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Forensic Odontology: Application in the Mediterranean Area

ASHREF DARDOURI

A thesis submitted to the University of Huddersfield in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy.

HUDDERSFIELD UNIVERSITY
School of Applied Science

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Abstract

The use of dentition for identification and age estimation has been well established in the field of forensics, however, the accuracy and validity of various methods has not been systematically investigated in a variety of ethnic groups. Dental analysis has been widely employed for personal identification and age estimation due to teeth durability and being resilient to change. The aim of this thesis is to evaluate the applicability and reliability of three major dental methods for age estimation, focusing on Libyan population. Three principal dental approaches (dental wear and on shading, third molar maturity index (I3M), and linear regression formula) are explored. Furthermore, cervical vertebrae analysis, which concomitantly used with I3M for age estimation of young people, is also applied in this study.

In the study of age estimation, dental wear and shading are two separate indicators that have to be combined together for best results. Herein, the score and shading data of 412 participants of known age and sex from North Africa (majority from Libya) and England were studied. The participants were classified into 14 age groups of 5-year intervals. A new table has been made for age estimation using shading wear. The results show a good agreement with real age of most participants with minimal errors associated with data analysis. The results also indicate the superiority of tooth wear level investigation over shading method in actual age estimation; 71% of the estimated ages are in agreement with the real age of the participants.

New samples were gathered for the purpose of validation of age estimates; a sample of 918 healthy living Libyan subjects (521 females and 397 males), aged between 14 and 23, was used to analyse the third molar development by assessment of the I3M. The obtained results highlighted the significance of the I3M-based approach to adult age estimation, as 86.4% of the females and 89% of the males were correctly classified. It was also shown that, by using an I3M cut-off value of 0.09 instead of 0.08, an increase of around 3% was achieved in the numbers of individuals correctly identified using the method of Cameriere et al. (2006), when estimating the age of children by measurements of open apices in their teeth. The authors provided a first formula for the Italian population and in 2007, a formula for the European population. In this study, a new formula has been produced for the Libyan populations.

According to the results, Libyan formula is the most accurate method compared with two methods tested in the present study, i.e. Italian and European formulae in Libyan population.

The performance of the age estimation formula developed in thesis for the Libyan population has been compared against two other formulae previously presented in literature for the Italian and the European populations. No statistically significant difference was found between the European and Libyan formula proposed in this thesis, however, a difference was found between when compared with the Italian formula. Nevertheless, the linear regression formula developed in this thesis performed exceptionally well in estimating the age of Libyan population.

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Nomenclature

ANOVA	Analysis of variance
BA	Biological age
C4	Fourth cervical vertebra
CA	Chronological age
CT	Computerised tomography
CVM	Cervical vertebrae maturation
CV	Coefficient of variation
EITALY	Italian estimated age
ELIBYA	Libyan estimated age
EUR	European estimated age
FDI	Fédération Dentaire Internationale
ICA	Iliac crest
IM ₃	Third molar index
IW	Iliac wing
JPEG	Joint Photographic Experts Group
KDM	Klemera and Doubal's method
KK-MS	Kreitner and Kellingaus main stages and substages
LR	Likelihood ratio
MACR	Minimum age for criminal responsibility
MAX	Maximum
MED	Median

MIN	Minimum
MLR	Multiple linear regression
MRI	Magnetic resonance imagin
N	Number of individuals
OPG	Orthopantologram
OPT	Digital panoramic radiographs
PC	Personal computer
PCA	Principal component analysis
PTR	Pulp-to-tooth area ratio
RAGE	Real age
SD	Standard deviation
SPSS	Statistical Package for the Social Sciences
TW2	Tanner-Whitehouse
TEM	Technical error of measurement
R	Coefficient of reliability
rTEM	Relative technical error of measurement
WMA	World Medical Association

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1 Introduction

1.1 General View

Age estimation is the process of estimating the age of a person through the use of biometric, social and psychological features (Chao et al., 2013), which plays an integral role within contemporary society. Currently, age estimation is utilised in many sectors, ranging from medical applications to forensic and legal matters. This thesis, therefore, aims to explore age estimation with particular attention to its actual definition, importance, and analysis of the age estimation methods by skeleton and teeth, with a focus on the Cameriere' methods (Cameriere et al., 2004, 2006, 2007a, 2007b, 2008a, 2008b, 2012, 2014, 2015).

Age estimation is imperative to the identification of missing subjects. With this process, investigators can narrow down their options of identifying who the missing person could be. Age estimation can also help to reveal the age of those people who try to conceal their actual age. For example, refugees may try to hide their age details and since they may not be having any documents, knowing their actual age will require an effective age estimation procedure (Chao et al., 2013). Age estimation is also important in determining the actual age of those who abuse the benefits system by falsifying their age or year of birth. For example, people might fake their age to qualify for a job that they would otherwise not be able to qualify for. In this case, age estimation can be used to help the employer identify those people who have increased or lowered their ages to benefit from a particular job opportunity.

Furthermore, age estimation could serve vital in validating an infant's biological age. For example, there can be claims that a few days old child is a new-born, when in fact it is not true. The truth of the claims can only be justified by performing an accurate age estimation procedure. Minors can also receive an age estimation procedure in order to find their true age and to be able to treat them accordingly. Without knowing their age, one cannot provide them with the basics such as shelter or asylum.

For many years, there has been open discussions in the judicial systems of Europe and elsewhere in the world regarding penal responsibility and culpability. Most countries have a system that assumes chronological criterion to define a minimum age for criminal responsibility (MACR) (Cameriere et al., 2015a); these are summarised in Table 1. Moreover, in cases where an individual's identification documents are not available or are of an unreliable origin, it has become almost a necessity to rely on accurate age estimation techniques (De Luca

et al., 2014). Depending on the requirements of the assessment body, all possible age estimation techniques should be combined, in an effort to produce a more reliable set of results with a specific range of confidence.

Table 1. Minimum age for criminal responsibility in different countries (Cameriere, et al. 2015a McGuinness, 2016)

8 years	10 years	11 years	12 years	13 years	14 years
Scotland	Australia	Barbados	Bolivia	Algeria	Bosnia Herzegovina and
	Cameroon	Turkey	Brazil	Benin	Bulgaria
	Cook Islands		Canada	Burkina Faso	Central African Republic
	Côte d'Ivoire		Colombia	Burundi	Croatia
	England		Costa Rica	Comoros	Democratic People's Republic of Korea
	Fiji		Dominica	Djibouti	Germany
	Guyana		Dominican Republic	France	Hungary
	Kiribati		East Timor	Gabon	Italy
	Malaysia		Ecuador	Guinea	Japan
	Nepal		El Salvador	Haiti	Libyan Arab Jamahiriya
	Niue		Eritrea	Madagascar	Liechtenstein
	Palau		Ghana	Mali	Macedonia

	Sierra Leone		Greece	Monaco	Marshall Islands
8 years	10 years	11 years	12 years	13 years	14 years
	Suriname		Honduras	Nicaragua	Mauritania
	Tuvalu		Ireland	Niger	New Zealand
	Vanuatu		Israel	Togo	Panama
	Wales		Jamaica	Tunisia	Paraguay
			Netherlands		Republic of Korea
			Peru		Romania
			San Marino		Rwanda
			Uganda		Slovenia
			Venezuela		Somalia
					Spain

Age estimation can also serve useful in the corridors of justice when requested by government entities. Forensics enters into play when the age of a person is unknown, and there is a situation that either involves criminal law, family law, immigration law, and social law (Schmeling et al., 2016). For the avoidance of wrong application of the law, courts dictate that forensic age examination to be carried out. Forensic age estimation involves the processes of physical evaluation, panoramic films of the jaws, a thin-slice of the medial clavicle, and X-ray of the hands. The processes can either be executed individually or combined together (Schmeling et al., 2016). Among the forensic parameters, the minimum-age concept is important to prevent flawed grouping of minors as of legal majority age.

In criminal justice, knowing the age of an individual is important as it speeds up the course of justice by sending the offender to the right place for trial. For example, if a person is an adult, they are subjected to adult discipline facilities, however, if it is confirmed through age estimation that the age of the offender is that of a minor, they are taken to minor correction facilities. Currently, under the Criminal Law in England and Wales, children of 10-13 years of age are treated in the same manner as those aged 14 and over. In Scotland, the age of criminal responsibility is pegged at 8 years old, making it the lowest age of criminal responsibility in the whole of Europe (McGuinness, 2016). Therefore, placing a minor in an adult correction facility can be a major problem (ethically incorrect) and the minor can suffer from distressful environment. Age estimation helps limit juvenile exposure to adult correctional facilities. This helps protect the youths from sexual abuse that can occur if they were to be put in adult correction facilities (Geng et al., 2007).

For years, humans have been in pursuit of prolonging their lives and in executing the goal successfully, researchers have dedicated their time to understand the mechanism of aging that is attributed to the loss of functions and increased susceptibility to diseases. Chronological age (CA) estimation¹ is the commonly used method when it comes to age estimation. However, the chronological age estimation is not full proof as there exists other equally competitive age estimation methods (Jia et al., 2017). What stands out as the major differentiating factor across all the methods is the role of CA and the selection standards of aging biomarkers.

Age estimation does not rely on a single method, rather, the process relies on several biomarkers that can apply mathematical modelling to come up with the biological age (BA) (Nakamura et al., 1989). The most used BA estimation methods include the multiple linear regression (MLR), the Klemera and Doubal's method (KDM), the principal component analysis (PCA) and the Hochschild's method. These BA estimation methods compare the biomarkers variables in order to deduce the estimated BA (Nakamura et al., 1989)

The multiple linear regression (MLR) is a basic and introductory BA estimation method that has been in use for more than 50 years (Jia et al., 2017). The MLR method determines biomarkers depending on their complementation with the CA (Hollingsworth et al., 1965). The

¹ Estimation is the process of determining a numerical value for one or more parameters of a population from a set of data samples reporting the accuracy associated with the value.

MLR method is simple to understand and implement, which makes it the method of choice for determining BA with CA as one of its linear constructs. However, the MLR method does not depict whether the estimated CA is due to a selection criterion or an aging biomarker. The applications of MLR are constrained to specific conditions that may include limited statistical capacity, and software/programming skills among other situations. Eventually, MLR will be replaced if the circumstances change by PCA and KDM (Jia et al., 2017).

The principal component analysis (PCA) is another method used for BA estimation used mainly by the Asian countries. Correlation analysis, redundancy analysis, and equation construction make up the primary approaches in the process of PCA estimation. The PCA method is used to identify patterns in biomarkers and express the same biomarkers in such a way that it points out the differences and similarities, which is useful in BA estimation (Krøll and Saxtrup, 2000).

In 2006, Klemmera and Doubal came up with the Klemmera and Doubal method (KDM) for age estimation, which is a graphical method that can be used to estimate BA even in young adults (Nakamura et al., 1989). The KDM BA method works under the assumption of comparability among CA, BA and aging biomarkers. The aging biomarkers include menopause in women, changes in cells, hormones, genes and behaviour. Grey hair and wrinkles can be considered as indicators of the CA and not as biomarkers for the functionality age (Cho et al., 2010). Nevertheless, the KDM is shown to be a more reliable predictor of mortality than any other methods (Jia et al., 2017).

The Hochschild's BA estimation method identified some shortcoming in MLR BA estimation method (Cho et al., 2010). This method seeks to streamline the defects exhibited in MLR. Hochschild proposed a method of selecting aging biomarkers with respect to their impact on life expectancy (Jia et al., 2017). The Hochschild's method puts more emphasis on selecting biomarkers based on their effect on the life expectancy, however, this method is not so popular as its substandard and complex procedure (Bae et al., 2013).

The structural equation modelling (SEM) is mainly focused on the environmental and mental factors. It is mainly centred on aging studies for mental health in elderly adults. However, SEM has never been applied in BA estimation as it does not show the comparable biomarkers, hindering the main concept of BA. Although not specialised in a particular age estimation method, SEM is often used in addition to help studying mental health.

Further studies are needed to refine the trial applications of BA estimation (Jia et al., 2017). Social and psychological evaluations require a clinician or social work practitioner who has had the correct and proper training in conducting such evaluations. Assessing the mental, rather than the subject’s physical maturation, is the goal of this procedure. The practitioner will conduct interviews, relative to events recall, around the life history of the individual and make up an opinion based on 1) individual’s response nature while their events are being discussed and 2) their outlook towards key events in their past. It is possible that upon the completion of the procedures mentioned, the need to move on to the physical age estimation may be deemed unnecessary (Black et al., 2011). However, SEM-based age estimation methods are less frequently reported in literature due to the complexity that they add to BA estimation.

Comparisons across the methods mentioned thus far have been cross-sectional² and hence cannot be conclusive; therefore, longitudinal studies³ are required to support the findings. In addition to the techniques mentioned thus far, several methods such as MLR, PCA, KDM and the Hochschild’s methods have also been used for BA estimations (Nakamura et al., 1989). The pros and cons of these methods are summarised in Table 2.

Table 2. Comparison among MLR, PCA, KDM and Hochschild’s methods (Jia et al., 2017)

Method	Concept	Advantages	Disadvantages	References
MLR	Identifies a relationship between aging biomarkers and BA	<ul style="list-style-type: none"> • Introductory method • Easy to conduct 	<ul style="list-style-type: none"> • Contradicts CA standards • Distorts BA and ignores aging rate discontinuity 	Hollingsworth JW et al.,(1965)
PCA	A correlation between some unrelated variables is used to explain biomarkers	<ul style="list-style-type: none"> • Unrelated variables of biomarkers • Puts off MLRs’ influence on regression edge 	Does not avoid shortcomings of MLR	Nakamura Eet al.,(2014)

² A cross-sectional study is a type of observational study that involves the analysis of data collected from a population, or a representative subset, at one specific point in time

³ Longitudinal studies employ continuous or repeated measures to follow particular individuals over prolonged periods. They are generally observational in nature, with quantitative and/or qualitative data being collected on any combination of exposures and outcomes, without any external influenced being applied

KDM	Reduces the distance between regression lines and biomarkers	<ul style="list-style-type: none"> • A more precise method compared to others • Outperforms CA • Provides solution to CA problems 	Uses complex calculations	Levine ME (2014)
Hochschild's	Aging biomarkers are chosen based on their effect on life expectancy	<ul style="list-style-type: none"> • Solve CA inconsistencies • Prone to MLR statistical problems 	<ul style="list-style-type: none"> • Complicated and substandard • Does not relate to definition of BA • Fails scalability 	Hochschild Ret al (1994)

Methods of age estimation vary, with the most common approach being the skeleton and teeth evaluation. The skeleton contains tissues that because of their differential development can help in the estimation of the age of an individual. Moreover, teeth consist of a suitable tissue which can survive harsh environmental conditions, even at the time of death, making them an ideal tool for age examination purposes. Additionally, it is a tissue that grows continuously from childhood to adulthood, thus, making it more suitable for examination and classification of age ranges (Schmeling et al., 2016).

Identification of human remains involves assessment of their actual age at the time of death (Schmeling et al., 2016). The skeleton of an individual plays a major role in identifying age-related changes. The process of estimating the age of an individual at the time of death through the use of skeleton and teeth only provides the person's age at the time of death, and not the actual calendar dates of birth and death for that person. Some of the methods that are used to obtain the age of an individual using skeleton include those relying on macroscopic morphological features. Assessing age is reliable mostly when dealing with sub-adults, where features associated with bone growth can be used to estimate age (Iskan and Steyn, 2013). In sub-adults, bones develop gradually and this method can be useful mostly in this stage.

Also, X-ray can be used for assessing epiphyseal fusion, dental development, and trabecular patterns, whereby quantifying the degree of trabecular bone loss, one's age can be estimated (Schmeling et al., 2016).

This method can also be useful when dealing with soft tissue covered by bone. Additionally, microscopy is another method of age estimation at death, whereby counting osteons, non-Haversian canals and osteon fragments, the age of the subject at death can be estimated (see

Figure 1). Using this technique, it is imperative to have comparative skeletal collections with access to larger samples, which can result in a better coverage of each age estimation method (Schmeling et al., 2016).

Table 3. Effective radiation doses of X-rays used for assessment of the age (Schmeling et al., 2016).

X-ray Examination	Effective Dose (mSv)
Hand X-ray	0.0001
Orthopantomogram	0.026
Computed tomography of medial clavicular epiphysis	0.4

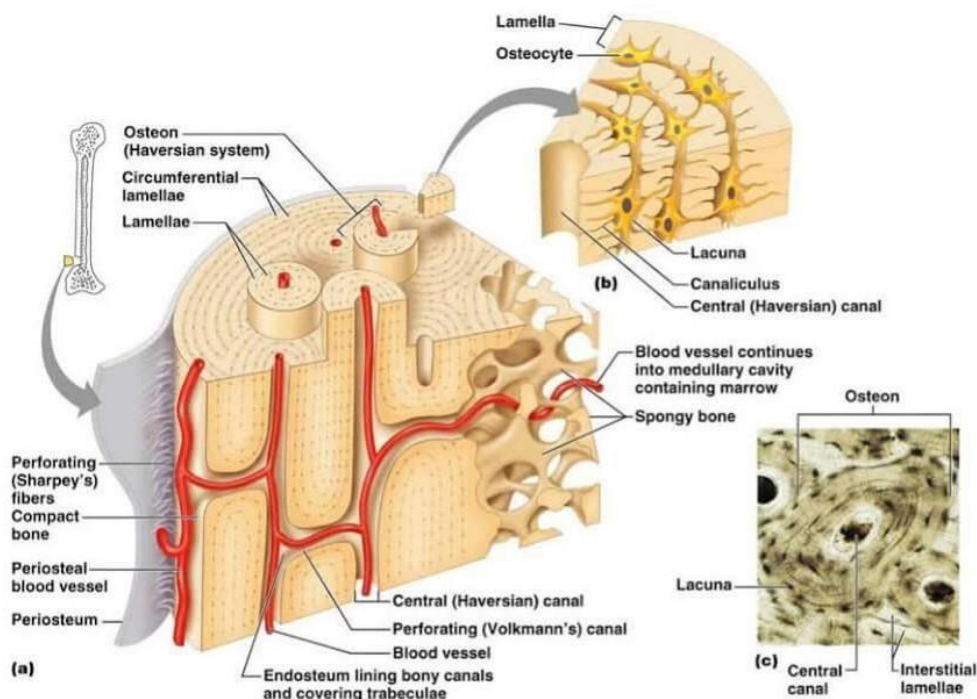


Figure 1. Microscopic structure of cortical bone, (a) 3D sketch of cortical bone, (b) cut of a Haversian system, (c) photomicrograph of a Haversian system (Cramer and Darby, 2017)

Age estimation performed by evaluating the teeth development and structure is a crucial part in forensic dentistry and anthropology (Schmeling et al., 2016). The development stages of teeth can be used for age estimation and estimating the age of children and adolescents. When it comes to adults, the method is based on the transparency of the root of the tooth, secondary dentin and root resorption. Teeth happen to be the most durable part of a skeleton and this makes them suitable for age estimation in individuals (Schmeling et al., 2016).

