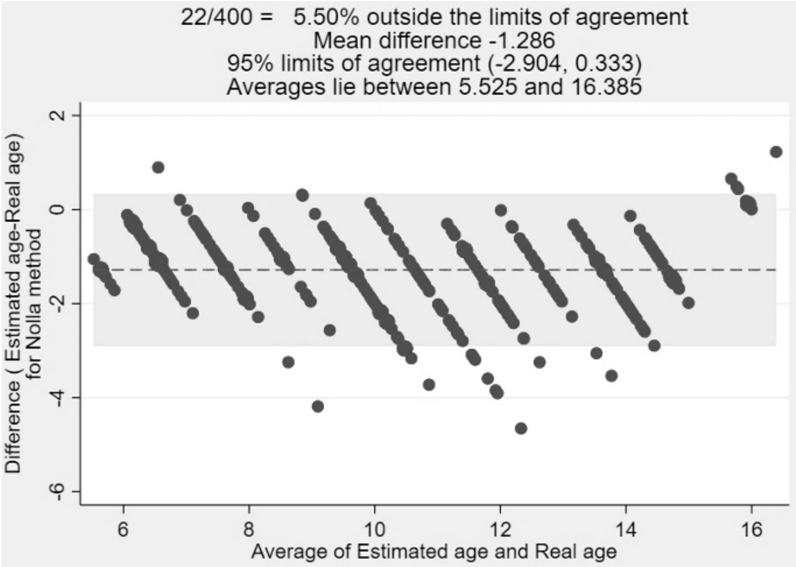


Figure 12. Box plot for the bias of Nolla method stratified by sex and age

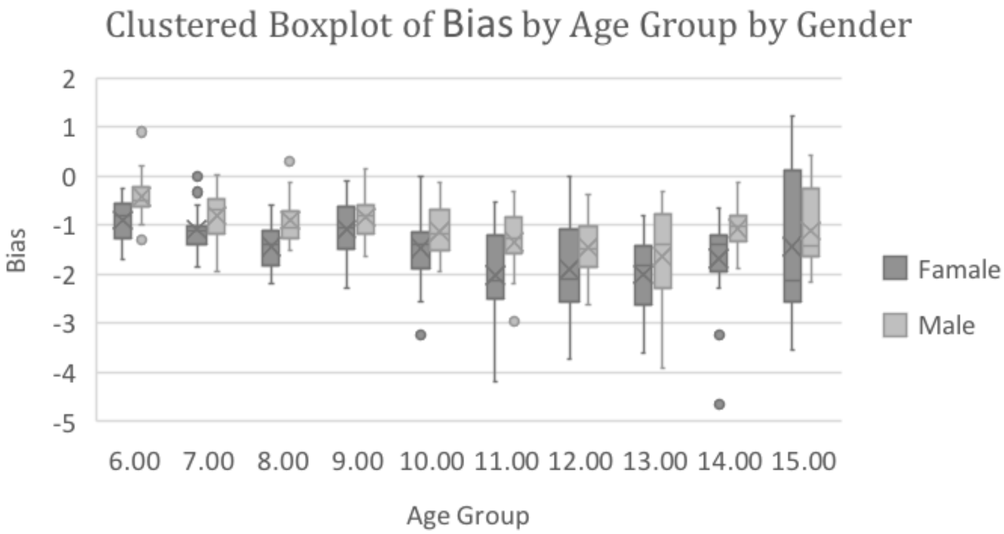


Figure 13. Bland-Altman plot the real age and estimated age for Gleiser and Hunt method

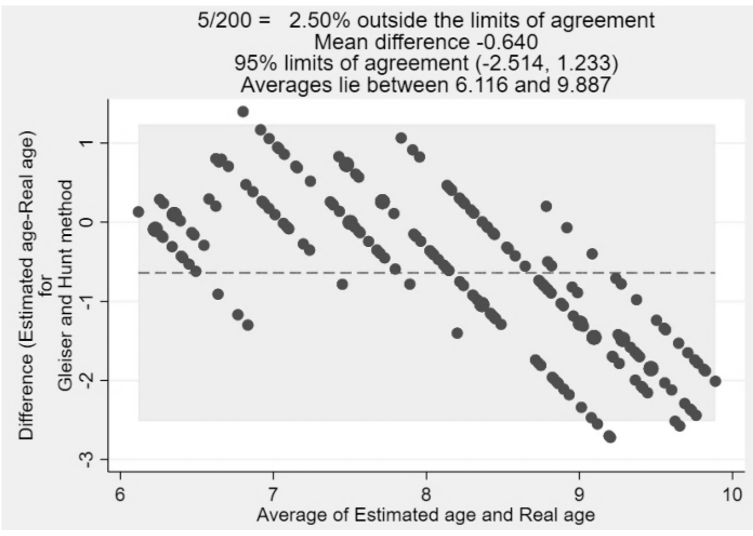


Figure 14. Box plot for the bias of Gleiser and Hunt stratified by sex and age

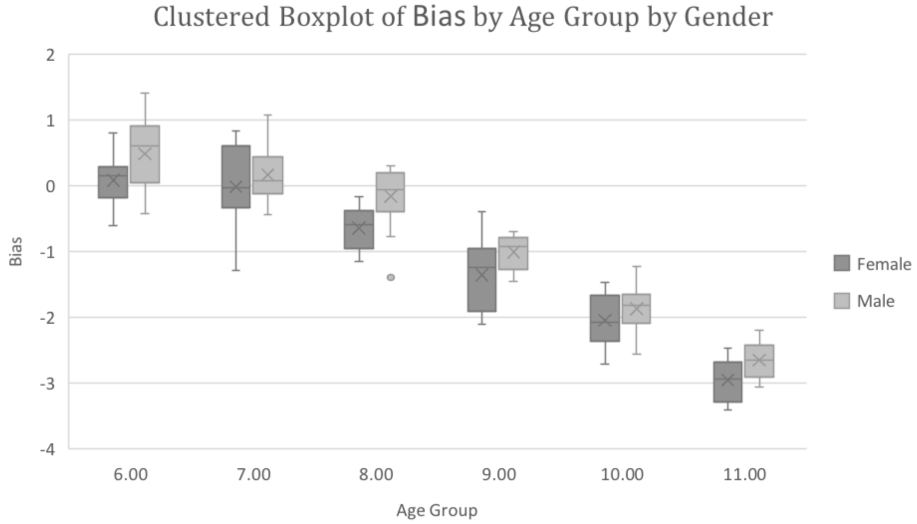


Table 5. Comparison of bias between different methods

	Mean bias	SD	95% C.I		P-value
			Lower Bound	Upper Bound	
Chaillet et al.	-2.19	0.98	-2.28	-2.09	<0.001
Demirjian	0.15	0.63	0.08	0.21	
MFH	-1.01	0.82	-1.09	-0.93	
Nicodemo et al.	-1.72	1.86	-1.91	-1.54	
Nolla	-1.29	0.83	-1.37	-1.20	

* The Gleiser and Hunt method was excluded from this comparison because all statistical analysis for this method was performed when the chronological age of subjects was ≤ 11.99, resulting in missing data above this age.

DISCUSSION

Age estimation is important in both forensic science and clinical practice. Knowing age could be helpful in several legal cases where it is necessary to differentiate a juvenile from an adult, cases of illegal immigration, and wrongly reported or manipulated ages in documents. According to the recommendations of the Study Group on Forensic Age Diagnostics, a forensic age estimate should consist of a physical examination, radiograph of the hand, and dental examination, including evaluation of a panoramic radiograph, if available.[17]

Dental maturation and emergence through the gingiva have long been recognized as the most useful criteria for estimating age. This approach is more favorable because teeth are less affected by environmental factors and hormonal disturbances than bones, and most techniques used are less invasive and simple to use [18]. Many methods have been developed, providing results with various levels of accuracy and using different statistical procedures for age estimation based on tooth development in children and adolescents.[19]

Accuracy and precision are essential in DA assessment. Accuracy refers to the proximity of DA to CA.[20] We presented accuracy as the mean difference between DA and CA (bias) and the absolute mean difference between DA and CA. When assessing DA, it is essential to consider the precision of the age estimation method. Precision, also called reliability, is used in intra- and inter-observer reproducibility [21]. The choice of tooth stage assessment is an important factor influencing reproducibility. Those described by Nicodemo et al. are the least detailed, thus showing lower precision in this study. The previous finding of lower precision for the Nolla's method was not observed in this study [22, 23], probably because the addition of fractions to the Nolla score (0.2, 0.5, and 0.7) was not considered in the DA estimation. This was done because an increased number of stages in the

Nolla method has been suggested to moderately decrease its precision while complicating the assessment and making it more subjective.[22] Moreover, in the present study, one examiner tested the performance of all the methods with almost perfect intra-examiner kappa values. One examiner controlled for errors attributable to inter-examiner differences.