The development stage of dentition is well established and when there is a disturbance during the period of teeth development, the change that occurs remains permanent throughout the lifetime of an individual (Schmeling et al., 2016). Using this method of age estimation, there are various parameters that are examined, including the degree of attrition, root transparency, secondary dentin deposition, cementum apposition and root resorption. Compared to other body tissues, age estimation methods centred around the teeth always result in a closer estimate to the subject's actual age. This makes the teeth the most efficient tool to use in estimating the age of an individual. Through the teeth, the age can be examined in stages of childhood and adolescence, by looking at the eruption of deciduous and permanent teeth. This can be determined for subjects of up to 14 years of age; thereafter, the third molar is used to estimate the age of youths up to the age of 20 years. After this particular period, age can then be estimated by visual examination, changes in the teeth and by process of biochemical methods (Schmeling et al., 2016).

Cameriere' method uses a particular formula to come up with the estimated age figures. This method is more reliable in age estimation as it measures the teeth with open apex, and through application of a formula, the chronological age of a child is established (Gulsahi et al., 2015). In children and young adults, the method used to estimate their age is through the developing teeth. Through different research across the world, it has been proven that this method is reliable to provide accurate results of estimated age in children. In this method, the relationship between the age of the child and open-apex teeth is crucial. Then, these figures are considered and a formula is applied to estimate the age of the individual. There is a good relationship between chronologic and dental age (Gulsahi et al., 2015).

The success of several disciplines relies on more accurate studies on biological age evaluation in both growing and adult subjects. In literature, research on several anatomic areas for biological age estimation have been presented (Singh et al., 2004). Along with many scientists that conduct extensive research on accurate biological age estimation, auxologists, paediatricians, forensic pathologists and dentists have also been researching on techniques that can produce the most accurate evaluation of biological age, both for clinical and forensic purposes. The diversity of opinions that have been put forward by the scientists from different disciplines have indeed created some problems in the applicability of the techniques for both known and unknown ages (Singh et al., 2004).

The static age of a subject is also of importance to auxologists or paediatricians, who are mostly interested to see whether or not the biological age of the subject corresponds to their actual age (Demirjian et al., 1973) and are mostly acknowledged for their works carried out on age estimation using wrists and teeth. These techniques are developed to estimate the biological age of growing subjects for clinical purposes, where qualitative studies are employed to allow for the identification of the subject's age through the stage conversions and look-up tables. Moreover, these techniques are also found useful in subjects of unknown age, for example, estimating the vital static age of a subject whose birth documents are not available. Most reviewed studies in the forensic field adopt these techniques in an effort to convert them into a forensic tool, though the authors of these researches might have not directly implied the forensic application of their studies (Demirjian et al., 1973).

1.1.1 Hand (wrist)

This method is commonly utilized using radiographs of the hand by checking the level of epiphyseal ossification as well as the size and form of bone elements. For purposes of determining the development stage, a given image is contrasted to the standard images of the relevant sex and age. This is also used to evaluate the level of maturity in cases of individual bones otherwise referred to as the single bone method. Cumulatively, the two methods tabulate the general stage of maturity.

Mohammed et al. (2014) have come up with a method based on Fishman's skeletal maturation assessment, which relies on skeletal maturity indicators reflected on hand (wrist) radiographs. The sequence of events offers a method for identifying maturation stage which covers the whole adolescent period. Skeletal maturation indicators enable easier observation of skeletal maturity. This approach uses eleven anatomical sites on adductor sesamoid, phalanx and radius (Figure 3).

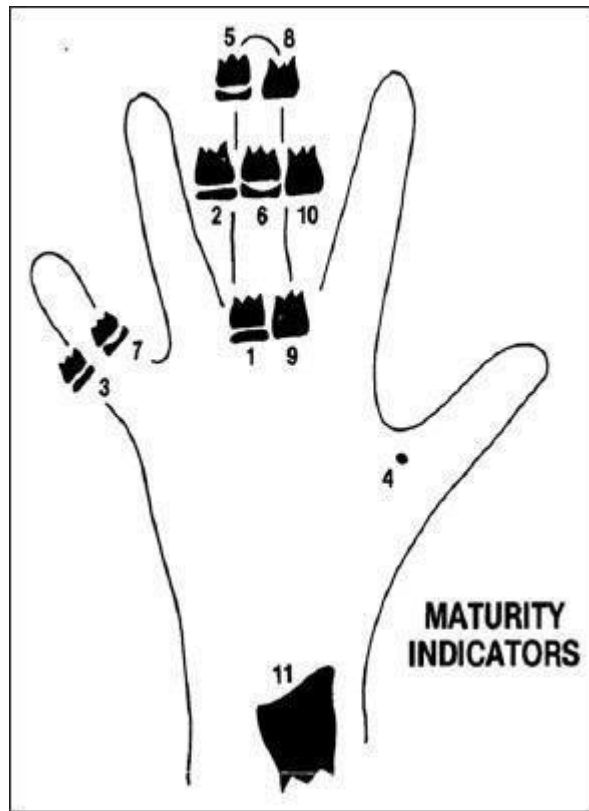


Figure 2. Fishman's eleven skeletal maturity indicators ⁴(Mohammed et al., 2014a)

Acheson (1966) was the first to publish work based on the qualitative method. This paper studied a sample of 500 children of pre-school age and defined a score method of analysis for hand, wrist and knee. With regards to hand and wrist, Archeson used the Greulich and Pyle maturity score (Greulich and Pyle, 1959) and evaluated a score for different stages of maturation. Each maturation stage was studied and a progressive score was used. Subsequent studies looked at the hip and pelvis using the same method. Acherson's work was the first study using a quantitative score for skeletal growth (Acheson., 1966).

⁴ Eleven skeletal maturity indicators Fishman's (1982); 1= third finger proximal phalanx shows equal width of the diaphysis and epiphysis; 2= The third finger middle phalanx shows equal width of the diaphysis and epiphysis; 3= The fifth finger middle phalanx shows equal width of the diaphysis and epiphysis; 4= Appearance of Thumb adductor sesamoid; 5= Distal phalanx epiphysis capping on the third finger; 6= Middle phalanx epiphysis capping on the third finger; 7= Middle phalanx epiphysis capping on the fifth finger; 8= Diaphysis and epiphysis fusion of third finger distal phalanx; 9= Diaphysis and epiphysis fusion of third finger proximal phalanx; 10= Diaphysis and epiphysis fusion of third finger middle phalanx; 11= Diaphysis and epiphysis fusion observed in the radius.

One of the most common methods used for processing skeletal maturation and development in children is the radiographic atlas of skeletal development of the hand and wrist, an approach that was proposed by Greulich and Pyle (1959). This is considered to be an appropriate method since its application is simple compared to other individual bone approaches and it is associated with a low systematic error. This method uses radiographs and by comparing them to standards, a close match is identified, which gives an age estimate that is the skeletal age (Dembetembe and Morris, 2012). With the help of standard light box, the development levels are assessed in a number of areas including ulnar and radial epiphyses sizes relative to their respective diaphysis, epiphyseal capping in phalanx and metacarpals, sesamoid bones size, and diaphysis and epiphysis fusion in all these bones.

The Tanner-Whitehouse (TW2) method, developed by Tanner et al. (1975), uses radiographs of wrist and hand in bone age assessment (Gilsanz and Ratib, 2005). The left hand and wrist is preferred because of several reasons including the fact that most of the people are right-handed and it has higher chances of being injured compared to left hand (Gilsanz and Ratib, 2005). Therefore, it is recommendable to perform physical measurement on left side instead of right side. There are 3 different TW2 approaches. The radius ulna short bones approach evaluates the 13 short or long bones (ulna, radius and short bones of the first, third and fifth fingers). The carpal approach evaluates the seven carpals, while the 20 bones approach evaluates the 13 short or long bones together with the seven carpals. Each bone maturity level is classified into a stage (ranges from A to H or I), then it is assigned a score and the summation is done to get the total score. The total score provides an estimate for the age. Area of the carpal bones, epiphyses of the ulna and radius, and the entire carpal area, together with the subject's sex, are the variables that can be employed by the regression formula to evaluate the chronological age of a sub-adult subject.

Many researchers have tried to correlate the formation of the bones and their development in humans. The first studies at the beginning of the 1900s were conducted by an anatomist at State College of Kentucky, who looked at the bones of the hand and wrist (Cameriere et al., 2008a). The first findings, still being valid, were about the difference in the development in males and females, and, that the maturation of the left and right sides were symmetric (Cameriere et al., 2008a). Carpals (see Figure 3) are often used as age indicators. In 2008, Cameriere *et al.* planned the use of the ratio between the total area of carpal and epiphyses of the ulna and radius (Bo), and carpals (Ca) as age indicators.

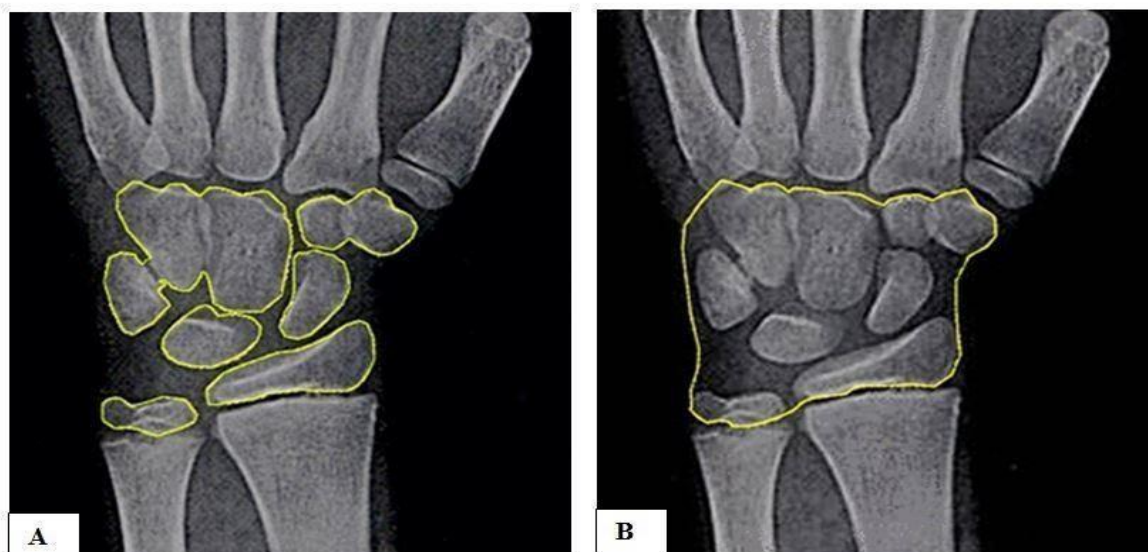


Figure 3. a) Correct selection of carpal bone, and b) carpal area selected using Adobe's Polygonal Lasso Tool Adobe®Photoshop® CS4 software (Cameriere et al., 2008a)

1.1.2 Knee Approach

This approach focuses on maturation of the knee's growth plate. Dedouit et al., (2012), evaluated the validity and reliability of the epiphyseal plate growth in the knee for age estimation, utilising an original magnetic resonance imaging (MRI) and precisely within 10-30 years age group (Dedouit et al., 2012). New valuable information is made available by the epiphyses of radiological analysis of the knee joint, with the ability of being utilised, in combination with these well-established methods, for maximisation of accuracy in making assessment of 18 year olds. Very encouraging results were derived through the study of Cameriere carried out in 2012, discussing the application of this method in making epiphyseal fusion analysis at the knee joint (Cameriere et al., 2012a).

There are three classifications involved in the assessment of ossification degree of distal femur and proximal tibia, together with proximal fibula which are as follows: in stage 1, there is no fusion of epiphysis (Figure 4 – left); in stage 2, there is complete ossification of epiphysis and visibility of epiphyseal scar (Figure 4 – middle); and in stage 3, there is complete ossification of epiphysis and no visibility of epiphyseal scar (Figure 4 – right).



Figure 4. Right – epiphysis is fully ossified and epiphyseal scar is not visible, middle – epiphysis is fully ossified and epiphyseal scar is visible, and left – epiphysis is not fused (Cameriere et al., 2012a)

1.1.3 Rib and Clavicle

This recent approach estimates the age of living subjects by analysing the level of ossification of the first rib. According to Garamendi et al, (2011), this approach can be utilised in combination with the clavicle ossification, given that it can be analysed using the same X-rays. Going by the specification provided by Michelson in 1934, the ossification of the costal cartilage of the first rib can be analysed using digital thorax X-rays and contrasted against known sex and age of the subject. In reference to determining the age of individuals that are more than 18 years old, the level of ossification of the cartilage at the clavicle’s external end is utilised. This is because by that time, all the developmental systems that are under evaluation have completely developed.

Furthermore, recently in 2017, Monum et al. pioneered a new method for costal cartilage ossification on chest plate radiographs, which is deemed as the one of the most useful methods in adult age estimation. The study was performed 136 remains, yielding a regression formula for the age estimation in Thai male population (Monum et al., 2017). This technique is based on the Garvin’s method, where eight features on chest plate imaging are scored. Ultimately, composite scores are calculated by summation of all the scores, which were then further analysed to generate a regression for age such that $\text{Age} = 16.664 \times e^{0.161 (\text{composite score})}$ with a 95% confidence interval. From the results, it was found out that the predicted age intervals in all composite scores overlapped each other, except for scores of 0 and 7; thus, it is

conclusive that, if all features are absent/present, the person is likely to be less/more than 29 years of age.

Figure 5 presents an exemplary chest plate X-ray image and score, where A is costal cartilage ossification of any of the sternal rib ends; B is costal cartilage ossification peri-sternally; C is costal cartilage ossification centrichondrally (mid-costal cartilage); D is irregularity or cartilage ossification to the costal manubrium notch; E is irregularity evidence of flaring, cupping, bony extensions, or bone degradation of the sternal rib ends; F is complete fusion of the sternal body; G is any bony fusion of the xiphoid to the sternal body; H is any bony fusion of the manubrium to the sternal body (Monum et al., 2017).

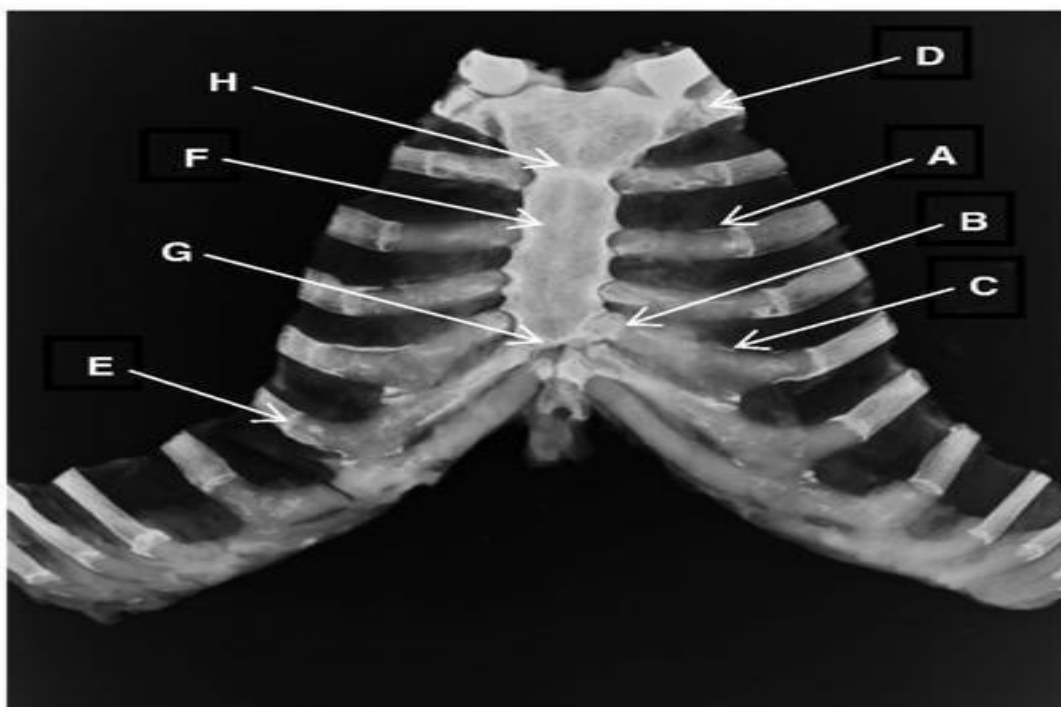


Figure 5. Exemplary chest plate X-ray image and score (Monum et al., 2017)

1.1.4 Cervical Vertebrae (or Vertebrae of Neck)

The growth and development of the cervical vertebrae as well as that of third molars was examined by (Thevissen et al., 2011) using cephalometric radiographs. This is a different age approximation technique that is fairly new in reference to living subjects. In view of this, there was a comparison of varied systems of grading, but only two of the most accurate were utilised in conjunction with the developmental stages of the third molar (Gleiser and Hunt, 1955).

The study of Predko-Engel et al. (2015) aimed at evaluating the reliability of the cervical vertebrae maturation (CVM) method in evaluating maturation of cervical vertebrae by a non-

calibrated panel of orthodontists having long clinical practice. The researchers used a sample of 50 randomly selected cephalograms and scanned them at 300 dpi resolution, cropped them to only visualize the cervical vertebrae. Finally, the scans were loaded into power points for rating by the 10 practicing orthodontists with a mean practice time of 12.3 years (Predko-Engel et al., 2015). The study also used a six-stage modification of the CVM method as described by (Nestman et al., 2011).

As presented in Figure 6, a new method was carried out by (Cameriere et al., 2015a), in order to assess the applicability of using the growth of the body of fourth cervical vertebra (C4) vertebra for the assessment of age in young and children adolescents. The proposed method relies on the fact that the proportions between the radiologic anterior and projections of the posterior sides of the C4 vertebral body, which form a trapezoidal shape, differ with respect to age such that in younger subjects, the posterior side is higher, whilst in older individuals, the projections of the sides of the vertebral body form a rectangular shape with the two equal sides or with the anterior side slightly above it. All in all, although the Bayesian calibration method might not be able to outperform the classical regression models in estimation accuracy, it provides a more robust estimation that minimises the typical bias in regression model approaches and enables for incorporation of multiple predictors (Cameriere et al., 2015a).

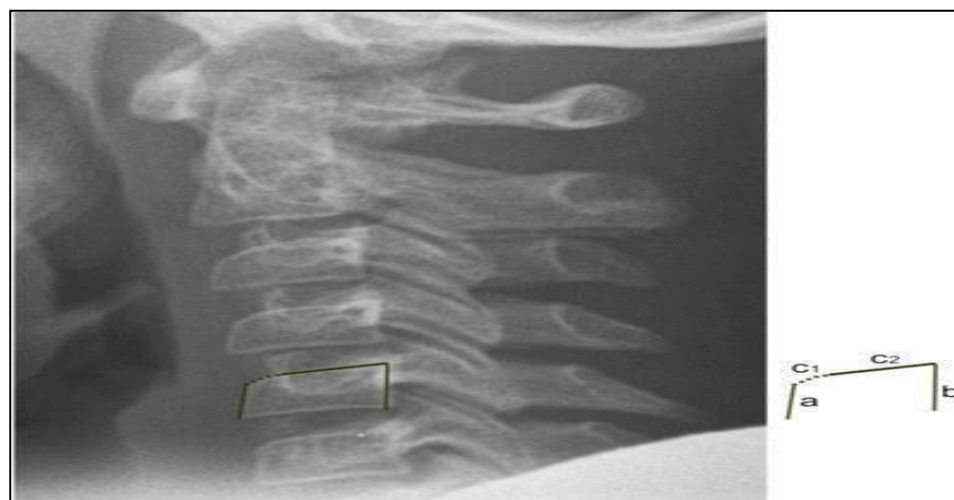


Figure 6. Example of the anterior (a) and posterior (b) sides of the fourth cervical vertebral body. Anterior side of the body is measured to the point where anterior side (a) curves, (C1) toward the superior side (C2) of the vertebral body (Cameriere et al., 2015a)

1.1.5 Iliac Crest

Also known as the hip method, the iliac apophysis of the pelvis can also be used to estimate the skeletal age. The outlook of the apophysis on a pelvic X-ray appears laterally but as an

individual is becoming an adult it edges towards the spine. Risser's sign has five defined stages that measure the growth left in the spine. The five stages are essentially categorized from 14-16 years of age for the case of girls and 15-18 years in the case of boys. As was the case in the earlier approaches, this approach is relatively new in the approximation of age among living individuals (Bartolini et al., 2017).

Furthermore, Bartolini et al. (2017) compared three methods in pelvis X-rays as a way of estimating the forensic age. The researchers used a sample of 354 Italian participants (168 females, 186 males) aged between 10-25 years, post exclusion of 143 due to artefacts or defects that prevented adequate pelvis visualisation, and bone disorders. The following methods were applied in assessing age estimation.

A) Risser sign staging method (both French and USA approaches)

Risser classification is based on the theory that during the process of ossification, the apophysis of the iliac crest development along the ilium begins from the anteromedial margin. However, the fusion of this apophysis with the ilium begins from the posteromedial side. In both Risser Fr and Us staging systems, the attribution of the ossification stage (in six stages from 0 to 5) is based on the assessment of the ossification progression along the iliac crest.

In Risser US classification, the iliac crest gets divided into quarters, which defines the next four stages (1-4). Absence of ossification corresponds with stage 0 and stages 1-4 relate to the ossification progression by the appearance to completion. However, fusion of the apophysis to iliac crest to the ilium corresponds to stage 5, from the start of the process until its completion.

The Risser Fr version provides for the division of the iliac crest in three parts. Moreover, the apophysis of the iliac crest fusion with the ilium is divided in two stages, that is, stage 4 where there is incomplete fusion of the apophysis of the iliac crest with the ilium, and stage 5 where there is complete fusion of the apophysis of the iliac crest with the ilium.

B) Kreitner and Kellingaus main stages and substages (KK-MS) system

This method describes eight stages and substages (1, 2a, 2b, 2c, 3a, 3b, 3c, 4), assessed according to the ossification of the apophysis progression and the fusion of the pelvic bone with the iliac apophysis. The assumption under this classification is that both of the two processes may start at any point without precise progression along the iliac crest.

1.1.6 AM Method

This method provides measurement of areas on X-rays in accordance to the Cameriere’s approach for estimation of age in the living. In contrast to the other staging methods, this method is based on measurements and ratios of particular areas and not on subdivision in stages. The method requires measuring of the areas of the ossification centre(s) of the iliac wing (IW) and the areas of iliac crest (ICA). Linear regression is used in analysing the ICA/IW ratio and using a formula, age estimation can be performed. Table 4 provides a summary of different approaches to age estimation through skeletal maturation.

Table 4. Different approaches of age estimation (Skeletal Maturation)

Name of Method	Approach	Age Range (years)	Notes	Reference
Fishman method	The left hand and wrist	9-20	Relies on skeletal maturity indicator reflected on the hand – wrist radiographs (Mohammed, 2014). The sequence of events offers a method for Identifying maturation stage which covers the whole adolescent period.	Mohammed, et al. (2014)
Greulich and Pyle approach	The left hand and wrist	13-22 years	Uses radiographs of wrist and hand in bone age assessment.	Dembetembe et al., (2012)
Tanner Whitehouse 2 methods	The left hand and wrist	1-16 years	Uses radiographs of wrist and hand in bone age assessment.	Gilsanz et al., (2005)
Cameriere et al.	Fourth cervical vertebra	5-15 years	Used the fact that the parts between anterior sides and the radiologic projections of the posterior of the C4 vertebral body.	Cameriere et al., (2015)

Cameriere et al.	Carpal area	-	Studied the use of the ratio between the total area of carpal bones and epiphyses of the ulna and radius (Bo) and carpals (Ca) as age indicators of the left hand	Cameriere et al., (2008)
Tawachai Monuma et al.	Chest plate radiographs	15-81 years	Radiographs of all chest plates were performed in antero-posterior orientation	Monum et al., (2017)
Cameriere et al.	Knee	14-24 years	Radiological analysis of the epiphyses of the knee	Cameriere et al., (2012)
Bartolini et al.	Iliac crest	10-25 years	Analysis of X-ray images of the iliac apophysis.	Bartolini et al., (2017)

1.2 Skeletal Maturation and Age Estimation

The growing process in humans is defined as a phase during which progressive changes occur in both morphology and size. It is possible to correlate skeletal changes with age, however, since many factors affect this correlation, it may not be as equal or identical in all subjects. Skeletal development starts from the mesenchyme, which undergoes a series of changes during its maturation process, including simple beginnings from the embryonic connective tissue, up to complete endings with typical characteristics of an adult individual (Cameriere, 2008a).

In forensic age estimation of unidentified skeletons and corpses, the mortal remains' quality and quantity are crucial, while in living subjects, certain factors such as the precise legally appropriate age threshold is taken into consideration (Geng et al., 2007).

There are three fundamental moments in studying skeletal age; these are, 1) the creation of ossification centres, 2) changes in morphology and the formation of ossification centres, and 3) the fusion of those centres. These centres are visible from birth and throughout the first decade of life, which can be used as indicators of skeletal age. Considering new-borns, they generally have six characteristics associated with their ossification centres; 1) the proximal epiphysis (head) of the humerus, 2) the distal epiphysis (chondyle) of the femur, the proximal epiphysis of the tibia, 3) the talus (astragalus), 4) heel calcaneus, and 5) cuboid bone. In literature, there are several tables which have been developed to show the times of appearance

of these centres. Early works on fusion in dry bone and on radiographs were carried out by Stevenson and Stewart (Pyle and Sontag, 1943). The condition and timing of the fusion of the main centres of ossification are summarised in Table 5.

Table 5. Average time of appearance (in months) \pm standard deviation (SD in months) of 61 ossification centres (Pyle and Sontag, 1943)

Order of Appearance	Male		Female	
	Average (months)	SD	Average (months)	SD
Distal femur	0	0.1	0	
Proximal tibia	0.1	0.3	0.1	
Cuboid	0.5	0.7	0.4	
Head of humerus	0.7	0.8	0.9	
Capitate	2.4	1.8	2.3	2.1
Hamate	3.4	2.2	2.5	2.3
Distal tibia	3.9	1.5	3.4	1.4
Head of femur	4.4	2.0	3.7	1.6
Lateral triquetral	4.4	4.3	3.8	4.4
Capitulum	6.3	4.3	4.1	3.6
Great tubercle of humerus	11.4	7.2	6.6	3.3
Distal fibula	12.5	4.1	9.3	2.6
Distal radius	13.0	4.7	10.4	3.1
Proximal phalanx – 3 rd / middle finger	16.2	5.3	10.6	2.8
Distal phalanx – big toe	16.8	5.6	10.8	4.4
Proximal phalanx – 2 nd / index finger	17.3	5.0	11.0	3.0
Proximal phalanx – 4 th finger	17.7	5.4	11.1	3.2
2 nd metacarpus	17.9	5.1	12.2	3.8
Distal phalanx – 1 st / index finger	18.4	6.2	12.8	3.7
Proximal phalanx – 3 rd toe	19.5	5.2	2.8	3.7

Proximal phalanx – 4 th toe	21.0	5.1	12.8	3.8
3 rd metacarpus	21.1	6.4	14.1	3.8
Triquetral medial	21.9	9.9	14.2	4.0
Proximal phalanx – 5 th / little finger	22.2	5.6	15.2	4.2
Proximal phalanx – 2 nd toe	22.2	5.8	15.8	4.8
4 th metacarpus	23.6	7.1	15.9	4.9
Medial phalanx – 3 rd / middle finger	24.9	7.6	16.0	4.1
Medial phalanx – 4 th finger	24.9	7.8	16.7	8.5
5 th metacarpus	26.0	9.0	17.2	4.7
Medial phalanx – 2 nd finger	26.9	7.5	17.3	5.2
Pyramidal	27.3	15.9	19.9	5.9
1 st metatarsus	27.7	4.7	20.1	3.3
Distal phalanx – 3 rd finger	27.8	6.4	20.2	3.9
Distal phalanx – 4 th finger	28.3	7.0	20.3	5.3
Median cuneiform	28.4	11.2	20.3	5.5
1 st metacarpus	29.8	7.3	21.3	7.6
Proximal phalanx- 1 st toe	29.9	5.8	21.3	4.8
Proximal phalanx- 5 th toe	32.0	5.9	21.6	5.1
Scaphoid (navicular)	33.4	13.4	23.6	13.7
Metatarsus	33.4	6.8	24.9	7.9
Proximal phalanx – 1 st finger	34.8	7.9	25.5	7.0
Distal phalanx – 2 nd finger	37.0	7.9	25.8	11.1
Distal phalanx – 5 th finger	37.4	7.4	25.8	6.1
Medial phalanx – 5 th finger	40.3	11.7	25.8	6.9
3 rd metatarsus	41.5	7.9	29.1	6.4

Greater trochanter of femur	42.6	7.6	29.8	6.4
Semilunar	46.0	19.3	30.7	7.9
Proximal fibula	47.0	11.8	32.6	9.3
4 th metatarsus	48.7	9.0	32.8	-
Distal phalanx – 5 th toe	51.2	10.1	34.0	-
Patella	51.9	11.6	34.6	-
Distal phalanx – 3 rd toe	53.5	11.2	34.8	-
5 th metatarsus	53.6	10.6	35.5	-
Distal phalanx – 2 nd toe	57.0	11.4	38.6	-
Navicular	60.1	14.1	41.3	-
Proximal radius	63.5	17.2	47.0	-
Trapezium	64.3	19.7	47.5	-
Trapezoid	64.4	15.2	47.8	-
Medial epicondyle of humerus	73.6	17.5	48.3	-
Distal ulna	82.4	10.6	63.2	-
Calcaneal epiphysis	89.6	14.0	63.7	-

Table 6. Time of initial fusion of epiphyses of long bones (Ubelaker, 2002)

Epiphysis	Age of Initial Fusion (years)	
	Male	Female
Medial extremity of clavicle	18-22	17-21
Acromion process of scapula	14-22	13-20
Humerus: - head	14-21	14-20
- Greater tubercle	2-4	2-4
- Trochlea	11-15	9-13
- Lateral epicondyle	11-17	10-14
- Medial epicondyle	15-18	13-15
Radius: - head	14-19	13-16
- distal border	16-20	16-19

Distal border of ulna	18-20	16-19
Iliac crest	17-20	17-19
Ischium-pubis	7-9	7-9
Ischial tuberosità	17-22	16-20
Femur: - head	15-18	13-17
- greater trochanter	16-18	13-17
- lesser trochanter	15-17	13-17
- distal border	14-19	14-17
Tibia: - proximal border	15-19	14-17
- distal border	14-18	14-16
Fibula: - proximal border	14-20	14-18
- distal border	14-18	13-16

Throughout their development, these ossification centres experience many variations, which often differ greatly from one another for a number of reasons including individual, sexual, ethnicity, nutritional, functional and pathological. Other concerns may arise as a result of the different study procedures in experimental examinations, sometimes based on osteology, ultrasound, radiological techniques, etc., and on technical difficulties in interpreting the morphological data (Cameriere, 2008a).

One of the most commonly used techniques in age estimation is monitoring continual changes in the shape and size of different bone structures such as those of hands and teeth, which mark the growth period of children and young adults. Considering the growth speed of the diaphysis of the long bones, it starts by developing fairly fast in the first year of life, then it slows down until the age of 6 years, which then slows down even further as the child reaches the age of 10 years. The progressive increase in growth time of the long bones' diaphysis with age has been a subject study by many scientists. During the second decade of life, the ossification centres begin to fuse, both in short bones (e.g. hands and feet) and long bones, excluding the head of the humerus.

These fusion processes, as shown in Figure 7 (Cao et al., 2004) and Table 6, follow a chronological order, though less reliable than the appearance of the ossification centres (Flores-Mir et al., 2006). The developmental stage of bones is essential in the estimation of skeletal

age. The maturation or fusion of precise bones helps in the approximation of the development stages. This is done through the different approaches.

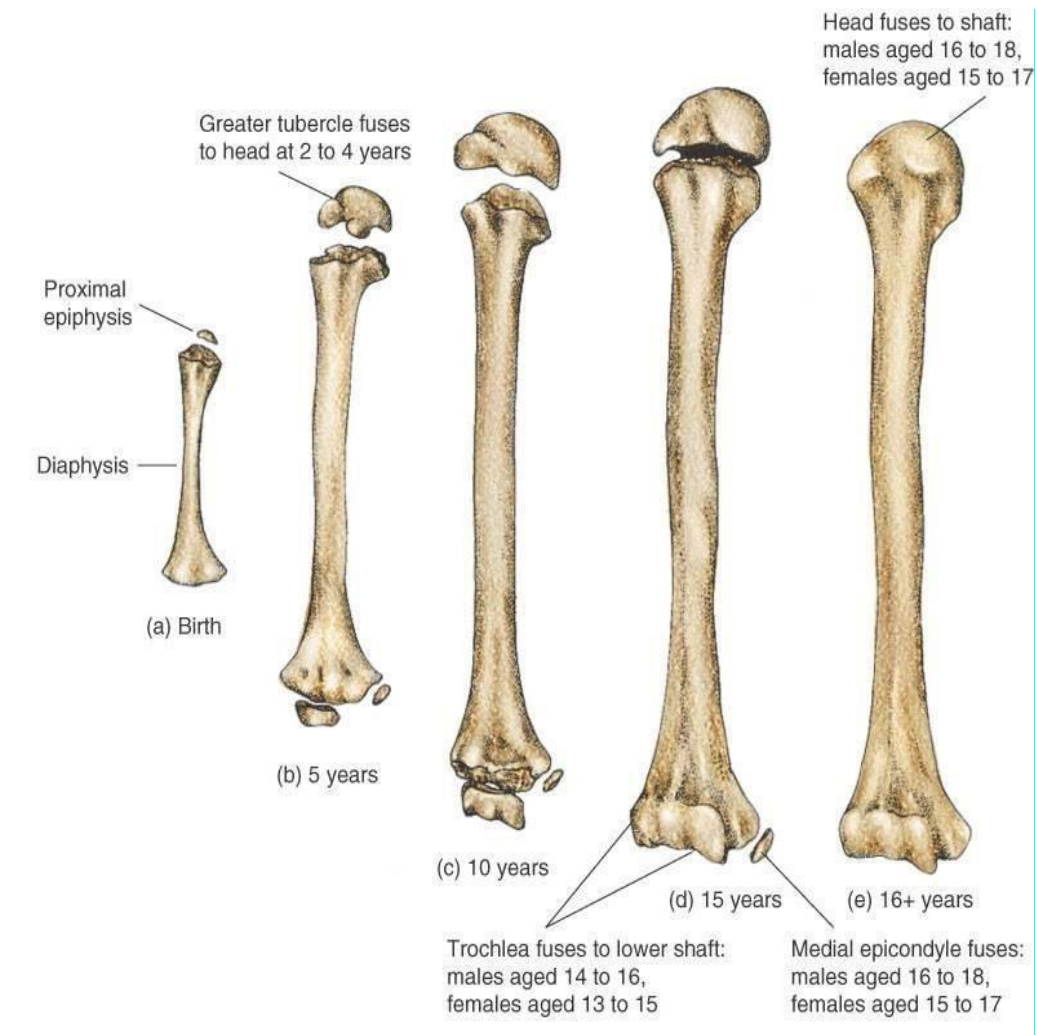


Figure 7. Development of humerus from the birth at the end of the growth, (Cao, 2004)

1.3 Tooth Development and Age Estimation

It is within the sixth week of embryonic life that the development of teeth commences through the buds. According to (Miles, 1963), there is a slight difference in tooth development between girls and boys. This is because tooth development is more advanced in girls as opposed to boys prior to puberty. Children begin to develop the deciduous teeth between 6 and 9 months. The eruption of teeth is progressive and begins with the anterior teeth through to the posterior ones. Consequently, the permanent dentition commences at the age of 6 years with the development of the first four molars. Further, the development of the permanent anterior teeth begins to erupt between the ages of 6 and 12 years. The eruption of the second molars occurs at around 12 years of age. The third molars, otherwise referred to as the “wisdom” teeth, are the last final

permanent teeth to emerge between the ages of eighteen and early twenties. This marks the end of tooth development with a final number of 32 teeth.