The Demirjian Method:
The Demirjian method has been tested and applied to different populations for several years. It has been found to consistently overestimate age in various populations such as Croatia, Brazil, France, Italy, Kuwait, North Germany, Northern China, Portugal, Romania, South America, Spain, Sri Lanka and Turkey.[24–33] Liversidge et al. believed that the overestimation of DA in recent findings when Demirjian's method was used in different populations may be partly explained by a positive secular trend in growth and development during the last 25 years.[36]
In contrast, in Eastern China, underestimation was generally demonstrated in boys and girls, except for the age group of 13–14.99 years in boys and 11–14.99 years in girls.[37] Additionally, Cruz-Landeira et al. reported an underestimation of age using this method in a Venezuelan Amerindian sample; however, they suggested that this finding may be due to the small sample size and ethnic origin of their sample.[38]
In Saudi Arabia, a study assessing DA in Riyadh in children between the ages of 8.5 to 17 years found an overestimation of 0.3 years for boys and 0.4 years for girls [39]. Similar results were reported in Saudi children aged 4 to 14 years; however, the overestimation was 0.77 years for boys and 0.83 years for girls.[40] A study performed by Alshihri et al. in the Western Saudi Arabian population concluded that girls are 0.059 ± 1.26 years and boys are 0.66 ± 1.14 years ahead of the French-Canadian children.[41] Alassiry et al. found that, in a sample of 298 Saudi children and adolescents between the ages of 3 and 15 years, the mean difference between DA and CA was 0.50 ± 1.57 years. The difference was 0.57 ± 1.48 years in boys and 0.44 ± 1.66 years in girls.[42]
In this study, Demirjian's method overestimated CA, consistent with the results of previous studies. The overestimation was more pronounced for the age groups of 6, 7, 12, and 14 years. The only underestimation was for the age groups of 8 and 15 years. The underestimation found in the age group of 15 years was similar to the results reported by Urzel and Bruzek. They explained that most children had reached a total maturity score of 100 and that no further scoring could be performed.[30]

The Nolla Method:
The Nolla method provided mixed results for various populations. When tested on Turkish children, Nolla's method reported an underestimation of CA, with the mean age differences being -0.003 years for boys and -0.32 years for girls.[27] Maber et al. reported similar results of underestimation of CA by −0.87 years for boys and −1.18 years for girls in their study on 3–16.99 years old children of Bangladesh and British Caucasian ethnic origin [22]. Hegde et al. reported a mean difference of -0.13 ± 0.80 years for boys and -0.30 ± 0.82 years for girls in Indian children aged 5 to 15 years.[23] Underestimations have also been found in South American, Portuguese, and Spanish populations.[31,32]
However, overestimation of CA has also been reported in studies on Malaysian and south Indian populations.[43,44] Moreover, in the Chinese population, overestimation and underestimation were observed in boys and girls, respectively.[26] In contrast to other studies, Nolla's method was suitable for estimating CA of Brazilian children with due care, considering that the growth spurt commences at approximately 11 and 12 years.[33]
For the Saudi population, Yassin et al. reported that Nolla's method underestimated CA in all age groups and both sexes, with an age difference of −2.68 months to −6 months in boys and −2.17 months to −4.24 months in girls.[45] This was similar to our study, where an underestimation was found in all age groups and sexes; however, the difference was more pronounced. The probable reason for these higher age differences in our results could be the different utilization of the method by not adding fractions to the staged scores of mineralization for each tooth.

The MFH method:
The MFH method underestimated CA in this study in all age groups and sexes, consistent with the results of several studies conducted in different populations. A study performed by Martínez GVM et al. in the Venezuela population found consistent age underestimation in all groups and sexes, with the variation ranging from 0.20 ± 1.14 to 7.61 ± 0.231 years.[32] Similar results

were reported in three samples of South African children aged 3–16 years.[46]

Additionally, when tested on Mangalorean children, the MFH method significantly underestimated CA, with mean age differences of -3 years for boys and -2.9 years for girls.[47] Underestimation was also found in Kuwaiti children aged 5 to 15 years, with mean age differences of 1.01 years for girls and 0.89 years for boys [48]. In a sample of American Caucasian children aged from 9 to 14 years, the MFH method underestimated CA by 2.3 years for girls and 1.9 years for boys.[49]

Contrary to other studies, Corral et al. concluded that the MFH method presented a high correlation coefficient between DA and CA, with a tendency to overestimate CA of Colombian children aged 5–16 years.[50] Although the MFH method has been tested in different populations, a literature search revealed that none of the studies had tested the accuracy of the MFH method for DA assessment in the Saudi population.

The Chaillet method:

In this study, Chaillet's original standards for Belgian children were used; to obtain an increase in reliability, the 95th percentile of dental maturity was used to calculate DA.

Studies testing the Chaillet's multi-ethnic international maturity standards method have reported overestimations of 0.28±0.90 and 0.37±1.04 years in boys and (0.09 ± 0.83) and (0.21± 1.07) years in girls of Bosnian-Herzegovinian and Spanish Caucasian populations, respectively.[38] However, underestimation has been reported in several populations, such as Venezuelan, Indians using Chaillet's original standards for Belgian children and Kosovar populations.[30,38,51,52]

In the present study, the underestimations were higher than those reported in previous studies that used Chaillet's original standards for Belgian children. This difference between our results and those of the previous studies may be attributed to the 95% percentile being considered in this study. The higher the considered percentile level, the stronger the reliability, and the accuracy decreases as the reliability increases.[7]

The Gleiser and Hunt method:

In this method, age estimation uses the calcification of the permanent mandibular first molar only. In our study, all statistical analyses using the Gleiser and Hunt method were performed when the CA was ≤ 11.99 years. Because DA estimation is limited by tooth maturation, the mandibular first molar achieves its final maturation at 11 years.[53] Unlike the present study, previous studies tested the applicability of the Gleiser and Hunt dental staging system modified by Kohler on the second and third molars.[54,55] Therefore, comparisons could not be made.

In this study, the Gleiser and Hunt method underestimated CA in both sexes, except for the age groups of 6 and 7 years, for which overestimations were obtained.

The Nicodemo et al. method:

The method proposed by Nicodemo et al. consistently underestimates age in various populations. When tested on Indian children, Nicodemo et al. reported an underestimation of CA for both sexes, and the differences were more pronounced in older groups [56]. Kurita et al. and Silva et al. reported similar results of CA underestimation in their studies on the Brazilian population.[57,58]

Our results are in accordance with previously published studies, where the Nicodemo et al. method underestimated CA for both sexes. Underestimations were also more pronounced in the older age groups. The only overestimation was for the age groups of 6 and 7 years.

Comparison between the methods:

Chaillet et al. considered a 1-year accuracy sufficient in forensic anthropology, whereas McKenna et al. commended ±0.5 years as more acceptable.[59, 60] An age estimation method is considered accurate if it predicts CA as closely as possible. In our study, the most accurate method was the Demirjian's method, followed by the MFH method, whereas the Nicodemo et al. and Chaillet et al. methods were the least accurate. Moreover, repeated measures ANOVA verified the significant differences among the tested methods.

Most studies on DA estimation have compared only two different methods; few have studied the accuracy of six different methods simultaneously. Kelmendi et al. evaluated the accuracy of four Demirjian, Chaillet, and Willems methods for age estimation in Kosovo children. Their results indicated that the Demirjian method from 1973 was the least accurate among the six methods.[61] Kumaresan et al. tested the accuracy of five DA estimation methods (Demirjian, Willems, Nolla, Haavikko, and Cameriere) in 426 Malaysian children aged 5–15 years. The Demirjian method exhibited the lowest precision and accuracy among those tested.[44]

Several studies have compared the Demirjian and Nolla methods. Melo and Ata-Ali compared these two methods in a Spanish population and stated that both methods were accurate in estimating CA in a Spanish population, with an overestimation of age using the Demirjian method and an underestimation using the Nolla method.[62] Tomás et al. reported similar results for the Portuguese and Spanish samples.[31] Duruk et al. found that Nolla's method was more accurate for CA estimation than Demirjian's method in an Eastern Turkish population [27]. Similar results were reported by Lopes et al. in Brazilian children aged 7–13 years.[33]

Additionally, Han et al. studied the accuracy of the Demirjian, Willems, and Nolla methods in a northern Chinese population. Among the three methods, the accuracy was the highest for the Nolla method.[26] However, Cortés et al. found that the Willems method was more appropriate when the three methods were tested in a Spanish ethnicity population.[63]

Additionally, Mohammed et al. concluded that Nolla's method was more accurate in estimating DA in southern Indian children than Demirjian, Willems, and Haavikko's methods.[43] Gutiérrez and Ortega-Pertuz studied the accuracy of three methods (Nolla, Moorrees et al. and Demirjian) in 512 Venezuelan children aged 6–18 years; their results indicated that the Demirjian method was the most accurate; whereas the Moorrees et al. method was the least accurate [32]. In contrast, Tony et al. stated that neither the Demirjian nor the Moorrees et al. methods accurately estimated CA in their sample of contemporary American Caucasian children aged 9–14 years.[49]

Furthermore, Chaillet's method was more appropriate for Spanish and Venezuelan children than the Demirjian method.[38] Nevertheless, Pinchi et al. found Willems and Demirjian methods as the most accurate, though they overestimated CA, compared to Cameriere and Haavikko's methods in the Italian population.[64]

The different results in various populations can be attributed to genetic variations, ethnicities, climate, and environmental factors, such as nutrition, dietary habits, and lifestyle, significantly influencing tooth development. Moreover, the uneven sample size of each age group may affect the accuracy of DA estimation. A previous finding that age can be more accurately predicted in younger children than in older children was observed in the present study for both sexes. This is mainly because more teeth continue to develop in this period, which can provide more information for DA estimation. For older age groups, most teeth had already completed their development; therefore, only a few teeth attributed to DA estimation, resulting in a large mean difference between DA and CA. Thus, DA estimation may be more accurate in studies with larger samples of younger patients.

Another possible reason could be age mimicry, a phenomenon in which the target population's estimates tend to mimic the reference population's age structure (the population upon which a method is based).[65] Liversidge et al. used this phenomenon to explain the poor performance of the MFH 1963 method in their study, which is one of the few radiographic studies from birth to the age of 25 years.[20]

Another explanation is the complexity of some of these methods. The methods of Demirjian, Chaillet, and Nolla involve a complex process of double numerical conversion. Additionally, not all maturity score values could be found in the conversion tables provided by Demirjian (1973), Chaillet (2004), and Nolla (1960); in these cases, we had to estimate the DA based on the closest smaller maturity score values, which might influence the accuracy of these methods. Moreover, the MFH method involves demanding steps for interpolating the attained values from the graphs, followed by calculating the predicted values from these interpolated values. The problem of inter-observer error in interpolation from graphs influences the accuracy of this method. However, the MFH method was more straightforward when the Smith tables were used.

One limitation of this study is that it was conducted in a geographically restricted sample. This study was conducted in Riyadh, Saudi Arabia and did not include other regions of Saudi Arabia. Hence, the results of this study cannot be generalized to the Saudi population. Further studies applying these methods to other Saudi Arabian regions would be beneficial. Additionally, computerized tools for calculating DA could be used instead of the classic method for a better workflow.

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Validation of radiographic visibility of root pulp in mandibular first, second and third molars in the prediction of 21 years in a sample of south Indian population: A digital panoramic radiographic study

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KEYWORDS

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ABSTRACT

This study examines the radiographic visibility of root pulp (RPV) in lower first, second and third molars to validate the completion of 21 years. RPV in all lower three molars of both sides was assessed using a sample of 930 orthopantomograms of individuals aged between 15 and 30. The scoring of RPV was done using the Olze et al. four-stage classification (Int J Legal Med 124(3):183–186, 2010). Cut-off values were determined for each molar using the receiver operating characteristic (ROC) curve and the area under the ROC curve (AUC). The selected cut-off values were stage 3 for the first molar, stage 2 for the second molar and stage 1 for the third molar. For lower first molar, the AUC was 0.702, and the sensitivity, specificity and posttest probability (PTP) were 60.1%, 98.8% and 98.1% in males, and 64.5%, 99.1% and 98.6% in females. For lower second molar, the AUC was 0.828, and the sensitivity, specificity and PTP were 75.5%, 97% and 96.2% in males, and 74.4%, 96.3% and 95.3% in females. For the lower third molar, the AUC was 0.906; the sensitivity was 74.1% and 64.4% in males and females, while specificity and PTP were 100% in both sexes. The accuracy of predictions for the completion of 21 years was high. However, the greater percentage of false negatives and inapplicability of this method in one-third of lower-third molars have been recommended for using this method in conjunction with other dental or skeletal methods.

INTRODUCTION

An essential aspect of age assessment practice is the correct discrimination of the subjects according to the age threshold in question and reducing false categorisation. The increase in the proportion of individuals who were unable to or unwilling to disclose their actual age made it necessary for age assessments to provide justice. Besides legal issues and issues related to undocumented migrants, age estimation practices in adolescents and sub-adults also concern the field of competitive sports.[1, 2] In such instances, the completion of the 18th year and 21st year of life is validated of paramount importance. In India, the completion of the 21st year of life is essential for marriage in males.[3, 4]

The development and the completion of third molars is a subject of interest in forensic and medico-legal practice as it provides proof of attainment of legal ages 16 and 18. However, the evidence of completion of third molar development even before 18 years makes it difficult to validation of the completion of legal

age thresholds.[5] This necessitates alternative markers associated with normal ageing to predict the completion of the 21st year of life. In 2010, Olze et al.[6] described a four-stage classification based on the root pulp visibility in lower third molars. It studies the changes in the appearance of root canals of lower third molars that could relate to secondary dentin deposition and appositional bone growth.[7] Later, several researchers tested this method in different populations and concluded that this method could be reliable, reproducible, and helpful in assessing ages over 18 and 21 years.[7-14] Few researchers tested the applicability of this method in first and second molars and recommended the use of this method for predicting the age over 14, 16 and 18 years in the absence of third molars.[15-18]
To date, researchers have only studied the applicability of this method in lower third molars to predict the attainment of the legal age of 21 years.[11] To the best of our knowledge, no studies were available in the literature studying this pattern in lower first and second molars to predict 21 years. Therefore, the present paper explores the potential application of root pulp visibility in mandibular first, second and third molars to determine whether or not a subject in question is under or over the 21-year threshold.

MATERIALS AND METHODS

Sample collection and selection criteria
A total of 930 OPGs (465 males and 465 females) from adolescents, young adults, and adults of South Indian origin aged between 16 and 30 years were collected. All the OPGs were obtained retrospectively from the archives of private dental clinics in 2017 and 2020. The inclusion criteria were radiographic images of good diagnostic quality with at least one mandibular first, second, and third molars. The most typical reasons for excluding radiographs were molars with caries, restorations, evidence of endodontic treatment, and dental anomalies. A few more reasons are one-rooted and molar teeth with root foreshortening due to perspective.
After meeting the selection criteria, each OPG was given a unique identification number (UIN). Details of sex, date of birth, and the date on which the radiograph was taken were entered against each UIN. The chronological age of each individual is obtained after calculating the difference between the date of birth and the date of exposure.

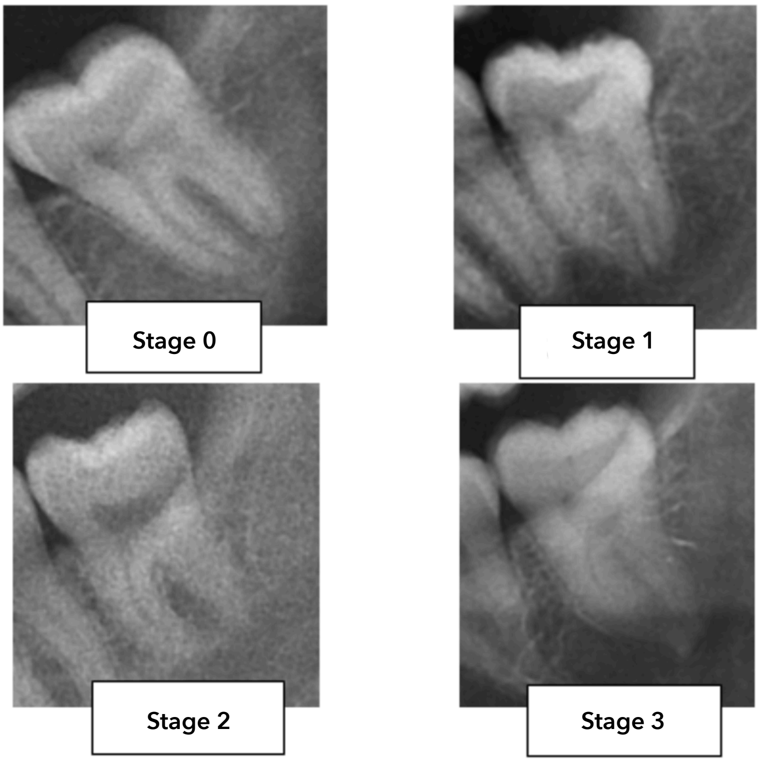
Method
In each OPG, the lower first, second, and third molars from both sides were categorised using the Olze et al. four-stage classification of root pulp visibility (Figure 1).[6] In stage 0, the lumen of the root canals is visible up to the apex. In stage 1, the lumen of one root canal is discernible up to the root apex; in stage 2, two root canals with incompletely visible lumen to the apex or one canal might be virtually not visible in entire length; and in stage 3, the lumen of two root canals is almost not visible in entire length.
All the OPGs were analysed and graded by a forensic odontologist with more than eight years of experience in forensic age estimation. To study the intraobserver variability, the researchers randomly selected 90 OPGs to re-evaluate. The minimum time interval between both examinations was one month. And to explore the interobserver variability, the same number of OPGs was evaluated by another investigator.