A tooth that is developmentally complete consists of a root and a crown. The development from crown to root takes place at the cement to enamel junction, also referred to as the cervical line (Miles, 1963). As illustrated in Figure 8, there are four tissues in human teeth including soft tissues of the pulp, as well as three calcified tissues namely enamel, dentin and cementum. In reference to the crown, there is the inner layer known as dentin and the outer layer known as enamel. Enamel acts as a resistant structure that allows chewing.

When compared to the enamel, the dentin is slightly harder than bone. The destruction of the enamel leads to rapid dental decay. The root is covered by cementum that provides an attachment location for the connective tissue fibres that are responsible for securing the root to the nearby alveolus, also known as a bony socket. The pulp cavity of a tooth consists of a connective tissue known as the dental pulp. There are two categories of dental pulp the first one is found in the central pulp chamber of the crown and is known as the coronal pulp, while the other one is found at the pulp canals of the root and is known as the radicular pulp (Miles, 1963).

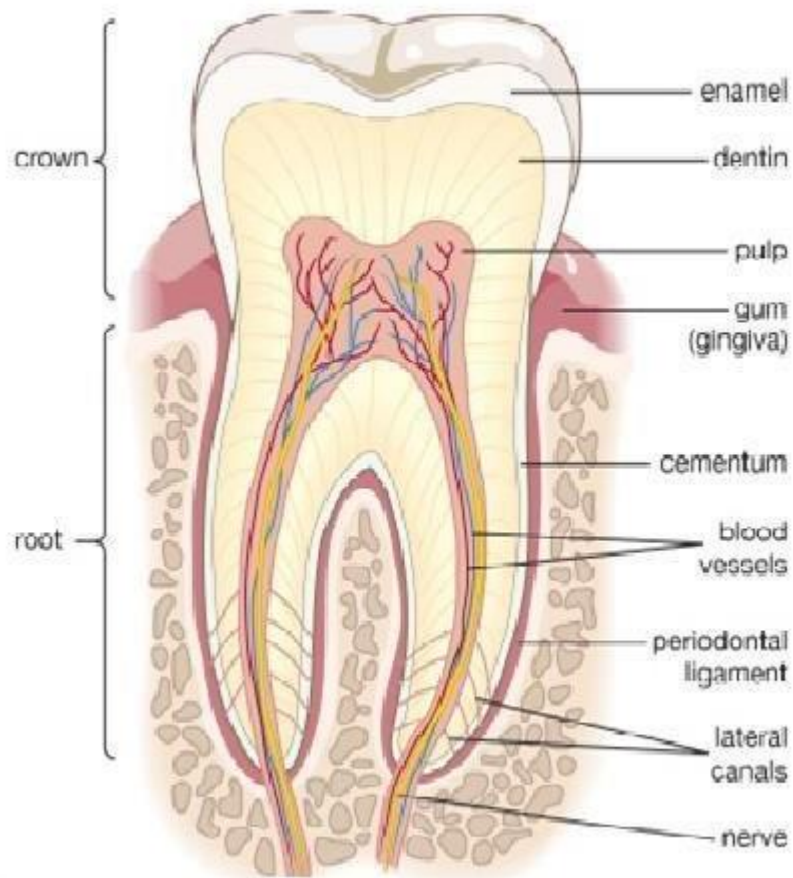


Figure 8. Tooth anatomy (Encyclopaedia Britannica, Inc2013.)

Age estimation is a procedure that has been adopted by several scientists like anthropologists, archaeologists and forensics in their research work (Singh et al., 2004). It provides help in identifying victims in crimes, where the rating of this method has been declared as being authentic and reliable in court (Singh et al., 2004). Some changes can be associated with the advancing age of people such as resorption and erosion, as well as periodontal disease, root translucency, secondary dentine deposition and cementum apposition around the root, and, nonetheless, changes in colour and rise in root irregularity (Singh et al., 2004).

There abound many techniques when it comes to age estimation (Ajmal et al., 200). A method for juveniles is teeth growth and development, as well as deciduous eruption and permanent teeth that are genetically-based, as displayed in Figure 9. This age estimation is based on radiographs and is for children and adolescents who have reached 14 years of age (Millard and Gowland, 2002, Ajmal et al., 2001). As an adolescent reaches the age of 14 years, according to Ajmal et al. (2001), only one tooth, which is the third molar, is in existence and its development continues well into the age of 20 years. This provides the only source by which dental age is estimated.

The adult's mouth includes 32 teeth, often two incisors, one canine, two premolars and three molars, as illustrates in Figure 10.

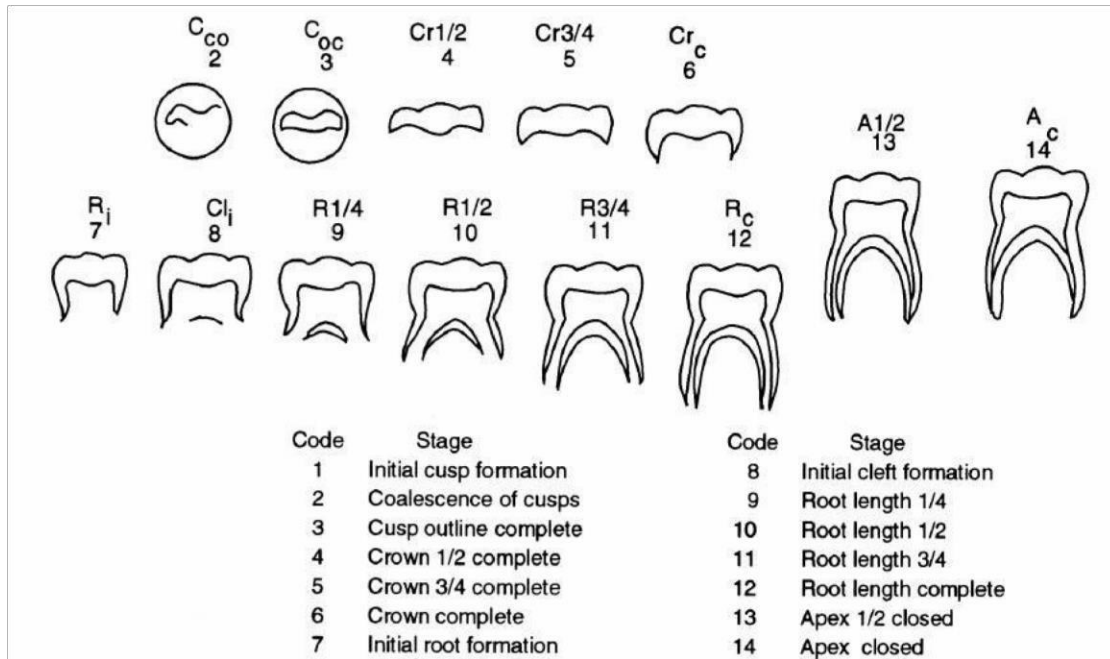


Figure 9. Tooth development adopted from (Millard and Gowland, 2002)

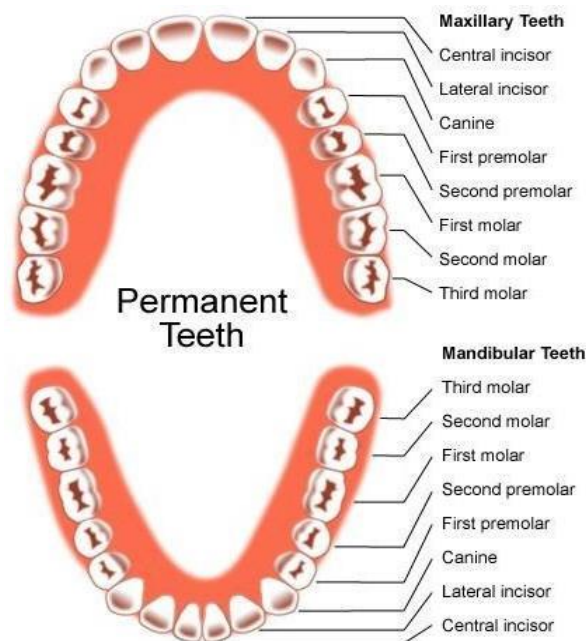


Figure 10. Permanent teeth (Studio Dentaire, 2008)

In adults, when teeth reach their maturity, fully developed skeleton can be utilised for age estimation. The discussion, when it comes to degeneration, is mainly focused on tooth wear while dental wear patterns are used in estimating the ages of adults (Millard and Gowland,

2002). Figure 11 displays the dental wear illustration as affected by age, where an individual who is approximately 18 years old is shown on the left side while an individual who is approximately 40/50 years old is shown on the right side. In their work, Millard and Gowland, (2002), show that the tooth wear age estimation method is not so accurate. There are differences in dental wear among populations, as well as several cultural and environmental factors that are not age-related but have an effect on the rate and the extent of age. In general, the major influences could be considered to be age and sex, together with diet, ancestry, and occupational history, lifestyle, inherited dispositions and jaw anatomies. Solheim (Ajmal et al., 2001) revealed another standard that proved the existence of significant relation between teeth colour and one's age.



Figure 11. Tooth wear of an 18 year-old and 40/50 year-old adopted from (Millard and Gowland, 2002)

Dental wear can be affected by two mechanisms, i.e. abrasion and attrition. Abrasion is caused by the teeth making contact with food or some other solid exogenous materials. This usually takes place when there is a forceful movement of food over occlusal surfaces (Larsen, 2015). Attrition, however, is the result of tooth-on-tooth contact. It is suitable due to different dental attrition consequences resulting from the types of food consumed allowing for a comparison to be made within the group of interest. It is possible for dental wear to appear like a precise indicator, specifically when it comes to prehistoric populations, since their type results in irregularities in attrition amounts of lifestyle (Lovejoy, 1985).

Erosion, meaning tooth surfaces superficial loss, in the form of chemical dissolution consequences, is sometimes seen as an additional kind of wear. Several methods have been developed for the purpose of determining age using wear patterns. Some of these methods are either time-consuming or cost-inefficient. Besides, a tooth extraction, required in certain situation may, for religious, ethical or cultural reasons, be an impediment. The simplicity and non-invasiveness of age estimation coming from dental wear makes it a convenient method, however, the limitations brought about by its low accuracy level must be considered (Kim et al., 2000).

Miles (1963), popularly recognised by everyone as Loma, is among the first authors who made proposals for the use of dental attrition method in the age estimation field. Miles, from 1980s, with his partner, conducted research on 416 individual remains that were exhumed from Outer Hebrides's chapel and burial mound, specifically on the Isle of Ensay (Miles, 1963). In 1963 and 2001, Miles determined how old young people were from tooth development so that he would be able to come up with the rate of wear for a precise archaeological population (Miles, 1963, Miles, 2001). This group of juveniles was established as "known age". Miles assumed that M1, M2 and M3 erupt in six-year intervals (namely; M1 at 6 years, M2 in 12 years and M3 at 18 years). Consequently, there was a possibility for the observation of dental wear after a period of occlusion. As a result, there exists the possibility for the dental wear to be observed after an occlusion period.

Miles (2001) further reiterated on the functional age differences present in the wear stage M1 and M2. The definition of the functional age ratio is assisted by subjective analysis for similar wear stages as 6: 6.5: 7 for M1: M2: M3. Further studies of similar subject have come up with reports of identical or equal wear rates which assumes continuous progress during an individual's lifetime. He made a classification of people whose ages were not supposed to be older than those of the "known age" group as estimated ages. Millard and Gowland (2002) also reiterated on "their serrated dentition and the progressively extrapolated ages, beginning from the known age group down to the rest." In spite of the fact that two tests of Miles's method were carried out with reliable results, some limitations were present. The underestimation of individuals who are fifty years old and above is a weakness in Miles method (Miles, 2001).

Figure 12 illustrates the upper and lower scale of Miles' system. Three intervals are in existence upon the marking of molars, with the first ones marked at 6-year intervals and the second marked at 6.5-year intervals, while the third are marked at 7-year intervals. Figure 13 is a report

of Miles' original histogram, a comparison of 416 individuals who were buried in Ensay. The revised age estimations of 451 individuals coming from the same burial site are on the right side. No changes have been discovered within the younger intervals. The biggest differences, in contrast, are seen in groups of above 45 years old.

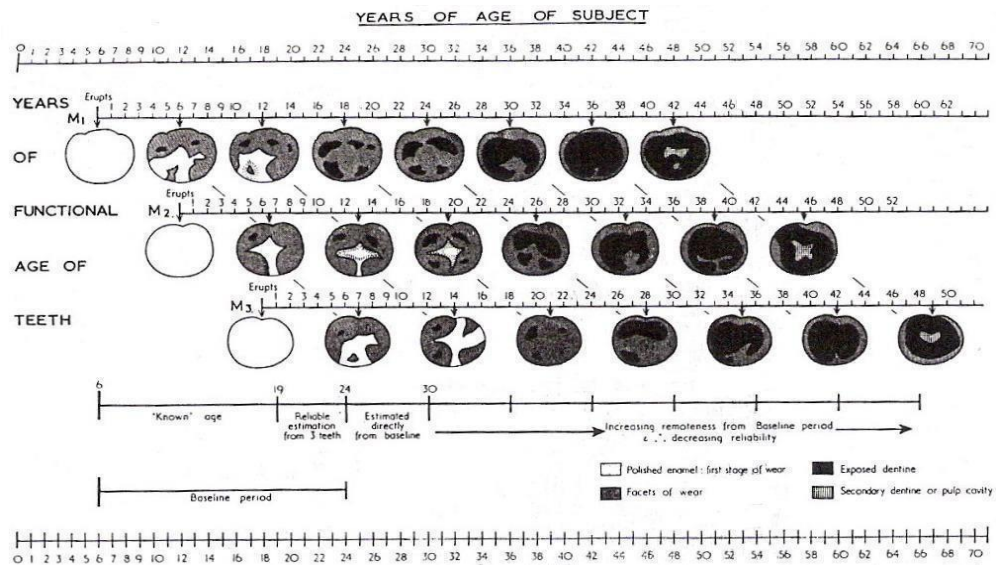


Figure 12. Miles' system (adopted from Millard and Gowland, 2002)

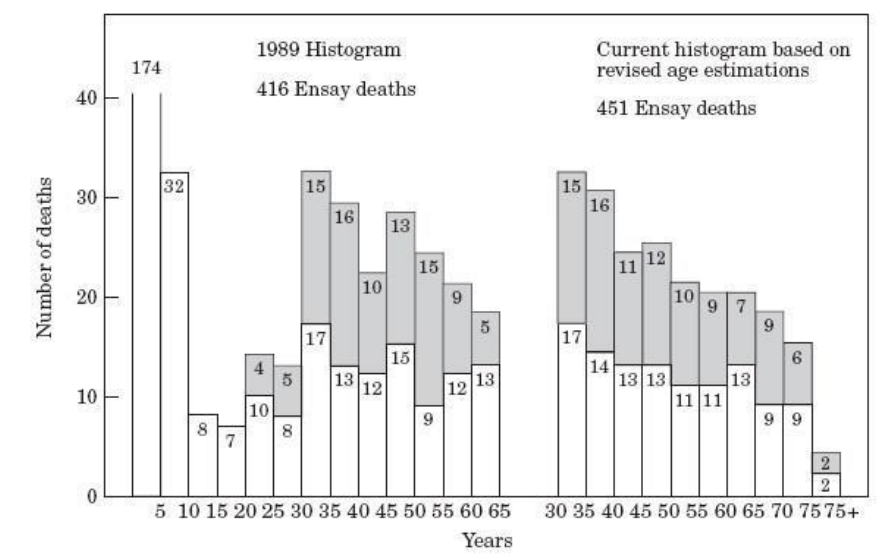


Figure 13. Miles' histograms from Ensay. The grey colour represents men; women are shown by white colour (Miles, 2001)

Brothwell (1981) made a report of his study that stemmed from identical data to that of Miles'. Assessment of his chart was made as one of the most utilised schemes in skeletal series when it came to age estimation in dead subjects. Figure 14 displays a chart that represents an uncomplicated ordinal scoring for classification of age in four categories, relative to large ranges of age, from 17 to 25 years, from 25 to 35 years, from 35 to 45 years, and 45 years or

above. Brothwell’s chart, in fact, has been criticised in some works for its less accurate criteria (Oliveira et al., 2006). Brothwell, responding to criticism regarding the inapplicability of his chart in all cases, refutes the argument that the tooth wear rates of the British population had not changed dramatically, and as a result of this, his chart is approximately accurate.

Age Period	About 17-25			25-35			35-45			45 or more			
Molar	M1	M2	M3	M1	M2	M3	M1	M2	M3	M1	M2	M3	
Wear pattern			Dentine not exposed. There may be slight enamel polishing.							Any greater degree of wear than in the previous columns. NB. Very unequal wear sometimes occurs in the later stages.			

Figure 14. Brothwell’s system for scoring surface wear in molars (Brothwell, 1981)

Oliveira et al., (2006), continued Miles’ research and suggested that in cases of individuals that are not complete, investigation of tooth attributes may be the only source in estimating their age. As previously stated, it is also the source for information about culture, health or diet of people (Oliveira et al., 2006). Scott (1977) went more with the research of Miles and proposed that the tooth attributes investigation may show to be the lone source in determining an individual’s age on cases of incomplete exemplars. As already stated, this is also the source of evidence when it comes to health, culture, or people’s diet.

According to Scott (1977)’s technique, the molar teeth are divided into four equal parts and measured through the scoring of current enamel in every quadrant within a scale of 1 to 10. The four wear scores, after that, are integrated so that a score between 4 and 40 for every tooth is obtained. This author asserted that the most applicable indicator with regards to the tooth’s functional life is the amount of enamel. Scott (1977) also considered the secondary dentine and the supposed “second enamel” – the enamel that forms during the second stage of tooth development – but it has to be taken into consideration that this kind of dentine is not exhibited by every individual, thus, the focus of the investigator should be more on the current enamel. The author made an identification of some vital steps so that the molars’ occlusal attrition could be recorded. The primary division of the molar’s occlusal surface into four parts and the succeeding scoring have been stated already. The amount of enamel scored in the section is worth remembering. Determining the amount of dentine relative to the amount of enamel

present is taken into consideration upon draining out the main occlusal features; it reveals scores from 1 to 4. The entire score for a precise tooth is represented by these four scores' summary. There is a scoring for each single quadrant based on these classes. The number 5 becomes the score in the event that one-fourth of a quadrant is covered by the worn patch. However, the score will turn out to be 6 if the dentine exposure becomes greater than one-fourth of the quadrant (although the area of patch is still surrounded by enamel). The situation is then represented by score 7 when the enamel does not entirely surround by a worn patch, indicating that this enamel is discovered to be only on two "flanks". Scott (1977), in a case that finds enamel being on one "side" of the quadrant alone and with thick to medium, had a score of 8 assigned to it. The next score is akin to a score of 8, except that the enamel is thinner. A score with the number 10 is given if there is complete dentine exposure with no enamel left (Scott, 1977).

Shykoluk and Lovell (2010) stressed that the molar wear description made by Scott's method is considered to be more thorough. This contrasts other methods that completely record the entire occlusal wear. They contend that by considering the additive scores, the possibility of occlusal wear patterns being hidden becomes apparent, and besides, every primary divided surface section has the ability to become thoroughly unconnected. As a result of this limitation, these authors succeeded in developing an improved Scott quadrant system that assigns each quadrant, together with the major molar cusps, to ensure an accurate scoring is in place. Moreover, the reporting of scores is made in an individual and sequential manner (Shykoluk and Lovell, 2010).

Smith (1984) used a tooth wear index, frequently used among investigators, to measure wear. This tooth wear index is made up of five levels, with level 1 indicating absence of wear while the highest score 4 indicates wear and where the secondary dentine and the pulp are exposed (Bartlett et al., 2011). The tooth wear index report is displayed in Table 7. On the basis of Bartlett (2003)'s statement, quite a huge divergence exists between scores 2 and 3 in terms of tooth wear severity.

Table 7. Smith and Knight Tooth wear index. B = buccal or labial; L = lingual or palatal; O = occlusal; I = incisal; C = cervical adapted from (Bartlett, 2003)

Score	Surface	Criterion
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0	B/L/O/I C	No loss of enamel surface characteristics No change in contour
1	B/L/O/I C	Loss of enamel characteristics Minimal loss of contour
2	B/L/O I C	Loss of enamel exposing dentine for less than 1/3 of the surface Loss of enamel just exposing dentine Defect less than 1 mm deep
3	B/L/O I C	Loss of enamel exposing dentine for more than 1/3 of the surface Loss of enamel and substantial loss of dentine but not exposing the pulp or secondary dentine Defect 1-2 mm deep
4	B/L/O I C	Complete loss of enamel, or pulp exposure, or exposure of secondary dentine Pulp exposure or exposure of secondary dentine Defect more than 2 mm deep, or pulp exposure or exposure of secondary dentine

Bartlett et al. (2011) later conducted a research that anchored on the index of Smith and Knight for the purpose of investigating tooth wear that is in line with the participant's dietary habits. Their new index made an evaluation of the buccal and cervical, as well as the incisal/occlusal and palatal/tongue surfaces, after which, an independent rating of the scores were conducted for dentine and for enamel. Bartlett et al. (2011) stressed on their interest in good lighting and drying in their study. The modified tooth wear index used by Bartlett et al. (2011) is displayed in Table 8.

Table 8. The exact tooth wear index adapted from (Bartlett et al., 2011)

(A) ETW index for enamel:	
0	No tooth wear: no loss of enamel characteristics or change in contour

1	Loss of enamel affecting less than 10% of the scored surface
2	Enamel loss affecting between 10% and 1/3 of the scored surface
3	Enamel loss affecting at least 1/3 but less than 2/3 of the scored surface
4	Enamel loss affecting 2/3 or more of the scored surface
(B) ETW index for dentine:	
0	No dentinal tooth wear: no loss of dentine
1	Loss of dentine affecting less than 10% of the scored surface
2	Dentine loss affecting between 10% and 1/3 of the scored surface
3	Dentine loss affecting at least 1/3 but less than 2/3 of the scored surface
4	Dentine loss affecting 2/3 or more of the scored surface, no pulpal exposure
5	Exposure of secondary dentine formative or pulpal exposure

(Gustafson, 1950) looked into the structural changes that tooth undergoes in relation to the abovementioned terms. This author identified a total of six changes that come in the form of attrition, secondary dentin and gingival recession, together with cemental apposition, root transparency, and root resorption. All the mentioned factors are age-related, despite having mostly a pathologic base.

Metzger et al. (1980) went further to state that Gustafson made an evaluation of these factors and provided a point value from 0 to 3 for each of them. Each tooth's total point values and the individuals' known ages derived from their extracted teeth were used for regression curve construction (Metzger et al., 1980). This curve was utilised to estimate what the ages of unknown bodies were, by conducting forensic science investigations. It is important to pay attention to the fact that the method of Gustafson requires precise training. The investigator should also take into consideration, and never underestimate, factors that come in the form of occlusal relations like caries and restorations, as well as non-vital teeth, attrition, periodontal condition and apical resorption (Gustafson., 1950).

The most common techniques in dental age estimation are based on developmental stage analysis, teeth eruption sequence and gingival emergence (see Figure 15). One of the most accurate approaches is the development stage analysis, proposed by Demirjian et al. (1973). This method takes into account the calcification of permanent teeth located on the left side of the mandible. Tooth calcification is divided into eight stages with each having a designated score, different in females and males. Numerous studies performed using this approach revealed that the main limitation of the approach is that it is time consuming and less friendly, considering that several tables have to be referred to in the process (Demirjian et al., 1973). Even though other approaches have been applied in age estimation, the most widely used approach is Demirjian method, mainly due to its simplicity.

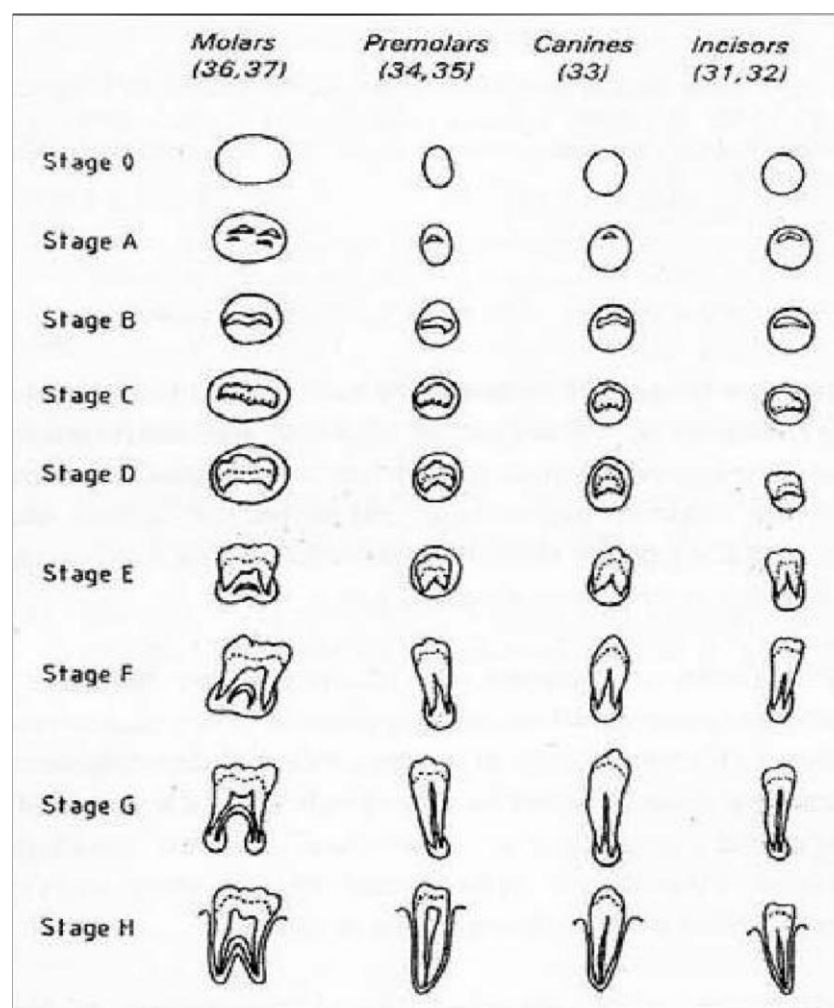


Figure 15. Graphical of the developmental stages as presented by Demirjian et al. (1973)

In 2001, Willems examined the accuracy of Demirjian's approach using a sample from Belgium and changed the scoring system as a result of a significant overestimation being reported. The new approach uses the eight steps developed by Demirjian et al. (1973), but a different scoring

system was proposed instead (Williams, 2001). The total score is obtained after adding the score for seven teeth to get the estimated dental age of the subject. Willem's approach uses the developing teeth in radiographs by assessing the status of dental maturity, which also accounts for changes among different communities. As a result, Willems' approach has proved to be more accurate compared to Demirjian's method. The accuracy of this approach is assessed by measuring the variation between the chronological age and the one deduced from dental age estimation.

The deciduous teeth microstructural analysis is one of the other deciduous teeth because of its investigation into the tooth enamel and dentine, precisely their histological marker since it makes the dental development record available (Katzenberg et al., 2005). This method is often used in the age estimation of people whose dental maturity has not yet completed the one tooth minimum. Four major steps, according to the report of Katzenberg et al. (2005), have to be taken into account, 1) identification of the neonatal line present in a tooth section set at zero, 2) determining what the average cross striation repeat interval is (representing the growth enamel of one day) along a prism that runs from the neonatal line (A) to the point at the enamel surface (B) where growth no longer takes place, 3) measurement of this prism's length, and, finally, 4) determining what the age is, in days, by using the average growth rate per day to divide the prism's length.

Pöllmann and other researchers (1987) discovered that as one advances in age, an increase in wear level also takes place, and they took notice of other studies that reported significant wear taking place in younger population. It has been reported that men, with regards to sex influence, demonstrate a higher tooth wear level compared to women. Pöllmann et al. (1987) further reiterated that the nature of occupation is one other factor put forward in influencing the level of wear. More wear is experienced when the nature of the subject's job is linked to physical stress. For example, miners and stonemasons, facing dusty environment and experiencing dust entering even into their mouth on a regular basis, exhibit high occlusal wear level and incisal tooth surface loss. Sad to say, the analysis, which specifically investigated these socio-economic factors and their effects, was not made available.

In 1925, Bodecker showed that there was a correlation between the apposition of the secondary dentine and the chronological age of a particular subject (Bodecker, 1925). Since then, more detailed studies have been carried out by various researchers on the pattern and rate of secondary dentine apposition in the upper and lower anterior teeth. In the work of Gustafson

(1950), secondary dentine deposition was included as an enabling method for age estimation where dentine transparency and secondary dentine values exhibited the highest correlation with age. Notably, Philippas (1961) was one of the first scientists who verified the influence of age on the formation of dentine by using the radiographic method.

In 1995, Kvaal et al. (1995) proposed a new technique for age estimation in adult subjects, where the estimation was realised based on the relationship between age and pulp size on periapical dental radiographs. Later on, Paewinsky et al. (2005) tested Kvaal's method using digital panoramic radiographs, however, specific regression formulae were required. Nowadays, owing to some conventional techniques, such as standard radiographs, or newly developed methods such as micro-focus X-ray computed tomography, apposition of the secondary dentine can be used as a useful tool in age estimation of adult subjects. Amongst these techniques, dental radiography is deemed a convenient, simple and cost-effective method for various situations where an accurate destructive method might not be permissible, for example, sacrificing a tooth in a living subject for forensic investigations.

Ramsthaler et al. (2014) investigated the reliability of a new digital odontological technique, based on different zones of dental root luminance, for estimation of a subject's age at death (see Figure 16). This technique was evolved based on an original method developed by Lamendin for applications in forensic anthropology (Ramsthaler et al., 2014). Multiple regression analysis studies have successfully demonstrated the strong significance of different statistical variables such as the arithmetic mean and standard deviation of luminance for deriving the regression formula. Using the aforementioned technique, the location of root translucency was shown to be an age-related phenomenon. The authors also take it a step further to show that, in addition to age, translucency could be a reflection of a number of other influencing factors.

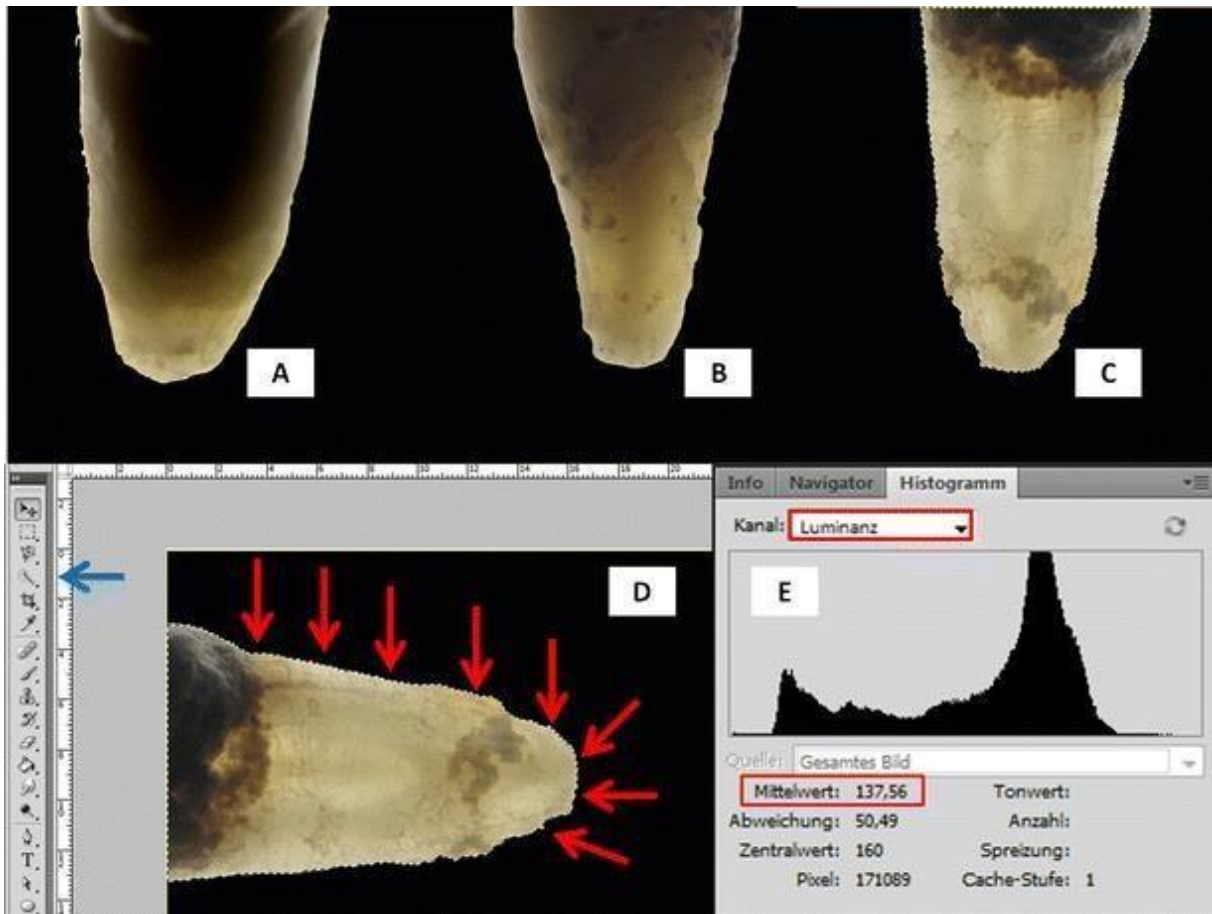


Figure 16. Increasing luminance depending on age (real age a 29 years, b 42 years, c 71 years)
 (Ramsthaler et al., 2014)

The work of Tardivo et al. (2014) proposed an age estimation technique that could be applied on both living and deceased individuals. Tardivo et al. (2014) used a 3D software to model the canines and perform calculations of pulp volume and total volume of each tooth, all in an automated fashion. The analytics were conducted using seven mathematical models, which appeared to be more efficient, relative to findings of previous studies. In general, regressions yield more accurate age estimates, however, Tardivo et al. (2014) recommended using more validated age techniques in a joint format, in order to reduce the fluctuation intervals (Tardivo et al., 2014).