Statistical analysis
IBM SPSS Statistics for Windows v20 (IBM Corp., Armonk, NY) was used to perform statistical analysis, and the level of significance was set at 5% ($p < 0.05$). Inter- and intra-observer agreements were calculated using Cohen's kappa test. Descriptive statistics, including mean, standard deviation, minimum age, maximum age, and median, were performed for each stage in all lower molars for both sexes. Chronological age was recorded as a binary variable with a cut-off at 21 years old, i.e., "0" for subjects under 21 years and "1" for those above 21 years. Chi-square tests were used to test the relationship between chronological age and stage attainment. Receiver operating characteristic (ROC) curve analysis was performed, and we sought optimal grade cut-offs to classify subjects into particular categories. As sex is a possible confounding variable, we performed separate ROC analyses for both sexes.
The performance of the cut-off values, i.e., stages of root pulp visibility of lower three molars, were evaluated by calculating the areas under the ROC curve (AUC), sensitivity, specificity, and likelihood ratios (LR+ and LR-). AUC is a measure of the accuracy of a quantitative diagnostic test. It represents the overall performance of the stages of root pulp visibility

in discriminating subjects under or over the 21-year threshold. The sensitivity indicates the probability that cases will be correctly diagnosed as being 21 or older; specificity suggests the possibility that controls will be correctly interpreted as younger than 21. The likelihood ratio combines sensitivity and specificity into a single value that indicates how much the test result will reduce the uncertainty of a given diagnosis.[19, 20] For example, LR+ indicates how many true positives will be observed per false positive. Therefore, an LR+ of >1 suggests a positive result is likely correct. The greater the value, the higher the probability that the outcome is correct. On the other hand, LR− indicates how many false negatives will be observed per true negative. The closer the LR− value is to 0, the higher the discrimination potential of the cut-off value.

The root pulp visibility stages in the first, second, and third molars may help discriminate between individuals under or over the legal age of 21 years by the Bayes post-test probability. Using the census data of India (http://www.censusindia.gov.in/2011census/C- series/C-13.html), we assumed the possibility of the individual in question being 21 or older in the target population between 15 and 30 years is 0.50 in males and females.

Figure 1. Diagrammatic representation of Olze et al stages of root pulp visibility in mandibular molars



RESULTS

Sample studied and excluded OPGs

Table 1 displays the age and sex distribution of the overall sample. The mean age of the males and females was 21.65 ± 4.33 and 21.64 ± 4.36 years, respectively.

Table 2 lists the reasons for excluding OPGs or third molars from evaluation. Approximately one-third of lower third molars (right and left) in females and 28% in males were not eligible for evaluation. The most common reason for their exclusion is the developing third molars with open apices; missing third molars (below 5% in both sexes) is another reason. Most developing third molars were seen below 20 years of age.

Intraobserver and interobserver agreements

Cohen kappa statistics revealed that the intraobserver agreement was 0.845 ($p < 0.001$) with a 95% confidence interval (CI) of (0.731, 0.946), indicating almost perfect agreement. On the other hand, the interobserver agreement was 0.762 ($p<0.001$) with a 95% confidence interval (CI) of (0.628, 0.891), indicating a substantial agreement.

Data analysis

Table 3 shows the results of descriptive statistics, i.e., mean, standard deviation, minimum age, maximum age, and median for each stage of root pulp visibility in lower first, second and third molars (right and left) in both sexes. Table 4 displays the proportion of subjects under and over the 21-year-old thresholds for each stage of root pulp visibility in both sexes.

Table 5 shows the performance of root pulp visibility stages at the optimal cut-offs, i.e., stage 3 in the lower first molar, stage 2 in the lower second molar, and stage 1 in the lower third molar for indicating the age above 21 years. ROC curve analysis showed that stage 3 of root pulp visibility in the lower first molar exhibited moderate discriminatory capacity (AUC, 0.702; 95% CI, 0.668- 0.736) and stage 2 in the lower second molar (AUC, 0.828; 95% CI, 0.801- 0.856) and stage 1 in the lower third molar (AUC, 0.906; 95% CI, 0.884- 0.929) has exhibited high discriminatory capacity (Figures 2 to 4).

Table 1. Age and sex distribution of the total sample

Age groups	Males	Females	Total
15- 15.9 years	40	40	80
16- 16.9 years	40	40	80
17- 17.9 years	40	40	80
18- 18.9 years	40	40	80
19- 19.9 years	40	40	80
20- 20.9 years	30	30	60
21- 21.9 years	30	30	60
22- 22.9 years	30	30	60
23- 23.9 years	25	25	50
24- 24.9 years	25	25	50
25- 25.9 years	25	25	50
26- 26.9 years	25	25	50
27- 27.9 years	25	25	50
28- 28.9 years	25	25	50
29- 29.9 years	25	25	50
Total	**465**	**465**	**930**

Table 2. Age and sex distribution of the total sample

Reason for exclusion	Tooth 38		Tooth 48	
	Males	Females	Males	Females
Developing	117 (25.1)	135 (29)	118 (25.3)	134 (28.8)
Missing	14 (3.1)	20 (4.3)	13 (2.8)	16 (3.4)
Total	131 (28.1)	155 (33.3)	131 (28.1)	150 (32.2)

Tooth 38, Lower left third molar; Tooth 48, Lower right third molar

Table 3. Descriptive statistics of chronological age according to sex and RPV stages in lower first, second and third molars of both sides

Teeth	Stage	Males						Stage	Females					
		n	Mean	SD	Min	Max	Median		n	Mean	SD	Min	Max	Median
36	0	45	16.69	1.11	15.10	18.98	16.59	0	42	16.25	0.83	15.05	18.43	16.25
	1	117	17.83	1.85	15.02	21.91	17.62	1	125	17.96	1.92	15.01	21.99	17.89
	2	219	22.71	3.41	15.50	29.91	22.31	2	188	22.05	2.93	16.23	19.22	22.12
	3	84	26.87	2.14	15.06	29.92	26.85	3	110	27.19	1.98	17.51	29.96	27.47
46	0	40	16.56	0.99	15.10	18.98	16.34	0	44	16.29	0.85	15.05	18.43	16.26
	1	123	17.82	1.83	15.02	21.91	17.62	1	125	17.99	1.96	15.01	22.71	17.89
	2	224	22.79	3.36	15.50	29.91	22.39	2	196	22.26	2.97	16.23	29.22	22.34
	3	78	27.02	2.10	15.06	29.92	26.99	3	100	27.34	1.95	17.51	29.96	27.55
37	0	163	17.53	1.82	15.02	22.59	17.33	0	158	17.43	1.86	15.01	22.45	17.08
	1	135	21.01	2.51	15.06	26.91	20.76	1	143	21.08	2.67	16.15	26.83	20.55
	2	141	25.80	2.53	20.03	29.91	26.09	2	130	25.63	2.51	20.28	29.94	25.65
	3	26	28.23	1.26	25.82	29.92	28.28	3	34	28.33	0.99	25.59	29.96	28.17
47	0	160	17.51	1.84	15.02	22.59	17.20	0	158	17.46	1.90	15.01	22.45	17.08
	1	142	21.02	2.52	15.06	26.91	20.71	1	148	21.17	2.81	16.15	29.94	20.56
	2	154	26.11	2.51	20.03	29.92	26.31	2	134	25.82	2.42	20.28	29.90	25.91
	3	09	28.66	0.53	27.88	29.43	28.66	3	25	28.50	1.04	25.59	29.96	28.31
38	0	143	20.08	1.99	15.06	26.50	19.79	0	136	20.55	2.35	17.05	26.81	20.23
	1	188	25.81	2.51	21.08	29.92	26.05	1	174	25.96	2.52	21.02	29.96	26.25
	2	03	29.24	0.15	29.14	29.43	29.17	2	--	--	--	--	--	--
	3	--	--	--	--	--	--	3	--	--	--	--	--	--
48	0	168	20.63	2.33	15.06	27.46	20.09	0	170	21.09	2.46	17.05	26.83	20.61
	1	163	26.09	2.48	21.08	29.92	26.36	1	144	26.51	2.33	21.02	29.96	26.93
	2	03	29.24	0.15	29.14	29.43	29.17	2	01	--	--	--	--	--
	3	--	--	--	--	--	--	3	--	--	--	--	--	--

n, number; SD, Standard deviation; Min, Minimum age; Max, Maximum age.

Table 4. Proportion of subjects under and over 21 years according to the RPV stages for all lower three molars in both sexes

Tooth	Age category	Males				Females			
		Stage 0 n (%)	Stage 1 n (%)	Stage 2 n (%)	Stage 3 n (%)	Stage 0 n (%)	Stage 1 n (%)	Stage 2 n (%)	Stage 3 n (%)
36	<21 years	45 (9.6)	110 (23.6)	74 (16)	01 (0.2)	42 (9)	116 (25)	71 (15.2)	01 (0.2)
	≥21 years	0 (0)	07 (1.5)	145 (31.1)	83 (17.8)	0 (0)	09 (1.9)	117 (25.1)	109 (23.4)
37	<21 years	152 (32.6)	73 (15.6)	05 (1.07)	00 (0)	149 (32)	75 (16.1)	06 (1.3)	00 (0)
	≥21 years	11 (2.3)	62 (13.3)	136 (29.2)	26 (5.6)	09 (1.9)	68 (14.6)	124 (26.6)	34 (7.3)
38	<21 years	106 (22.8)	00 (0)	00 (0)	00 (0)	89 (19.1)	00 (0)	00 (0)	00 (0)
	≥21 years	37 (7.9)	188 (40.3)	03 (0.6)	00 (0)	47 (10.1)	174 (37.4)	00 (0)	00 (0)

Table 5. Performance measures of root pulp visibility (RPV) in lower first, second and third molars for discriminating legal age 21 years

Quantity	Tooth 36	Tooth 37	Tooth 38
Males			
Accuracy	67.1 (62.6- 71.3)	83.2 (79.5- 86.5)	88.9 (85.1- 92.1)
Sensitivity	60.1 (54.9- 65.06)	75.5 (70.2- 80.3)	74.1 (66.1- 81.1)
Specificity	98.8 (93.5- 99.9)	97.01 (93.1- 99.02)	100 (98.1- 100)
Positive LHR	50.49 (7.18- 354.87)	25.22 (10.61- 59.94)	--
Negative LHR	0.40 (0.36- 0.46)	0.25 (0.21- 0.31)	0.26 (0.2- 0.34)
PTP	98.1 (87.8- 99.7)	96.2 (91.4- 98.4)	100
Females			
Accuracy	72.7 (68.3- 76.7)	82.1 (78.3- 85.5)	84.4 (80.3- 88.6)
Sensitivity	64.5 (59.2- 69.5)	74.4 (69.1- 79.2)	65.4 (56.8- 73.3)
Specificity	99.09 (95.04- 99.9)	96.3 (92.2- 98.6)	100 (97.9- 100)
Positive LHR	70.96 (10.07- 500.04)	20.34 (9.25- 44.74)	--
Negative LHR	0.36 (0.31- 0.41)	0.27 (0.22- 0.32)	0.35 (0.27- 0.44)
PTP	98.6 (91- 99.8)	95.3 (90.2- 97.8)	100

LHR, Likelihood ratio; PTP, Posttest probability

Figure 2. ROC curve for stage 3 root pulp visibility in lower first molar (Tooth 36) for 21-year threshold

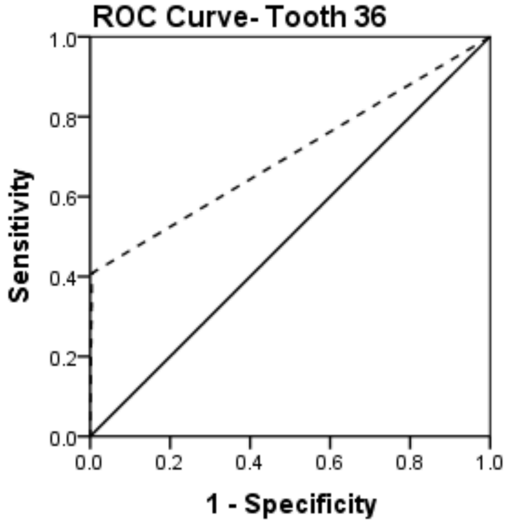


Figure 3. ROC curve for stage 2 root pulp visibility in lower second molar (Tooth 37) for 21-year threshold

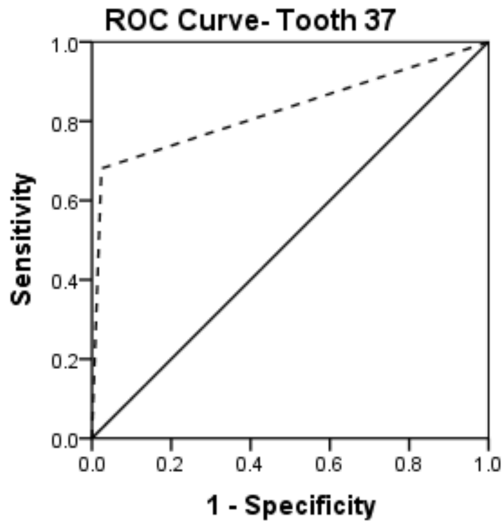
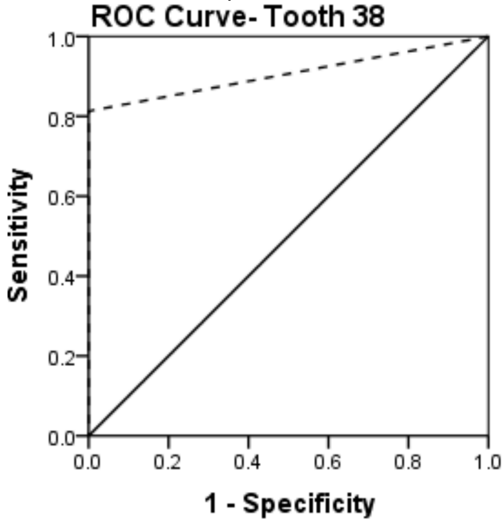


Figure 4. ROC curve for stage 3 root pulp visibility in lower third molar (Tooth 38) for 21-year threshold



DISCUSSION

It is essential to reduce false categorisation in age assessment practice. According to Akkaya N et al.,[11], a perfect "gold standard" age estimation method does not exist as all methods are associated with some errors. Furthermore, using techniques in conjunction with other methods is recommended, mainly while categorising individuals as above or below the age threshold of medicolegal importance. For example, a four-stage classification of root pulp visibility in lower third molars by Olze et al. [6] seems to be accurate in discriminating individuals older than 18 and 21 years. However, Al Qattan et al. [7] have stated multiple reasons (developing third molars, third molars with single roots, buccolingually tilted molars, missing teeth, positional or morphological anomalies, etc.), suggesting that it is not a suitable method. Therefore, it is essential to test this parameter, i.e., root pulp visibility in alternative teeth in the absence of third molars, to predict the legal age of 21 years. Therefore, the present investigation assessed the root pulp visibility in lower first, second, and third molars for the discrimination of individuals over 21 years.

Our study findings confirmed that repeatability (intraobserver differences) was almost perfect, while the reproducibility (interobserver differences) was substantial. These substantial findings could be related to the measurement of pulp visibility in an ordinal manner with four possible scores. One factor that could influence reproducibility is subjectivity which could result in observer errors. And, also the resolution of the radiographic images could affect the display of anatomical details. Therefore, observer training and calibration are essential.

Excluded OPGs

The present investigation selected OPGs based on a strict inclusion criterion, especially for lower first and second molars. Therefore, radiographic images with tilted molars, positional anomalies, and images that challenge the analysis of the molars due to superimposition or perspective factors were excluded beforehand. On the other hand, we have included OPGs with developing molars with incomplete roots and missing teeth for lower third molars. It shows how many samples were excluded from analysis and the percentage

of third molars the method could predict age above 21 years.
Our results showed that approximately one-third of the OPGs in females (tooth 38, 33.3% and tooth 48, 32.2%) and 28.1% OPGs (tooth 38 & 48) in male subjects were excluded from analysis due to developing third molars. Around 3 to 5% of the excluded sample in both sexes were due to missing teeth (developmentally missing or extracted).

Data analysis- Third molars
Few researchers have studied the applicability of this method in discriminating subjects over 21 years.[6, 9-12] In the original study by Olze et al. [6], the authors stated that if stage 1 was observed in the lower third molar of the examined individual, then he/ she is most probably 21 years of age, and the presence of stages 2 and 3 can safely put them above 21 years. Guo et al. [12], in their study among Northern Chinese subjects, found that individuals who were found to be in Stages 2 and 3 were at least older than 21 years. Timme et al. [10] reported very similar values. In another study by Gok et al., [21] individuals under stage 3 of root pulp visibility are over 21.
Similarly, Perez- Mongiovi et al. [9] also found stage 3 a helpful marker in Portuguese females. However, differences were noticed when our study findings were compared with others. In the present study, the earliest appearance (minimum age) of stage 1 was 21.08 years in males and 21.02 years in females. Very few male subjects (n= 3) were graded with stage 2 root pulp visibility with a minimum age of 29.14 years. None of them was graded with stage 3 in the studied age range. The possible explanation for the differences among studies could be research design, statistical approaches, sample age ranges, population differences, and inter-observer variations.
Based on our findings in lower third molars, when stage 1 root pulp visibility was observed in the studied subjects, they were at least above 21 years. However, when tested as a cut-off value for indicating age above 21, it resulted in a sensitivity of 74.1% and 65.4% in males and females, specificity, and posttest probability of 100% in both sexes. These lower sensitivity values indicate that one in four males and one in five females older than 21 could be wrongly identified as subjects below 21 years, resulting in false negatives.

Data analysis- First and second molars
To the best of our knowledge, this is the first Indian study to verify the stages of root pulp visibility in lower first and second molars to predict the legal age of 21 years. In our research, the earliest observation of stage 0 in males and females for lower first molars were 15.1 and 15.05 years, 15.02 and 15.01 years for stage 1, 15.50 and 16.23 years for stage 2, and 15.06 and 17.51 years for stage 3, respectively. When stage 3 of root pulp visibility in lower first molars was used as a cut-off value to predict the completion of 21 years, it resulted in a sensitivity of 60.1% and 64.5% in males and females, specificity of 98.8% and 99.1%, and posttest probability of 98.1% and 98.6% in both sexes.
The earliest observation of Stage 0 for lower second molars was observed at 15.02 in males and 15.01 in females, stage 1 at 15.06 and 16.15, stage 2 at 20.03 and 20.28, and Stage 3 at 25.82 and 25.59 years, respectively. When stage 2 of root pulp visibility in lower second molars was used as a cut-off value to predict the completion of 21 years, it resulted in a sensitivity of 75.5% and 74.4% in males and females, specificity of 97.01% and 96.3%, and posttest probability of 96.2% and 96.3% in both sexes.