Aboshi et al. (2010) proposed an age estimation technique based on the ratio of the three-dimensional volume of the pulp chamber with respect to the total tooth volume. The pulp-tooth volume ratio is an age-dependent variable that can be used to estimate age with reasonable accuracy. In order to calculate the pulp chamber volumes, micro focus X-ray computed tomography of the three-dimensional digital radiographic images of teeth were used, as shown in Figure 17, where each specimen was imaged by a micro-CT to reconstruct the three-

dimensional structure. Six different age groups were studied and their ratio (PTVR1-4) values were utilised as input for the multiple regression analysis. In all age groups, the coronal one-third of the root (L2) posed the greatest ratio, followed by L3, L4 and L1. During the examination of the subjects, morphological changes in the pulp cavity of different age groups were observable, where PTVR gradually reduced in value with increasing age. The steepest reduction occurred in the 20-50 age range, and most noticeably at the L2 level, as shown in Figure 17.

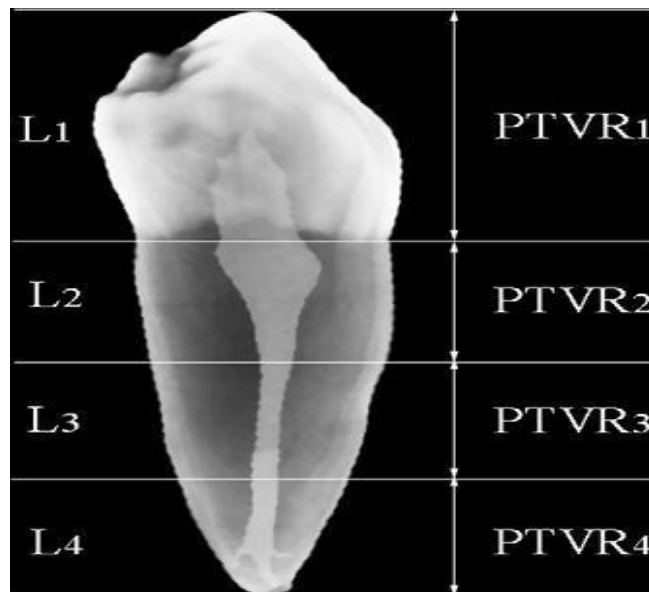


Figure 17. Transparent reconstructed image of a lower premolar showing the areas used for volume measurements (Aboshi et al., 2010)

González-Colmenares et al. (2007) aimed their study at comparing the accuracy of the Lamendin group and Prince and Ubelaker (2002) formulas in the estimation of age of the Spanish Caucasian population, and based on the obtained results, to come up with a new specific formula that can be applied to the Colombian racially mixed (mestizo) population. The first phase of the study had a sample of 79 teeth (34 females, 45 males) from subjects aged between 25 to 90 years. The second phase had 78 teeth (7 females, 71 males) from mestizo population aged between 25 to 87 years (González-Colmenares et al., 2007).

The researchers separated the teeth from their alveoli, washed them with water, digested in 0.05% sodium hypochlorite for 5 minutes, dried and placed them in plastic bags. Applying the Lamendin technique recommendations, González-Colmenares and his colleagues used a digital calliper with ± 0.02 mm precision in measuring periodontitis height, root height. Negatoscope

was also used in measuring the translucency height, before applying the proposed general equations from Lamendin and Prince (2002).

Cameriere et al. (2004, 2006, 2007a, 2007b, 2012) have been actively studying the pulp/tooth area ratio of the canines for a better age estimation technique. In their work, Cameriere et al. (2012) studied the relationship between age and age-dependent changes in the pulp/tooth area in monoradicular teeth, except for the canines, using orthopantomography. It was shown that using age-related variables in lower premolars and applying new regression formulae on the captured orthopantomography data led to the development of an accurate age estimation technique. In their analysis, Cameriere et al. (2012) used only orthopantomographs of high quality with clear radiological images. As demonstrated, by using high-quality images, a narrower age estimation error bar was achieved, improving the accuracy of the reported age estimation technique (see Figure 18).

It is known that the rate of physiological secondary dentinal secretion is not constant throughout life (Murray et al., 2002), therefore, it is of great interest to divide and examine the different patterns of secondary dentinal apposition as per age group. This allows for a more accurate identification of the age indicators in older individuals, where the reduced size of the root canal can also be stemmed from various age-related diseases such as arthritis, gout, kidney stones, gallstones, atherosclerosis and hypertension (Stanley et al., 1983).

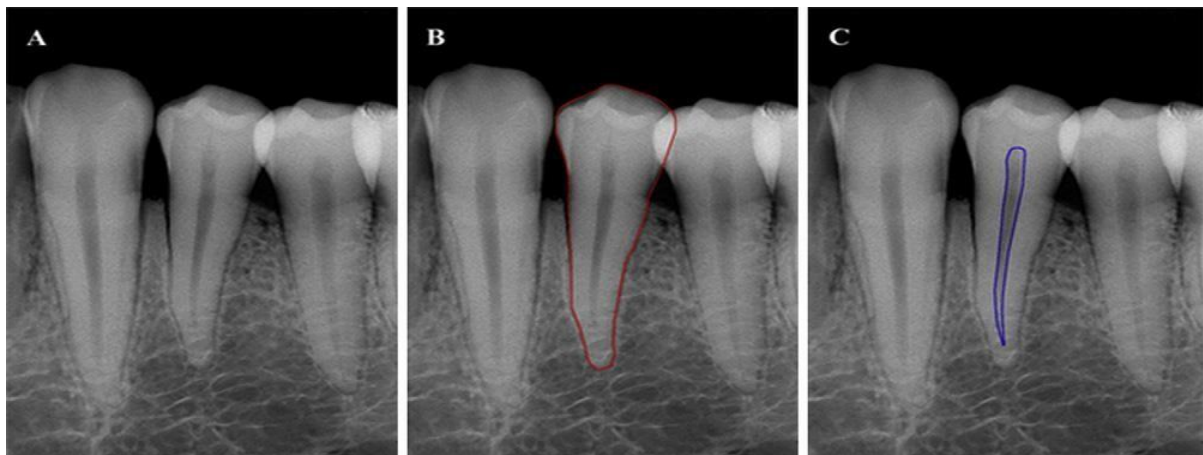


Figure 18. Radiographic image of left lower premolar, after processing and measuring pulp and tooth areas with line tool: red line, tooth area; blue line, pulp area (Cameriere, 2012)

To investigate the age-related changes in the pulp/tooth area ratio, Cameriere et al. (2012) studied per-apical X-ray measurements of both upper and lower incisors; their findings indicate that the variability in the age estimate, as obtained by the pulp/tooth area ratio in incisors, is

affected by the subject's sex. Moreover, their work showed that it is possible to obtain a more accurate age estimate by analysing the subject's upper lateral incisors. This is due to the reduction of blood flow in the pulp chamber of these teeth, which is already twice as fast as that in the lower incisors Cameriere et al. (2012). According to the results, it can be said that, in general, incisors are less reliable than canines or lower premolars. Furthermore, the work of Cameriere et al. (2012) reports on the possibility of using the pulp/tooth area ratio in incisors as an age-dependent variable that can produce reasonably accurate age estimates, especially when applied in combination with other age indicators or in cases where other single-rooted teeth are absent.

As Cameriere et al. (2013) showed in their work, teeth in their development stage can be used to provide an age estimate in children and adolescents, while their regressive changes can be applied in age estimation of adults. On the other hand, permanent teeth, except the third molars, generally complete their development between 12 and 14 years of age, although the third molars, which appear relatively late (around 8 years of age), are variable in development timing and can take until the age of 22 years to mature. However, when using the third molars for age estimation, the resulting confidence interval of the estimated age is significantly broader, compared with other permanent teeth (Cameriere et al., 2013).

Cameriere et al. (2006) introduced a new approach to estimation of chronological age in children, where they measured the open apices in seven mandibular teeth on radiographs of children of Italian origin. The Cameriere's method showed a causal relationship between sex and estimated age and was tested on a large sample of orthopantolograms (OPGs) from Italian, Kosovan and Slovenian decent children (Cameriere et al., 2006). The results showed that sample variation from different European origins did not have any significant influence on the estimated age. Later on, Cameriere et al. (2007a) established a European formula that was more generic and useful for all European origins. The formula was developed by regression analysis of OPGs from European children coming from Croatia, Germany, Kosovo, Italy, Slovenia, Spain and the United Kingdom (Cameriere et al., 2007a).

Age estimation in children has always been a challenge for forensic medicine, paediatric endocrinology and orthodontic treatment. The work of Galić et al. (2011) has shown that the evaluation of dental age in children of a particular regional group in Europe was only of interest until the end of the last century. The authors then stretch their argument by example of the war in Bosna and Herzegovina and its implications that led to an increase in need of identifying

missing and dead children. In their work, Galić et al. (2011) drew a comparison between three radiographic-based methods, namely the Cameriere (2007), Haavikko (2006) and Willems (2001) methods, where the developing teeth of Bosnian-Herzegovinian children were used. Ultimately, it was verified that all the radiographic methods proposed by the three authors above were applicable, however, the Cameriere's method based on a European formula was found to be most accurate when applied to both sexes. Later on, Cameriere's formula was assessed by De Luca et al. (2012), on a Mexican sample. The findings demonstrated that the formula is equally valid for age estimation of Mexican individuals. As stressed out by De Luca et al. (2012), Cameriere's formula can serve as a tool with broad applications in all cases of crimes and asylum proceedings (De Luca et al., 2012).

Cameriere et al. (2007) carried out a similar study on evaluation of the Cameriere's formula accuracy, when used in assessing chronological age of children based on the relationship between age and measurement of open apices in teeth. In addition, the study performs a comparison of the obtained accuracy with two other widely-adopted methods, namely Demirjian (1973) and Williams (2001). Using the Demirjian method it is possible to calculate a maturity score that is a function of age, useful for clinicians who already have knowledge of the child's real age and simply want to examine whether there are any abnormalities in the child's dental maturity. Despite the adoptability of the Demirjian's method for age estimation of unknown age individuals, it remains an unsuitable method for chronological age estimation. According to the findings of Cameriere et al. (2007), the Demirjian's (1973) method, as well as the Willems' (2001) method, are significantly less accurate, where more than 90% of the absolute residual errors were less than one year.

Cameriere et al. (2008) measured the third molar open apices of subjects between the age of 12 and 16 years old, taken from Italian, Croatian and Slovenian descents, in order to be able to identify the age of 14 in children for legal prosecution purposes. By applying Cameriere's regression formula (Cameriere et al., 2006), it was determined that, if all the apices are closed, a child is almost older than 12 years of ages (see Figure 19). Subsequently, the authors estimated dental maturity by using the seven left permanent mandibular teeth with completed root development and normalised the measurements of the third molar open apices. According to their findings, a subject was considered to be 14 years old if all the seven left permanent mandibular teeth had closed apices and the normalised measurement of the open apices was lower than 1.1. The results also proved that the findings were independent of the sex and nationality of the children.

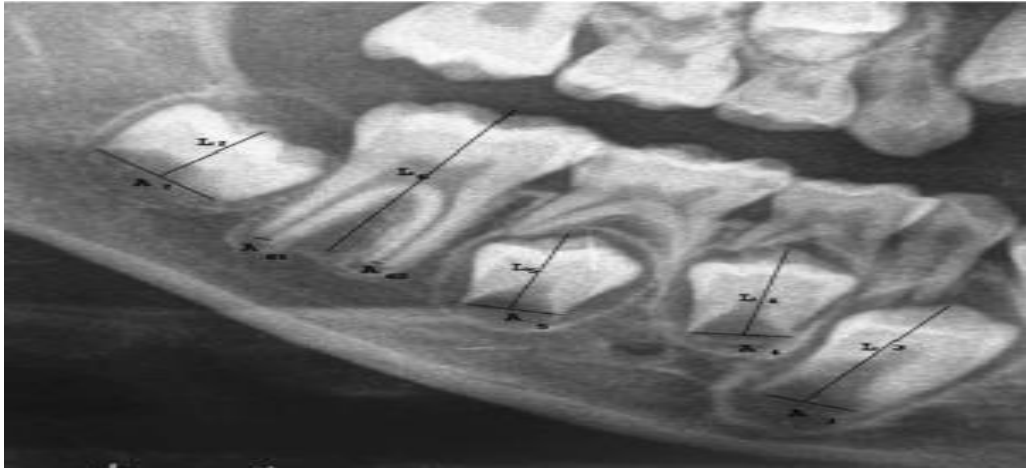


Figure 19. Example of tooth measurement. A_i , $i=1... 5$ (teeth with one root) presents the distance between the inner sides of the open apex; A_i , $i=6, 7$ (teeth with two roots) is the summary of the space between the inner sides of the two open apices; and A_i , $i=1... 7$ is the length of the seven teeth (Cameriere et al., 2006)

Another effective indicator of age evolution is tooth mineralisation. As Cameriere et al. (2007) reported, several factors play part in the growth of teeth, for example, sex, type of food, climate, hygiene, health, education and ethnicity, while nourishment is a controversial factor. In Cameriere et al. (2007), the authors investigated the hypothesis of whether there is any significant association between nutritional status, tender and the process of tooth mineralisation; the findings showed that nutrition had no apparent effect on the process of tooth growth.

The current non-destructive methods include wear and the apposition of the secondary dentine. The secondary dentine apposition occurs progressively, which can be related to the formation of the third molar, since the pulp is surrounded not only by harder tissue (e.g. enamel), but also by dentine that is prone to changes during lifetime. Subsequently, the analysis of the results on the apposition of the secondary dentine provides an improved and useful tool for age estimation in adult subjects, particularly in elderly subjects (Cameriere et al., 2007a). As another study example, Azevedo et al. (2014) focused their technique on the radiographic images of the canines, since canines are single-root teeth with the greatest pulp area, thus, allowing for an easier assessment. In this example study, the procedure involved a computer-aided drafting program, present the outlines of the proposed technique. As pointed out by Cameriere et al. (2004), multiple linear regression is the most frequent statistical method used for age estimation in forensic science, with focus on dental medicine.

In adults, the third molar tooth is used for age estimation, as this particular tooth continues to develop even after the age of 14. Since the application of the third molar tooth in age estimation

by Mincer (1993), this method has been widely adopted and continues to be the most frequently used age estimation technique for living subjects. However, Cameriere et al. (2004) states that this technique is not a perfect identification method for individuals of adult age, based on the fact that even after reaching the age of 18 years old, there is a possibility that some of the third molars might not have matured completely. The findings presented in the work of (Cameriere et al., 2004), emphasise on the importance of measuring the pulp/tooth area ratio of the second molar tooth as well. Cases where the second and third molars were taken into consideration, findings showed that sex plays no significant role in estimating the probability that an individual is 18 years of age. Table 9 summarises all the approaches to age estimation based on teeth development.

Table 9. Approaches of age estimation based on teeth development

Approach	Age range (year)	Notes	Reference
It uses calcification of permanent teeth located on the left side of mandible.	5-16	Tooth calcification is divided into 8 stages with each having a designated score, different in girls and boys.	Demirjian <i>et al.</i> (1973)

<p>The approach uses the eight steps proposed in Demirjian <i>et al.</i> (1973) approach but a different scoring system proposed by Willem's <i>et al.</i> The total score is obtained after adding the score for seven teeth to get the estimated dental age of the subject. The approach uses the developing teeth in radiographs</p>	<p>12-22</p>	<p>-</p>	<p>Willems <i>et al.</i> (2001)</p>
<p>there by assessing the status of dental maturity.</p>			
	<p>18-30</p>	<p>Completed a previously validated questionnaire containing 50 questions about current and historical dietary habits. Data were analysed at the tooth level using odds ratio.</p>	<p>Bartlett <i>et al.</i> (2011)</p>
<p>It uses teeth from either the left or the right side were chosen, whichever were best suited for measurement</p>	<p>20-87</p>	<p>Measurements from mandibular lateral incisors, canines and first premolars and maxillary central and lateral incisors and second premolars,</p>	<p>Kvaal <i>et al.</i> (1995)</p>

Single root tooth based on the original Lamendin method	28-85	A new digital odontological technique, measurement of the luminance of the teeth's translucent root zone	Ramsthaller <i>et al.</i> (2014)
Four healthy canines	15-85	four healthy canines present in the mouth and calculation of pulp volume (PV) and total volume (TV) of each tooth (CT scans)	Tardivo <i>et al.</i> (2014)
Left and right teeth premolar	20-78	Pulp chamber volumes were calculated using microfocus X-ray computed tomography of the three-dimensional digital radiographic images of teeth.	Aboshi <i>et al.</i> (2010)
Upper canines	19-74	Peri-apical radiographs	Azevedo <i>et al.</i> (2014)
Monoradicular teeth, with the exception of canines	18 -75	Examine the relationship between age and age-related changes in the pulp/tooth area ratio in monoradicular teeth,	Cameriere <i>et al.</i> (2012)
lateral and central incisors	18-74	Peri-apical X-ray images of upper and lower incisors, both lateral and medial, to examine the application of pulp/tooth area ratio as an indicator of age	Cameriere <i>et al.</i> (2013)

Seven left permanent mandibular teeth	5-15	Present a method for assessing chronological age based on the relationship between age and measurement of the open apices in teeth	Cameriere <i>et al.</i> (2006)
Left permanent mandibular teeth,	4-16	X-rays in digital form and measurement of open apices in teeth	Cameriere <i>et al.</i> (2007)
except the wisdom tooth			
The seven left permanent mandibular teeth were evaluated using Cameriere's method.	5-15	Measurement of open apices in tooth roots	De Luca <i>et al.</i> (2014)

2 Aims and Objectives

The need of age estimation for both living and death humans is becoming increasingly important in the clinical dentistry and forensic science, especially nowadays when Europe is facing increasing numbers of immigrants arriving without acceptable identification papers or with uncertain birth data. Some of these immigrants come from North Africa, namely from Tunisia, Egypt and Libya. The still-ongoing conflict in Libya began with the Arab Spring protests in 2011 and led to the First Libyan Civil War that erupted into violence and instability across the whole country that is one of the largest countries in terms of area and fourth country in size in the entire African continent. Figure 20 shows the migratory trends.



Figure 20. Migratory routes map (<http://abouthungary.hu/blog/frontex-prepares-forhttp://abouthungary.hu/blog/frontex-prepares-for-everything-but-border-protection/everything-but-border-protection/>)

Central Mediterranean route - The green route, for irregular migrants, has been an important point of entry to the EU, with approximately 40,000 of them detected in 2008, mostly close to Lampedusa and Malta. These were nationals of Tunisia and Nigeria, together with Somalia and Eritrea. Nonetheless, this movement completely came to a halt after the Italian government and Libya signed a bilateral agreement in 2009. The civil unrest eruption in Tunisia and Libya in 2011 resulted in the creation of massive spike with regards to the number of migrants totalling above 64,000 passing this route. More than 20,000 Tunisians came to the small Italian island of Lampedusa from January to March alone.

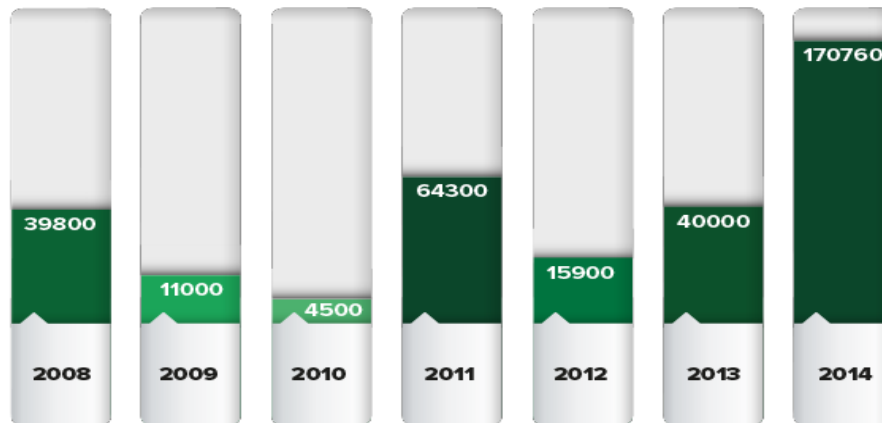


Figure 21. Increase in the number of migrants with the Years

The green yellow Apulia and Calabria route – a steady increase in the number of migrants that reached its peak took place during the time of the Arab Spring in 2011 since data collection began in 2009. The entire 2012 and 2013 saw a huge number of migrants crossing the Mediterranean Sea from North Africa and Middle East to enter the countries of Greece and Italy, as well as other European nations.

The smugglers, in 2014, began using much larger boats in crossing the sea that led to Italy. Most of the migrants were Syrians arriving in Calabria after departing from Turkey and Egypt. Among these migrants were also nationals from Pakistan and Egypt. A significant peak in the number of migrant arrivals departing mostly from Egypt was witnessed in 2013.

In Libya, the need for reliable and accurate age estimation techniques has never been greater than in the last years, mainly due to armed conflicts within the country lead to the lack of a validated method for assessing age in Libyan population is fundamental in criminal proceedings relating to irregular immigration and emigration movements at both national and international levels.

This PhD project aims to develop a descriptive reliable method in order to estimate age from the teeth, mainly focusing on the pulp/tooth area index in circum-Mediterranean populations. Moreover, the aim of this study is to test the reliability and applicability of pulp/tooth area ratio (PTR) in all teeth as an indicator of age by X-ray Panoramic Radiographs.

3 Materials and Methods

3.1 Dental Wear and Shading

Inferring from Lovejoy (1985), dental wear is an accurate indicator, more so in prehistoric populations, as lifestyles cause irregularities in attrition levels. Erosion, which involves a superficial loss of tooth surfaces due to chemical dissolution, is an additional form of wear. Tooth extraction, which is done following cultural, ethical and religious reasons serves as an obstacle in some cases. Estimating age using dental wear is a convenient approach not only for its simplicity but also for non-invasiveness. Nevertheless, it is vital to take into consideration the limitation of its low accuracy level (Kim, Kho et al., 2000).

Therefore, this study was carried out in order to estimate the unknown age of a human body following the shading tooth method. This method is based on approximating people's age by investigating their teeth surface wear; the wear of a human's teeth varies depending on age. According to that, data was collected from people of variable ages and 419 human participants of a known age were questioned in order to gather supportive data from them as a part of this research. 223 of those participants were North Africans (majority from Libya) and 196 were from Europe (majority from Britain). 43% of the participants were females and 57 % were males (see Table 10). In the study, two separate indicators had to be used; score and shading.

Table 10. Total Number of individual with different age, ethnicity and percentage of male and female of North African and British (M: Male, F: Female, B: British, NA: North African)

	M	F	Total
B	104	92	196
NA	136	87	223
Total	240	179	419
%	57%	43%	100%

The tooth wear index modification, which Smith and Knight (1984) introduced, is the first indicator score, and it has, these days, become the most widely utilised index in the world. Five severity levels, ranging from 0 (where there is no wear) to 4 (where there is severe wear that exposes secondary dentine and the pulp) are utilised. A comprehensive description of this index

has been previously stated, and it has been selected as a result of its effectiveness and reliability in determining wear over a long time (Bartlett, 2003).

Further, an existing index, which Hugoson et al. (1988) performed specifically in Sweden, was studied. They made an evaluation of incisal and occlusal wear and to accomplish this, they made use of the scores from 0 (where there is either no enamel wear or there is negligible wear) to 3 (where the wear of dentin is more than 1/3 of the crown height and/or excessive tooth wear restorative material). As a result of such a limited scale of scores, the sensitivity of wear severity measurement is deficient. It is also worth mentioning that authors have never utilised this index beyond Sweden. As a result, the use of this index as a basis for this project is not considered.

A scoring index of 0 to 6 is used in this thesis as an addition, so that a more precise set of results could be derived over a wider range of scores. It is possible for the evaluation of buccal and lingual, as well as occlusal surfaces, to be made in terms of enamel loss, by having dentine exposed by scores of 2 (below 1/3 of the surface), 3 (above 1/3) and 4 (below 2/3). The index of Smith and Knight's (1984) reveals just "above 1/3 of the surface" and later, "complete loss," as a result of a scale that is not all that narrow. In taking into account cervical surfaces, scores had been added for the purpose of detection of contour loss of 2-3mm deep and above 3mm deep. The tooth wear index scale that is broadened makes it possible for researchers to take measurement of the upper extreme of tooth wear in order to make assessment of more accurate findings. Table 11 shows the modified tooth wear index. The scale reported in Table 11 has been used when recording the degree of wear on the individual teeth.

Table 11. Modified Tooth Wear Index (B = buccal or labial; L = lingual or palatal; O = occlusal; I = incisal; C = cervical, additional categories developed by the author*)





Score	Surface	Criteria
0	B/L/O/ I C	No information available polished enamel No loss of enamel surface characteristics No change in contour
1	B/L/O/ I C	Loss of enamel surface characteristics (very small) Minimal loss of contour

2	B/L/O I C	Loss of enamel exposing dentine for less than 1/3 of the surface Loss of enamel just exposing dentine Defect < 1 mm deep
3	B/L/O I C	Loss of enamel exposing dentine for more than 1/3 of the surface Loss of enamel and substantial loss of dentine but not exposing the pulp or secondary dentine Defect 1-2 mm deep
4*	B/L/O I C	Loss of enamel exposing dentine for less than 2/3 Loss of enamel and substantial loss of dentine but not exposing the pulp or secondary dentine Defect ~ 2 mm deep
5	B/L/O I C	Complete loss of enamel, or pulp exposure, or exposure of secondary dentine Pulp exposure or exposure of secondary dentine Defect > 2 mm deep, or pulp exposure or exposure of secondary dentine
6*	B/L/O I C	Complete loss of enamel, and pulp exposure Pulp exposure Defect > 3 mm deep.





In Figure 22, the Tables 1, 2 and 3 provide report of the surface wear in Incisors, Canines and Premolars adapted from Smith and Knight (1984). The diagram displays the chart representation of an uncomplicated ordinal scoring for age classification in four different categories. Furthermore, the table reveals the different enamel levels affected by ageing. The condition of the teeth is good with no enamel or dentine loss and they still remain polished, although there is a gradual and more significant enamel reduction as a result of ageing. This procedure is ongoing until enamel is completely worn. The complete enamel loss, which exposes dentine, is relative to pulp exposure loss and secondary dentine exposure. Moreover, increasing changes in the cervical surface contour can also become apparent.

Table 4 of Figure 22 reveals the surface wear in Molars adapted from (Brothwell, 1981), but with slight modification. The diagram displays the chart representation of an uncomplicated ordinal scoring for age classification in four different categories. The procedure, as can be observed from the table, is identical to the case of incisors, canines and premolars, although with a slight difference as young individuals displayed no loss of enamel. It can also be said that the teeth have remained polished but as ageing sets in, appearance of the enamel wear becomes progressive. With enamel wear, exposed dentine can be observed until it results in pulp cavity.

• Table 1: System for Surface Wear in Incisors

INCISORS	INCISORS	INCISORS	INCISORS	INCISORS
				



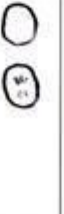







• Table 2: System for Surface Wear in Canines

CANINES	CANINES	CANINES	CANINES	CANINES
				

• Table 3: System for Surface Wear in premolar

premolar	Pre1	pre2	Pre1	pre2	Pre1	pre2	Pre1	pre2
								

• Table 4: System for Surface Wear in Molars

Molar	M1	M2	M3	M1	M2	M3	M1	M2	M3	M
										

Enamel :

Enamel wear :

Exposed dentine :

pulp cavity :

Figure 22. (Tables 1, 2, 3, and 4)

This method was planned to be carried out on 419 individuals of a variable age range and a different ethnicity (Smith and Knight, 1984). Figure 23 provides comprehensive summaries of all detailed information gathered from participants and their scores, created by the researcher. These data were then utilised for the investigation of the presence of enamel and dental wear, as well as pulp exposure for every tooth. Nonetheless, for individual ages to be determined, an accurate observation of an individual participant's teeth was made to obtain data with regards to his/her teeth surface wear. Upon determining the teeth surface shape, its recording was made as a reference along with data gathered from other participants in the same age group by shading special marks of their enamel on the collective data sheet. Again, performing this with known ages as a way of defining the surface teeth change in various age stages is an indication that testing should be conducted to identify whether there is the possibility of utilising it for humans with unknown age.

The authority of ethic permission has been approved since 2012 and the current study is corresponding to this approval.

Figure 23. Data collection form

3.2 Third Molar Index by Measurement of Open Apices

In Cameriere's method, taken orthopantomographs were made into X-ray images, which were digitalised on a scanner, recorded on computer files and processed by a computer-aided drafting program (Adobe Photoshop 7). The left permanent mandibular teeth, except the wisdom tooth, were valued with the apical ends of the roots completely closed (N0) and were calculated. Teeth with incomplete development (i.e. with open apices) were also taken into account. For teeth with one root, the distance A_i , $i=1, \dots, 5$, between the inner side of the open apex was measured. In case of teeth with two roots, A_i , $i=6, 7$, the sum of the spaces between the inner sides of the two open apices was assessed. To consider the effect of possible differences in magnification and angulation among X-rays, measurements were normalised by dividing the sum by the tooth length (L_i , $i=1, \dots, 7$). Finally, dental maturity was evaluated using the normalised measurements of the seven permanent left mandibular teeth ($x_i=A_i/L_i$, $i=1, \dots, 7$), the sum of normalised open apices ($s=x_1 + x_2+x_3+x_4+x_5+x_6+ x_7$), and the number (N0) of teeth with completed root development (Cameriere et al., 2007b, Cameriere et al., 2006).

This section aims to evaluate the accuracy and effectiveness of the threshold value of 0.08, where the third molar maturity index is measured in the determination of a person's age, that is, whether the individual is 18 years, younger or older. To achieve this, a sample of living young adults and children from Tripoli, a city characterised by different ethnic groups, is analysed.

In this research, digital panoramic radiographs comprising 420 healthy living individuals from Libya aged between 14 and 22 years were studied. A random sampling technique was used in selecting the sample from the Academic Dental Center in Tripoli. The sample was gathered solely for clinical purposes from January to March 2015. Before using the information for the study purpose, the consent was first sought from all the individuals involved, that is, from the individuals themselves, and in the case of those who had not attained the age of 18 years, the consent was sought from their guardians.

Patients' age, sex and identification numbers were recorded, but no ethnic details were collected. During the time digital panoramic radiograph (OPT) was obtained, the inclusion criteria were to collect the sample of healthy people of precise age and known sex. The images were expected to be of high quality implying high resolution with minimal distortion. On the

other hand, there was an exclusion criterion. This included panoramic X-ray images with indicated extracted or lost teeth, those with fillings, severe caries, crown restorations and abnormal dental anatomy as all this would lead to inaccurate measurement. A total of 307 OPTs were examined where all the children belonged to the middle socioeconomic class (see Table 12).

Table 12. Panoramic radiograph from Libyan subject according to age and sex group

Age (years)	Females	Males	Total
14	11	17	28
15	22	17	39
16	15	21	36
17	18	12	30
18	25	18	43
19	19	14	33
20-23	53	45	98
Total	163	144	307

Digital radiographs were stored in JPEG format. Image improvement tools were applied in adjusting the contrast, brightness and grey scale. The Fédération Dentaire Internationale (FDI) two-digit system notation was applied (Thevissen et al., 2013). Cameriere approach was used to access the dental age. Multicollinearity issues in the regression models were identified due to development that the right and left third molars is strongly correlated. Following this, the left side was evaluated for standardisation (Yusof et al., 2015). The apical ends of the third molar were studied where the measurements were done with the help of a computerised image-processing program.

The researcher started by coding the orthopantomographs using a numerical identity to avoid any kind of bias. This ensured that observers had no information relating to the sex and age of the subject. In assessing the reliability of the measurements, the concordance correlation coefficient was computed randomly on a selected number of subjects. Later, the measurements were re-evaluated. Random selection was applied to give each individual an independent and

equal chance of participating in the study. Analysis of covariance was conducted in studying the interaction between sex and the third molar index (I_{M3}). In estimating the age, the predictive variables were I_{M3} , sex, and age. By use of SPSS, all statistical analyses were conducted with a significant threshold of 5% and 1%. The cut-off value of 0.08 for the third molar index was analysed to determine the age.

According to the results of Cameriere et al (2008b), a similar cut-off value of 0.08 was applied for both male and females in order to consider an individual to be 18 years, if I_{M3} is 0.08 and above 18 years in instances where the I_{M3} is less than 0.08. Third molar index can be used in discriminating between individuals who are 18 years of age with those that are over and under through post-test probability (\square). The test sensitivity (means the proportion of people who are 18 years or older and have an I_{M3} of less than 0.08), together with specificity (individuals who are less than 18 years of age and have I_{M3} greater than 0.08) were examined. Additionally, a cut-off value of 0.09 was used with the intention of improving the discrimination model.

Based on the theory proposed by Bayers, the post-test probability is presented as:

$$\square = \frac{\square_0 + (\square_1 - \square_0) \square_1}{\square_0 + (\square_1 - \square_0) \square_2}$$

\square stands for the post-test probability; \square_0 is the probability that the individual being examined is 18 years or more; \square_1 is the sensitivity of the test; and \square_2 is the specificity. The \square_0 value was computed as the percentage of the individual between 18 and 22 years living in Libya and individuals between 15 and 22 years based on World Bank demographic data. For males, the probability was 54.4% for males and 55.6% for females.

The authority of ethic permission has been approved by E Mail from the Academic Dental Center in Tripoli and Benghazi.

The authority of ethic permission has been approved by E Mail from the Academic Dental Center in Tripoli and Benghazi

3.3 Validation of Ages Estimation in Libyan Population

Cameriere et al. (2008) developed a new approach based on the relationship existing between the age of a person and the normalised measures of the apices and third molar height. A threshold value of 0.08 was applied to differentiate between the people who are above 18 years

of age and those who are below (Ambarkova et al., 2014). Furthermore, in most nations, the

age of 18 is the legal majority. The criminal responsibility age in Africa differs by jurisdiction. It ranges from 14 to 18 years. In the last five years, more than ever, there has been a growing need for an accurate and reliable approach for age estimation, especially in Libya, due to increased cases of armed conflicts. Failing to have a validated approach to establishing the age of people in Libya in a criminal proceeding has been identified as a major issue (Altunsoy et al., 2015).

In the country, dental age estimation has not been thoroughly invested, as only one research has mainly focused on this topic (Liversidge et al., 1999). There is not even a single previous study that has used Cameriere's approach in studying the Libyan population. In addition, in the whole of the African continent, there is a lack of systematic studies on age estimation. Only limited researches have been carried out in establishing the age of people who have no documentation or records of their chronological age.

In contribution to the literature, this study aims to validate the accuracy of the threshold value of 0.08 as implied by the measurement of I_{M3} in defining if a subject is 18 years of age. For this purpose, a sample of living children and young adults from both Benghazi and Tripoli is to be analysed. Note that the main objective herein is to statistically validate the accuracy of the Cameriere's technique (Cameriere *et al.*, 2008), preliminarily tested in a small Libyan population (Dardouri et al., 2016). In particular, the cut-off value for determining the age of majority ($I_{M3} = 0.08$) in a Libyan sample consisting of children and adults will be verified. The findings will be useful in forensic practice and will aid the broader forensic community towards achieving multi-regional proof of the proposed technique (Dardouri et al., 2016).

A selection of 1137 OPGs was performed in the Libyan cities of Tripoli and Benghazi. They were taken for therapeutic purposes in two significant radiographic centres. The sample was indiscriminately selected from the National Centre for Disease Control and Academic Dental Centre in Tripoli. Moreover, OPGs of 918 were chosen and recorded, of which 397 were males, 521 were females, while 758 individuals were from Tripoli (324 boys and 434 girls) and 160 from Benghazi (73 males and 87 females), aged between 14 to 23 years old.

During data collection from each patient, without any information to reflect on the patient's ethnicity, the followings were taken:

- Identification number;
- Sex;

- Date of birth (day, month and year); and
- Data of data collection.

The inclusion criteria that was required of each individual was sound health, known sex and precise age (14-23 years) at the time the OPT was achieved. The images had to be of an appropriate quality/resolution with minimal alteration.