False categorisation/ errors
In forensic age estimation, two types of errors can occur, i.e., ethically unacceptable errors/ false positives (Type I) and technically unacceptable errors/ false negatives (Type II).[22] Although all errors should be kept to a minimum in forensic age estimation, it is essential that type I errors must be eliminated.
In the present study, a more significant percentage of type II errors was observed when stages of root pulp visibility in lower first, second and third molars were used as age markers for ages over 21 years. When stage 3 root pulp visibility in the lower first molar was used, it resulted in less than 1% false positives and 59% false negatives. Stage 2 root pulp visibility in the lower second molar resulted in 2.4% false positives and 32% false negatives. On the other hand, stage 1 root pulp visibility in the lower third molar resulted in zero false positives and 18.7% false negatives.

Strengths, limitations and future considerations
One of the strengths of the present study is the equal distribution of samples (with matching

males and females samples) among the age groups studied. Secondly, our study findings have provided an alternative parameter for determining the completion of the 21st year of life without third molars. These were crucial for predicting legal ages 18 and 21. Our study has some limitations. Mainly, the presence of a higher percentage of developing third molars or missing third molars is excluded from evaluation. Further studies are warranted to improve the interobserver differences using continuous markers, such as root pulp area, which may better the predictive performance. Future studies should investigate the influence of ethnicity and dietary habits on root pulp visibility.

CONCLUSION

Based on the study findings, the following conclusions can be made:

1. Subjects graded with stage 3 root pulp visibility in lower first molars were at least older than 21.
2. Subjects graded with stage 2 and 3 root pulp visibility in lower second molars were at least older than 21 years of age.
3. Subjects graded with stage 1, 2, and 3 root pulp visibility in lower third molars were at least older than 21 years of age.

Therefore, all the lower three molars have resulted in better specificity and posttest probability values (>90%), indicating that they could be reliable in forensic age estimation. However, it is advised to proceed with caution. They should be used in conjunction with other age estimation methods owing to the more significant percentage of false negatives with first and second molars and the inapplicability of this method in approximately one-third of the sample in lower third molars.

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Geometrics Morphometrics in Craniofacial Skeletal Age Estimation - A Systematic Review

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KEYWORDS

Geometric morphometrics,
Age estimation,
Skeletal age,
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Age determination by Skulls,
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ABSTRACT

Geometric morphometrics is a novel statistical shape based technique used as an additional approach to the currently used methods in forensics for the assessment of age. Various craniofacial units are used for the estimation of age using this technique. The aim of this systematic review was to assess if Geometric Morphometrics is an accurate and reliable method in craniofacial skeletal age estimation. A literature search was conducted for cross-sectional studies on geometric morphometrics in craniofacial skeletal age estimation using various search engines such as Pubmed, Google Scholar, and Scopus using specific MESH terms. AQUA (Anatomical Quality Assessment) tool was used for the quality assessment. A total of 4 articles were included for qualitative synthesis as they met the objectives of this review. The results of all the included studies suggested that geometric morphometrics can be used for craniofacial skeletal age estimation. The centroid size calculated using digitized images or CBCT scanned images is said to be the highest predictor of age.This systematic review summarises the merits and demerits of this technique and suggests that it is rapid and accurate method for age estimation even in instances of single skeletal remains of craniofacial units and can be performed on a digitized image or a CBCT scanned images. However, further studies are needed to derive reliable data and meta-analysis can be performed effectively.

INTRODUCTION

Aging is a gradual, continuous and uncertain process of natural change that begins in early adulthood as there are continuous variations occurring in human skeleton, ligaments, muscles, skin etc[1].However, it is been noted that at the end of skeletal development, few features which are age dependent (e.g., ossification centers, bone anatomy and fusion of epiphyses etc) [2] remain unchanged [3]and can be used for skeletal age estimation.

Several studies have utilized various craniofacial units such as frontal sinus [4] palatal sutures[5] sphenooccipital synchondrosis[6] mandible [7] for estimation of age and have shown that these units can be used for better assessment of age. In conventional morphometrics technique size of an object is measured and linear distances are compared and detection of the morphological similarities or differences in a sample is done. However this technique has several disadvantages including size and orientation differences within the sample [8] so a new metrics approach called as geometric morphometrics was developed.

Geometric morphometrics is, "A well-established statistical shape method which can be used to quantify the biological forms in landmark-based analysis."[9-12]
In this technique, landmark points are placed on the images to quantitatively analyze the shape so as to capture the geometry of the morphological structures and to preserve their information for further statistical analysis.[8, 13] Another important contribution of this technique is that it clearly defines the definition of shape and size (centroid size).[13, 14] The centroid size is defined as, "The square root of summed squared distances from each landmark to the configuration centroid." Further the cartesian coordinates of semi landmarks and landmarks are captured in their geometric form.
Landmarks are anatomically recognizable areas which are selected properly to capture the shape and is capable of being replicated. The semi-land marks [15], are used when the location of a landmark along a curvature might not be identifiable or repeatable. Thus, with these landmarks and semi-landmarks, a three-dimensional image can be created which aids in assessment of age.[13-15]
There is no systematic review which evaluates the accuracy of using geometric morphometric method for craniofacial age estimation. Thus, the objective of this review is to summarize the results of the studies done for age estimation by geometric morphometric method using craniofacial units and to assess its accuracy and reliability.

MATERIALS AND METHODS

Protocol and registration
The review is registered in PROSPERO (International prospective register of systematic reviews) with the number CRD42020206250.
This systematic review used the Preferred Reporting Items for Systematic Review and Meta Analyses (PRISMA) guidelines.[16]

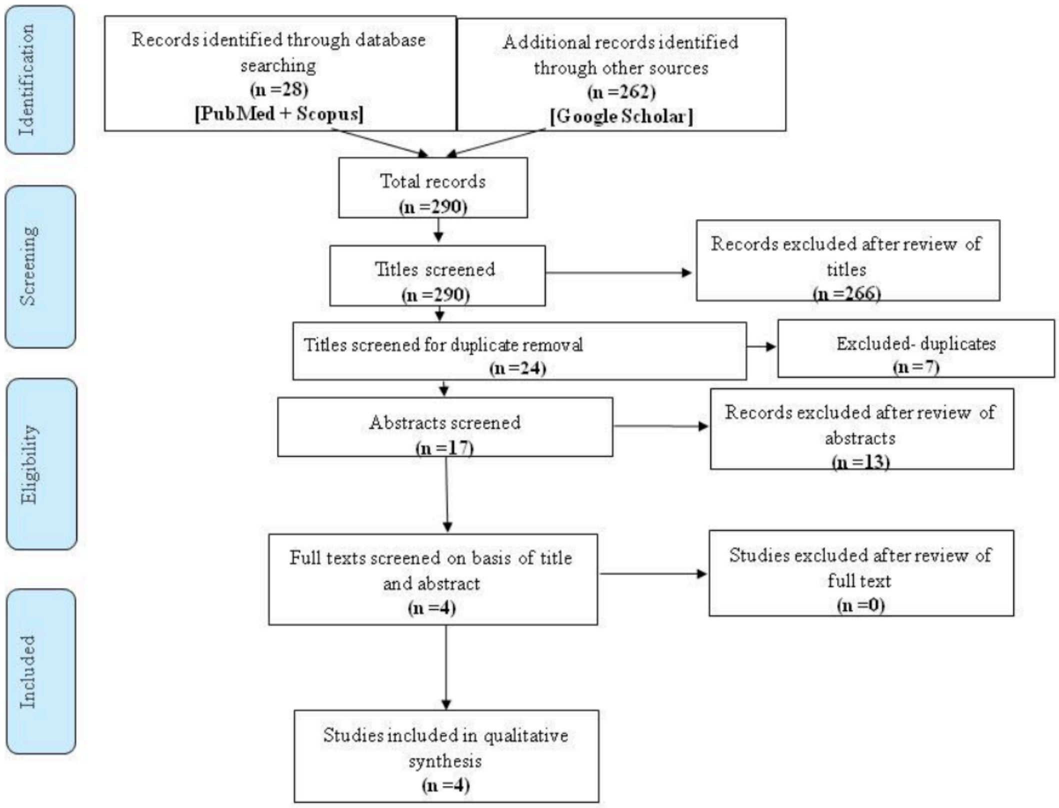
Search strategy:
The comprehensive data search was performed in 'PUBMED', 'GOOGLE SCHOLAR' and 'SCOPUS' data bases for publications till 1st September 2021. Language restrictions were applied and only studies done in English language were included.
The search strategy used Medical Subject Heading (MeSH) terms like "Geometric Morphometrics AND Craniofacial Skeletal Unit" OR "Skulls, Cranium, Calvaria, Calvarium AND Age Estimation". Original studies done on geometric morphometrics in craniofacial skeletal age estimation were included and Review articles, Case reports & case series, Conference abstracts, Editorials, Commentaries Animal studies, Studies published in other languages were excluded.(Table 1)

Table 1. List of search engines used to retrieve articles along with keywords and search terms

Data base	Keywords and search terms	Number of articles Retrieved
PubMed	(("geometric"[All Fields] OR "geometrical"[All Fields] OR "geometrically"[All Fields] OR "geometrics"[All Fields]) AND ("morphometric"[All Fields] OR "morphometrical"[All Fields] OR "morphometrically" [All Fields] OR "morphometrics"[All Fields]) AND ("humans"[MeSH Terms] AND "english"[Language]) AND ("craniofacial"[All Fields] OR "craniofacies"[All Fields]) AND ("humans"[MeSH Terms] AND "english"[Language]) AND ("skeletal"[All Fields] OR "skeletals"[All Fields])) AND ((humans[Filter]) AND (english[Filter]))	**24**
Google scholar	Geometric morphometrics AND craniofacial age AND skeletal age	**262**
Scopus	(TITLE-ABS-KEY (geometric) AND TITLE-ABS-KEY (morphometrics) AND TITLE-ABS-KEY (cranial) AND TITLE-ABS-KEY (skeletal) AND TITLE-ABS-KEY (age) AND TITLE-ABS-KEY (estimation))	**4**
	Total	**290**

Two review authors screened the titles and abstracts obtained by search strategy and included them if they met the inclusion criteria. Based on this, Full texts of 24 studies were obtained. Finally, the search yielded 4 studies to be included in systematic review. (Fig 1). Any disagreement between the authors was resolved by discussion

Figure 1. Flow diagram depicting the process of selection and exclusion of articles at each step



Risk of bias assessment

AQUA (Anatomical Quality Assessment) tool.[17] was used to assess the quality of the articles. The tool contains 5 domains and 20 signaling questions. The answers of these signaling questions are, "Yes", "No" or "Unclear". These answers indicate Risk of bias which is Low, High and Unclear, respectively. "Low", Risk of bias was judged when all the signaling questions were answered as "Yes". A consensus point was met by the authors when an answer was obtained as "No" as it indicated potential bias. Indication of "High" risk of bias suggested that the data obtained from the studies was insufficient and the "Unclear" option was used.

Among these 20 signaling questions 2 questions were eliminated as they were not applicable for the study design. (DOMAIN 4 and 5 the 4th signaling question)

RESULTS

Study selection

262 records were identified through data search using search strategy in google scholar, 24 records from PubMed and 4 records from Scopus. Finally, 4 articles were selected for qualitative synthesis as they were fitting in the inclusion criteria of the study.

Data Extraction and Study characteristics

A summary of the 4 studies included in the final systematic review is provided in (Table 2). Individual study characteristics and the outcome extracted from each included study are given below:

Table 2. Individual study characteristics and the outcome extracted from each included study are given below

Author	Jose Braga et al[18] 2007	Antoine Saade et al[8] 2018	Melissa Niel et al[19] 2019	Danniel Franklin et al[20] 2007
Title	Estimation of pediatric skeletal age using geometric morphometrics and three-dimensional cranial size changes	Predictability of Craniofacial Skeletal Age with Geometric Morphometrics	Maturation of the human foetal basioccipital: quantifying shape changes in second and third trimesters using elliptic Fourier analysis	Mandibular morphology as an indicator of human subadult age: geometric morphometric approaches
Location and race	France and North Africa, Toulouse,	lebanon	Marseilles, France	South African Bantu and African American
Study design	Cross sectional	Cross sectional	Cross sectional	Cross sectional
Imaging technique	CBCT	CBCT	CBCT	CBCT
Craniofacial skeletal unit	SKULL	SKULL	Basico occipit- CT-Skull	MANDIBLE
Age	Females- 3days to 17.67 yrs, Male- 3 to 16.5 yrs	7 - 15 yrs	Foetus between 18 - 41 Gestational weeks	1- 17 yrs
Sample size	127	48	221	79
Landmarks / location	supraorbital canal, supraorbital fissure,infraorbital canal ,round foramen and mental foramen and basicranial skeleton	Right and left supraorbital foramina , right and left superior orbital fissures, right and left foramen rotundum canals, oval foramina . Right and left infraorbital foramina . right and left mental foramina	Basico occipit	Coronion, Mandibular notch, Condyle, Posterior ramus. GonionMandibular body. Lateral gnathion infradentaleMentale, Posterior alveola Anterior ramus,Gnathion,Pogonio.symphysis Infradentale
Software used	Gamme Cépha © http://cepha.free.fr/gammecepha.php)	AVIZO 3D analysis software (version 8.1.1; FEI Visualization Sciences group, Merignac, France)	AVIZO Standard Edition software (v.7.0.0, Visualization Sciences Group, SAS)	Microscribe G2X portable digitizer running Inscribe-32 software
3D coordinates For calculating centroid size	Morphologika © (http://www.york.ac.uk/res/fme/resources/software.htm)	MorphoJ Software, version 1.06d	Morpho, Geomorph, TPSDIG2 v.2.17 digitization Programme., Elliptic Fourier analysis	Morphologika2, NTSYS-pc 2.2f
Statistical tests for Removal of shape variations	Generalized Procrustes analysis	Generalized Procrustes analysis	Generalized Procrustes analysis, Principal component analysis,	Generalized Procrustes analysis, Principal component analysis

Author	Jose Braga et al[18] 2007	Antoine Saade et al[8] 2018	Melissa Niel et al[19] 2019	Danniel Franklin et al[20] 2007
Statistical test used	Conventional least square linear regression analysis	Kolmogorov–Smirnov and Shapiro-Wilk statistics Quartile-Quartile plots. Pearson's coefficient Cook's distances, Mahalanobis distances, Residuals, Multiple regressions using SPSS v22.	Procrustes ANOVA RStudio using the software packages -Momocs, Morpho, Geomorph, factoextra, efourier and iefourier functions	Linear regression analyses Multiple regressions SPSS 11.5.0 TPSSmall 1.20
Outcome results	. Accurate results can be obtained when it is based on 3D facial size changes and study suggested that centroid size of the facial skeleton can be used as an age-related variable without any loss of accuracy with increased age.	. This study developed a new equation for determining craniofacial skeletal age was using the centroid size of the craniofacial frame, gender, and the known chronological age.	The study first quantified overall shape changes of the basioccipital between gestational ages and suggested that the morphological shape changes throughout the foetal period can be useful for anthropological studies and provide new perspectives for immature age estimation methods.	The study results showed that the mandible can be used to predict age in the subadult skeleton with accuracy comparable to standards based on the dentition (standard error rates arebetween ±1.3 and ±3.0 years) and will be accurate when adolescents are Included in the sample.

Jose Braga et al, [18] studied geometric morphometrics and its application on skeletal age using 3D shape changes occurring in the cranium of pediatric samples. The study was conducted on CT scan samples from different geographic locations like North Africa and France, Neuroradiology Unit, the Clinique Pasteur, Toulouse (France). Two major cranial components were considered the i.e, the face and base and were represented by skeletal landmarks. A cross-sectional sample of 73 non-adult females and 54 non-adult males ranging from 3 days-17.67 yrs and 3 days-16.5 yrs respectively were considered. Further using the Gamme Cepha software three dimensional points were marked on the CT scans.

The landmarks were distinguished in two configurations, both representing a major cranial component such as, 1. Facialand 2. Basicranial wire frame. Using the Morphologika software the centroid size was calculated for each wire frame and individual. Conventional least square liner regression and standard error at 95% confidence limit was used to indicate the accuracy. Standard error at 95% confidence level were, lower or equal to 2.1 years i.e., for the facial wire frame-1.27 to 2.09 and for the basicranial wire frame-1.52 to 2.64 years. The study showed more accurate results with the use of 3D facial changes only and the facial wire frame showed more significant and accurate results than the basicranial wire with an increasing age and the study concluded that use of geometric morphometrics gave more accurate results with an increasing age, contrary to most methods used in pediatric age estimation. This method has been reported to be reliable because it has demonstrated greater accuracy in centroid measurements of the facial skeleton with increasing age. This method is applicable from the early post-natal age to the end of adolescence and can be used on cranial remains.

To predict the craniofacial age Antoine Saadé et al,[8] conducted a study using geometric morphometrics technique and CBCT scans of 48 participants, which included 18 males and 30 females. This was further correlated with skeletal age which was obtained from hand and wrist radiograph. Six bilateral anatomical land marks were selected based on the method used by Wilson-Pauwels et al using a AVIZO 3D analysis software on the CBCT scans.The landmark analysis was performed using the MorphJ software and the 3D coordinates were obtained. Further, a Procrustes analysis was performed and the centroid size was calculated for each configuration. Mean skeletal age assessed was 11.9 ± 2.4 years and centroid size 151.5±7.2 was significantly correlated with chronological age and skeletal age. The study further determined a new equation for calculating craniofacial skeletal age using centroid size of the craniofacial frame , gender and craniofacial age . The study also highlighted the use of centroid size of craniofacial frame based on trigeminal landmarks as a good predictor to assess the skeletal age. The study emphasized on adding additional landmarks and a bigger sample size to allow better accuracy and possible results divergence related to gender.

Mellissa Niel et al, [19] conducted a study on shape changes occurring in the basioccipital bone of human fetus during third and second trimesters to understand the maturational changes with the help of geometric morphometrics method. The study identified the precise shape changes between gestational ages that is from 18 to 41 gestational weeks and included 221 foetal CT scans with no pathologies among which 75 were from girls, 110 from boys and 36 unknown sexes.The landmarks were assessed on the Basioccipital bone using ImageJ, AVIZO software and landmark points were further digitized with the TPSDIG2.

Using these landmarks, geometric morphometrics analysis was performed with Elliptic Fourier analysis and Principal components analysis (PCA), Procrustes ANOVA was performed for selection of harmonics and calculation of error, the morphological disparity among the stages were calculated with the individual. The study results showed that the youngest foetuses have the highest intra-stage shape variation.

Thus, the study results showed that the impact of measurement error was very low, indicating that the protocol was reliable and reproducible. The study concluded that the morphological shape changes throughout the foetal period can be useful for anthropological studies and by geometric morphometric method it is possible to quantify shape changes, assess interstage shape variability and precisely identify the shape changes between gestational ages. Daniel Franklin et al [20] studied effectiveness of geometric morphometrics using three dimensional multivariate descriptors of size and shape for subadult forensic age estimation . 79 known age and sex subadult mandibles ranging from 1-17 yrs of age were used. The sample comprised of 43 males and 36 females of South African Bantu and African American origin. The portable Microscribe G2X digitized scanner was used and 38 mandibular landmarks were recorded in three dimensions. The centroid size was calculated for individual configuration and further generalized Procrustes analysis (GPA) was done to standardize each coordinate to remove any size variation. The geometric morphometric analysis was performed with morphologica and NTSYS-pc 2.2f. Linear regression analyses and multiple regressions was performed for shape variations, cross-validate of the regression models was performed using jackknife procedure and TPSS mall 1.20 and SPSS 11.5.0 software's were used for other statistical results. A standard error ±1.3–2.2 years for size and ±1.7–3.0 years for shape, ±1.4– 1.8 and ±2.0–3.0 years for age was noted. The study concluded that mandibular morphology can be used to predict subadult age with a high degree of expected accuracy. Age prediction standards based on geometric morphometric data, are suitable for children (Below 10 years of age) or subadults (1– 17 years of age). It was also noted that prediction accuracy was better when the two populations and/or sexes were treated separately.

Risk of bias

The included studies have shown low risk of bias thus suggesting that the studies have good quality.

DISCUSSION

Skeletal age is considered as the gold standard for assessment of maturation and growth in infants, children and adolescents.[21,22] Geometric morphometrics is a latest approach to shape analysis which enables to visualize and quantify accurate morphological variations.[23-25]

In literature studies have been conducted on geometric morphometrics and its application in skeletal age estimation, sexual dimorphism, shape changes due to growth etc. Geometric morphometrics is a relatively newer technique and this advancement and increased approach the concept of age estimation has provided a new avenue for research and its various stake holders in different fields such as forensic odontology, anthropology, paleontology, law enforcement etc.

Chatzigianni et al [26] conducted a study on the shape of cervical vertebrae using geometric morphometrics and concluded that chronological age and centroid size were better predictors when used as independent variables along with vertebral shape and hand-wrist ossification. Y. Scholtz et al [27] conducted a study on sexual dimorphism of the human scapula and found that use of geometric morphometrics in estimation of sex using the shape of scapula can act as a good indicator and also suggested that better statistical results were obtained when the complete scapula was analyzed. San-Millán et al [28] studied the variability in shape of acetabulum fossa and acetabulum of humans using geometric morphometrics and correlated it with sex and changes related to age.

A preliminary study conducted by Gleim [29] using geometric morphometrics for juvenile dental age estimation demonstrated that tooth shape and size when measured with geometric morphometrics, statistically corelated with the chronological age of individual. Also, principal component analysis reveals that mandibular third molars have the highest correlation between age and shape.

There are a few studies available on application of geometric morphometric method for craniofacial age estimation. Due to lack of consensus in the uniform application of geometric morphometric method for craniofacial age estimation the need to perform this review was percieved. Hence, the review aimed to evaluate the accuracy and reliability of geometric morphometric technique for craniofacial skeletal age estimation.

The four studies included in this review assessed geometric morphometrics and provided a cumulative data with an all-inclusive picture, its applicability, accuracy and reliability in assessment of skeletal age. Two studies included samples from Toulouse [18] and Marseilles, France[19], other two studies were from Hadath, Lebanon[8] and included collection of skeletons from two different populations. One from the Raymond A. Dart- Bantu tribe of south Africa and second from Todd Osteological collections- Hamann tribe of African American's [20] respectively. As the sample sizes were taken from different genetic groups, which did not include all the 4 major groups, the influence of genetic origin cannot be assessed.

The estimations of age was done using CBCT scan and digital images of males and females using craniofacial skeletal units such as skull and mandible ranging in age from 18-41 Gestational Week to 18 years. The selected units were- CT scan images of Skull- face and base, CBCT scan images of basioccipit and digitized images of the mandible. This poses an advantage as there are no limitations for the age group to be studied on and even one bone from the craniofacial skeleton can be digitized and used.

There were methodological disparities among the included studies which were identified in this review. The software's used among the studies were different. Various analysis software were used like AVIZO 3D analysis software [18] ,Gamme Cepha and Microscribe G2X [20] portable digitizer for procuring the cartesian 3D coordinates.

Morphologika [18,20] and MorphJ [8] software's were used in three studies for obtaining the 3D landmark configurations or wire frames for centroid size calculation. Outline digitization and normalization was done using the TPSDIG2, Morpho and Geomorph software's for centroid size calculation in another study. These software's are technique sensitive, expensive and need prior training. Yet as the technological progress is rapid newer and cheaper options are being explored so that they would sufficiently aid in exploring geometric morphometrics.

AQUA tool [17] is a tool used for quality assessment of anatomical specimens. In our systematic review This tool was modified according to the included studies and applied on the CT scans, digitalized images of various craniofacial skeletal units for risk of bias assessment. The tool has 5 main domains with each domain having their separate signaling questions. One question each, from domain 4 and 5 were excluded for our review as they were not applicable for the study design. It was found that after the quality assessment of the four included studies all the

studies have shown low risk of bias thus suggesting that the studies have good quality.
The Generalized Procrustes analysiss[3] was done in all the studies which superimposes a population of shapes, and removes non-shape related differences like, size, orientation and position. Finally various statistical tests were performed in all the included studies for assessment of skeletal age. Two studies [18,20] performed linear regression analysis and other two studies performed multiple regression analysis[8] and Procrustes ANOVA, Principal component analysis.[19]
Intra and inter-observer agreement and error, were done for the selection of landmarks in two studies [18,20], one study [8] performed Inter and intra- observer agreement for selection of the radiographs and in another study the error was calculated for the validation of the samples[19]Standard error at 95% confidence interval for estimation of age was reported in three studies [8,18,20] and one study [19] did measurement error for repeatability and reproducibility.
It was necessary to read the papers several times to understand the methodology and how the accuracy was reported. In all the four studies included in this systematic review, geometric morphometric method could accurately estimate age of craniofacial units and reliability was usually associated with highest accuracy in all the studies.
To our knowledge, this is the first systematic review conducted on predicting the accuracy and reliability of using geometric morphometric technique in craniofacial skeletal age estimation. Limitations noted in the included studies were high heterogenicity in craniofacial units used for assesment, age groups analyzed, sample size, approaches in the steps performed in geometric morphometrics method, software's used and statistical tests performed.
We limited our search only to papers published in English language and unpublished studies, Conference abstracts, Editorials etc. were not included in this review so the interpretation and representation of the global literature on geometric morphometric was limited.
Due to varied interpretations and in consistencies of findings between studies included with regard to different age groups and sexes, it was not possible to perform a meta-analysis.
We recommend the future researches to conduct more studies on this technique and report the study results in an elaborate and specific way in terms of sample size and gender, mean, standard error, standard deviation. Also, studies are required to give a steady opinion about the software's used in the methodology, its ease in use and availability. Thus, in order to warrant the technique of geometric morphometrics and to allow its better application in craniofacial skeletal age estimation to obtain accurate and reliable outcomes.

CONCLUSION

This systematic review on geometric morphometrics in craniofacial skeletal age estimation has highlighted all the merits and demerits of this technique. It was noted that this technique is applicable and facilitates rapid, accurate and reliable identifications of a single bone or skeletal remain of the craniofacial skeletal unit, even with a digitalized image or a CBCT scan. The centroid size calculated using these images is said to be the highest predictor of age. However, the reliable data which was necessary for performing the meta-analysis was insufficient. Hence there is a need for conducting more studies that can estimate the craniofacial skeletal age using geometric morphometrics.

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