The selected digital radiographs were saved in JPEG format for ease of transfer onto a host PC, where image grey scale, brightness, contrast and overall quality were improved using the Adobe® Photoshop® CS4 image processing software. In this work, the method of Cameriere *et al.* (2008) was adopted to assess the patient's dental age, where the apical ends of the roots of the left lower third molar of each individual were analysed. Moreover, the measurements were performed using a computerised image-processing program (Image J).

In literature, the I_{M3} is defined such that, if the root development of the third molar is complete, i.e. the apical ends of the roots are completely closed, then I_{M3} equals zero. Otherwise, I_{M3} is evaluated as the sum of the distances between the inner sides of the two open apices, divided by tooth length. Note that the evaluation of I_{M3} takes a similar approach to calculation of A_i to L_i ratio, when $I = 6.7$. This is in accordance with the work of Cameriere *et al.* (2008), which reports on the other two teeth with two roots.

In this work, two experts provided evaluations of I_{M3} for each OPT, working independently, without exchange of any information. The other expert was a dentist with good experience and his observations were collected in an Excel spreadsheet.

Each individual's age was attained by calculating the difference between the date-of-birth declared by the individual on the records and the date on which the radiograph was taken, which could be found on the panoramic radiographs, using lead markers. In order to study the relationship between I_{M3} and sex, an analysis of covariance (ANCOVA) was conducted in Statistical Package for the Social Sciences (SPSS) 22.0 for Windows (SPSS Inc., Chicago, IL, USA). In accordance with the findings of Cameriere and De Luca *et al.* (2012), the I_{M3} for both sexes has been found to be 0.08. Therefore, those individual with an I_{M3} of 0.08 or lower could be considered to be 18 years of age or older. On the other hand, if the individual is under 18 years of age, a post-test probability can be carried out to examine the hypothesis of being 18 years of age or older, i.e. what proportion of the individuals with an I_{M3} of lower than 0.08 are

older than or equal to 18 years of age. Furthermore, the same I_{M3} cut-off value of 0.09 was used for both sexes in order to improve the discrimination model.

According to Bayes' theorem, post-test probability can be expressed as:

$$\square = \frac{\square_1 \square_0}{\square_1 \square_0 + (1 - \square_2)(1 - \square_0)}$$

where \square is the post-probability; \square_1 is the sensitivity of the test, the proportion of the individuals with 18 years of age or more whose I_{M3} were less than 0.08; \square_2 is the specificity, describing the proportion of the individuals younger than 18 whose I_{M3} were calculated to be less than 0.08; and, \square_0 is the probability that the subject under examination is 18 years or older, given that they fall within the age range of 14 to 23 years. The probability \square_0 was calculated by using the data from the World Bank (<http://data.worldbank.org/country>) and given as the proportion of Libyans aged between 18 and 23 years who live in Libya compared to those aged between 14 and 23 years, which was considered to be 58.8% for males and 60.1% for females.

During data sampling, a total of 918 OPTs from the middle socioeconomic class (521 females and 397 males) were analysed, whilst applying the following exclusion criteria:

- Panoramic X-ray images with lost or extracted single-rooted teeth;
- Fillings;
- Crown restorations;
- Severe caries or other abnormal dental anatomy that may result in inaccurate measurement;
- Agenesis and/or extraction of the third molars third molars with growing anomalies (e.g. abnormally short roots, dysmorphology); and
- Asymmetric root formation between left and right side and/or unclear emergence direction.

Estimation of intra- observer and inter- observe error encountered for each experiment also has been used in this thesis for both adults and children, more specifically minimum of 30 both sex male and female samples were measured and scored twice by an independent observer in this thesis (Edward F, et al 2009, M. Arroyo, et al 2010).

To define intraobserver precision, four different widely used precision estimates were calculated; the relative technical error of measurement (rTEM), the technical error of measurement (TEM), the coefficient

of reliability (R) and the coefficient of variation (CV). The TEM is the most commonly used measure of

precision, which is the square root of measurement error variance, TEM was calculated by the following formula, where $\sum \square^2$ is the summation of deviations raised to the second power and N is the number of volunteers measured.

$$\text{TEM} = \sqrt{(\sum \square^2)/2N}$$

The absolute TEM was transformed into relative TEM (rTEM) in order to obtain the error expressed as calculation corresponding to the overall average of the variable to be analysed. Therefore, the next equation was used, where VAV is the variable average value (Edward F, et al 2009, M. Arroyo, et al 2010).

$$\text{rTEM} = (\text{TEM} / \text{VAV}) \times 100$$

The coefficient of reliability (R) was calculated as percentage with the following equation, where $\square\square^2$ is the total intra-subject variance for the study, including measurement error.

$$R = 1 - (\text{TEM}^2 / \square\square^2)$$

As a final point, the coefficient of variation (CV) was calculated through the following formula,

$$\text{CV} = \frac{\square\square * 100}{\square}$$

where SD is the standard deviation and X is the average of measurements. The CV expresses sample variability relative to the mean of the sample, and all statistical analysis was performed with the software package SPSS (Edward F, et al 2009, M. Arroyo, et al 2010).

The authority of ethic permission has been approved by E Mail from the Academic Dental Center in Tripoli and Benghazi.

3.4 Age Estimation of Libyan Children by Open Teeth Apices

319 OPTs of healthy living Libyan subjects, aged between 5 and 15, were analysed retrospectively. The sample was selected from the Academic Dental Centre in Tripoli (Libya) from January to July 2015. The consent to use them for research and educational purposes was obtained directly from the patients; this information included patients' identification number, sex, age, date of birth and date of collection but no further information related with the ethnicity was collected. Panoramic X-ray images with lost or extracted single rooted teeth, as well as those with fillings, crown restorations, severe caries or other abnormal dental anatomy, which

might cause difficulty with measurement, were excluded from this analysis. Impacted third molars were also not included in this sample. A total of 319 OPTs (171 girls and 148 boys) was finally analysed.

The method is fully explained in (Cameriere et al., 2006). The seven left permanent mandibular teeth were valued. The number of teeth with root development completed, that is, apical ends of the roots completely closed (N0), was calculated, as showed in Figure 24.

In addition, consideration was given to the teeth with incomplete root development and, thus, with open apices. With regards to teeth having one root, there was measurement of the distance (A_i , $i=1, \dots, 5$) between the inner sides of the open apex, and with regards to teeth having roots (A_i , $i=6, 7$), evaluation was made for the sum of the distances between the two open apices inner sides. Normalising the measurements is accomplished with the use of tooth length (L_i , $i=1, \dots, 7$) to divide, so that the effect of possible differences in magnification and angulation among X-rays could be taken into consideration.



Figure 24. Measurement of inner side of the open apex and tooth length

Finally, the normalised measurements of the seven remaining permanent mandibular teeth ($x_i=A_i/L_i$, $i=1, \dots, 7$) were utilised in dental maturity and the normalised open apices sum(s) and

the number of teeth (N0) without complete root development. The same observer carried out all the measurement.

The conduct of the study adhered to the ethical standards stipulated by the Declaration of Helsinki (Finland). The Declaration of Helsinki was developed by the World Medical Association (WMA) as an ethical principles statement for medical research where human subjects that include research on identifiable human material and data are involved.

The selected digital radiographs were saved in JPEG format. In order to adjust the grey scale, brightness and contrast, image quality improvement tools in Adobe® Photoshop® CS4 were used.

The FDI two-digit system notation was used. According to the previous studies the left side was evaluated (Garamendi et al., 2005). Dental age was assessed according to the method of Cameriere et al. (2008).

Estimation was made of intra- and interobserver error encountered for each experiment in this thesis; more specifically, a minimum of 30 samples from both males and females were measured and scored twice by an independent observer.

To define intraobserver precision, four different widely used precision estimates were calculated; the relative technical error of measurement (rTEM), the technical error of measurement (TEM), the coefficient of reliability (R) and the coefficient of variation (CV). The TEM is the most commonly used measure of precision, which is the square root of measurement error variance, TEM was calculated by the following formula, where $\sum \square^2$ is the summation of deviations raised to the second power and N is the number of volunteers measured.

$$TEM = \sqrt{(\sum \square^2)/2N}$$

The absolute TEM was transformed into relative TEM (rTEM) in order to obtain the error expressed as calculation corresponding to the overall average of the variable to be analysed. Therefore, the next equation was used, where VAV is the variable average value (Edward F, et al 2009, M. Arroyo, et al 2010).

$$rTEM = (TEM / VAV) \times 100$$

The coefficient of reliability (R) was calculated as percentage with the following equation, where $\square \square^2$ is the total intra-subject variance for the study, including measurement error.

$$R = 1 - (TEM^2 / \square \square^2)$$

As a final point, the coefficient of variation (CV) was calculated through the following formula,

$$CV = \frac{SD * 100}{X}$$

where SD is the standard deviation and X is the average of measurements. The CV expresses sample variability relative to the mean of the sample, and all statistical analysis was performed with the software package SPSS (Edward F, et al 2009, M. Arroyo, et al 2010).

The authority of ethic permission has been approved by E Mail from the Academic Dental Center in Tripoli and Benghazi.

4 Results

4.1 Age Estimation by Dental Wear and Shading

The average age of the British males in the studied sample is 38.3 and 30.2 years for the British females, whereas it varied from 25.9 years for the North African males to 33.7 years the for North African females (Figure 25) [Apendix-1].

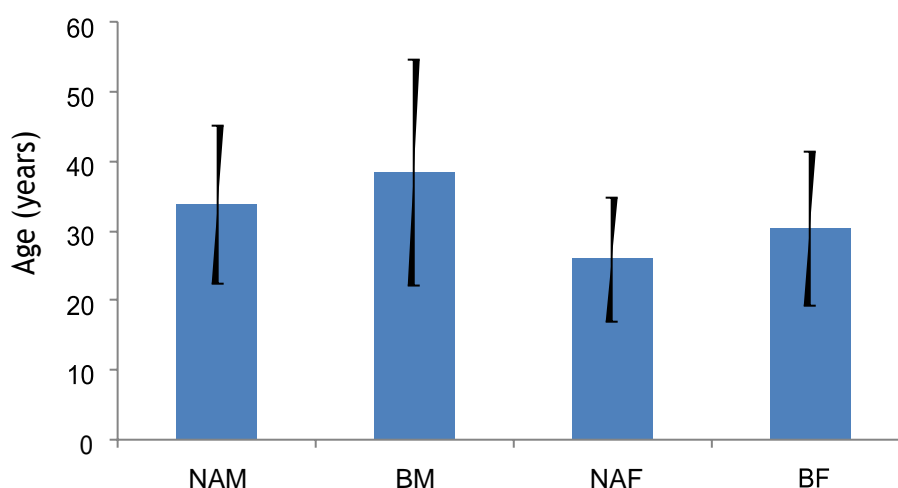


Figure 25. Age (\pm standard deviation) for males and females (North African and British), M: Male, F: Female, B: British, NA: North African STD

The surface wear in incisors, canines, premolars and molars is displayed in Figure 26, which is a form of an average taken from the data collected from all the participants in the study in every age group. They also reveal the age estimation procedure performed by tooth attrition and by occlusal surfaces for every tooth. Various teeth classified in the same classes are illustrated in the form of Brothwell chart as utilised when this study is being conducted. This is an indication

that the drawing of each of the individual's teeth was made by this author while integrated for the average tooth production for every age group and every tooth category.

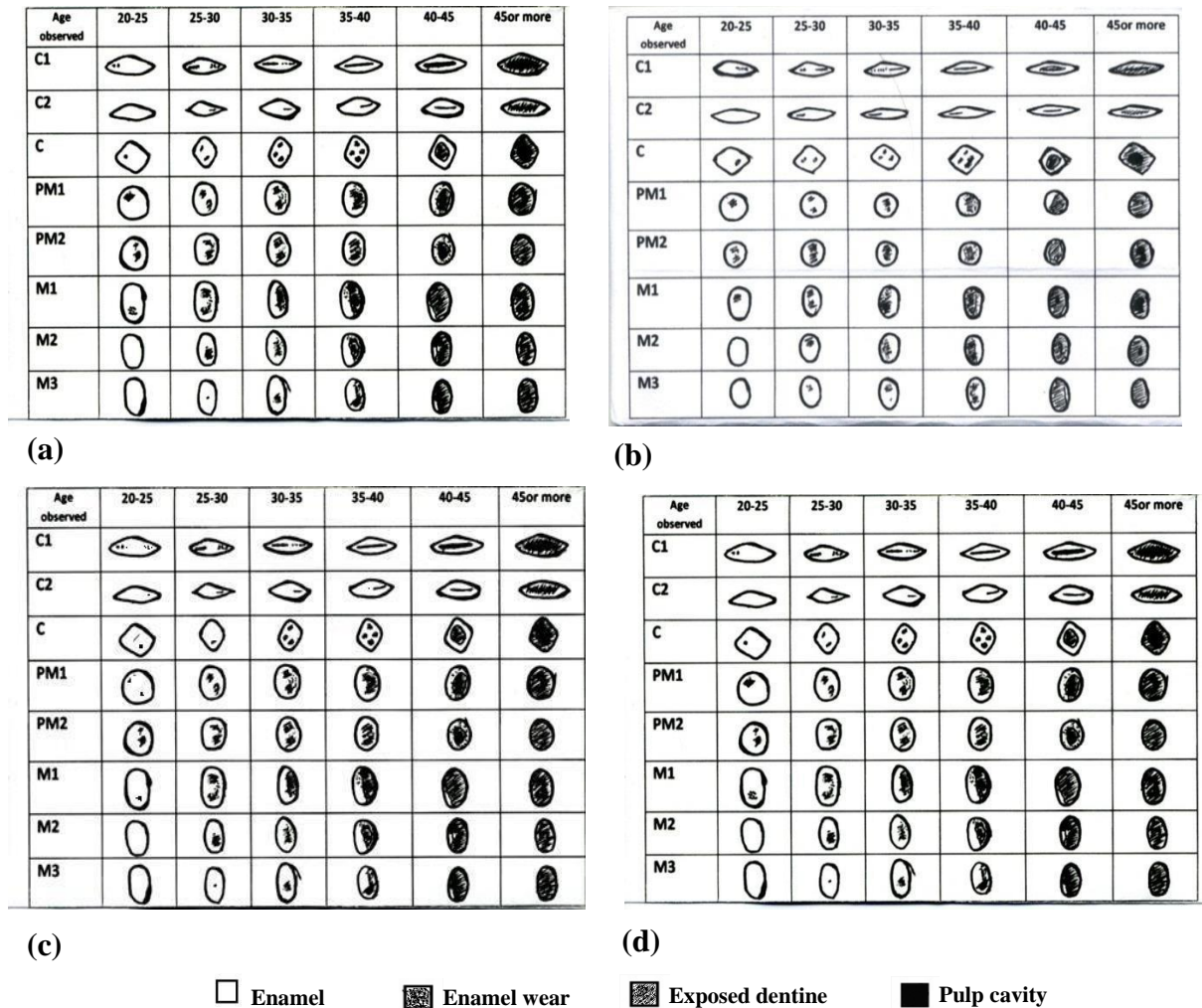


Figure 26. Age-related changes in all teeth (incisors, canines, premolars and molars) across all parts of the mouth; (a) age categories (right lower), (b) 7 age categories (left lower), (c) age categories (right upper), and (d) age categories (left upper)

The development of a new table in Figure 27 has been made. The data came from 419 human participants whose age is unknown and forty of them hailed from Africa, while the rest hailed from Europe. This data was utilised in successful estimation of the ages of the participants.

Age observed	20-25 years	25-30 years	30-35 years	35-40 years	40-45 years	45 or more years
C1						
C2						
C						
PM1						
PM2						
M1						
M2						
M3						

Enamel
 Enamel wear
 Exposed dentine
 Pulp cavity

Figure 27. Average estimation of the age using dental wear

Finally, the integration of the data derived from the first four tables have been made for the production of a complete average picture of the age-related changes in every tooth (incisors, canines, premolars and molars) across every part of the mouth as displayed in Figure 27. The intention is to utilise the presented chart in order to make it possible for the age estimation of an individual through comparison of his/her teeth and illustrations produced in the research of this study.

Data from 14 people of unknown ages from Libyan and England have been collected to estimate their ages using Figure 27 as a method of estimation. 71% of the estimated ages are in agreement with the real age of the participants and 29% not in agreement. In detail, the outcome of this study showed that according to the data obtained using Figure 27, four persons' ages were estimated to be in the range of 45 years old or above, which were in agreement with the actual ages taken after their teeth were analysed. The actual ages for them were 49 years old for two persons and (46 and 51 years) for the other two; one of them was not covered in the age range estimated. On the other hand, six other persons' ages were estimated to be between 20-25 years old, which were in agreement with their actual ages (21 years old for two persons, 24 years old for three persons and 26 years old for one person). One of them was clearly not covered in the age range estimated. Finally, four persons' ages were estimated to be between 30-35 years old when their actual ages were later known to be 36, 37, 32, and 34. Although,

two of them were clearly not covered in the age range estimated. However, this does not suggest that the method used is inaccurate because the reason behind the incorrect estimation obtained could be due to an error while performing the analysis. The results are presented in Table 13.

Table 13. Estimated age for fourteen people, (😊=agreement in a 5-year range, 😞=not agreement)

Real age	Sex	Estimated age	Agreement
21	Male	20-25	😊
21	Male	20-25	😊
22	Male	20-25	😊
24	Male	20-25	😊
24	Female	20-25	😊
26	Male	20-25	😞
32	Female	30-35	😊
34	Female	30-35	😊
36	Male	30-35	😞
37	Female	30-35	😞
46	Male	45-50	😊
46	Female	45-50	😊
49	Male	45-50	😊

51	Male	45-50	
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4.2 Age Estimation by Measurement of Open Apices in Libyan Population

To determine the reliability of the data gathered, an analysis was conducted and the concordance correlation coefficient was computed. The result revealed that there was no significant variation between paired sets of measurements.

In this study conducted on 307 individuals, sample I_{M3} scores were between 0.00 to 1.34 based on the age group. Distribution of real age gradually decreased as I_{M3} increased, in both boys and girls (see Figure 28)

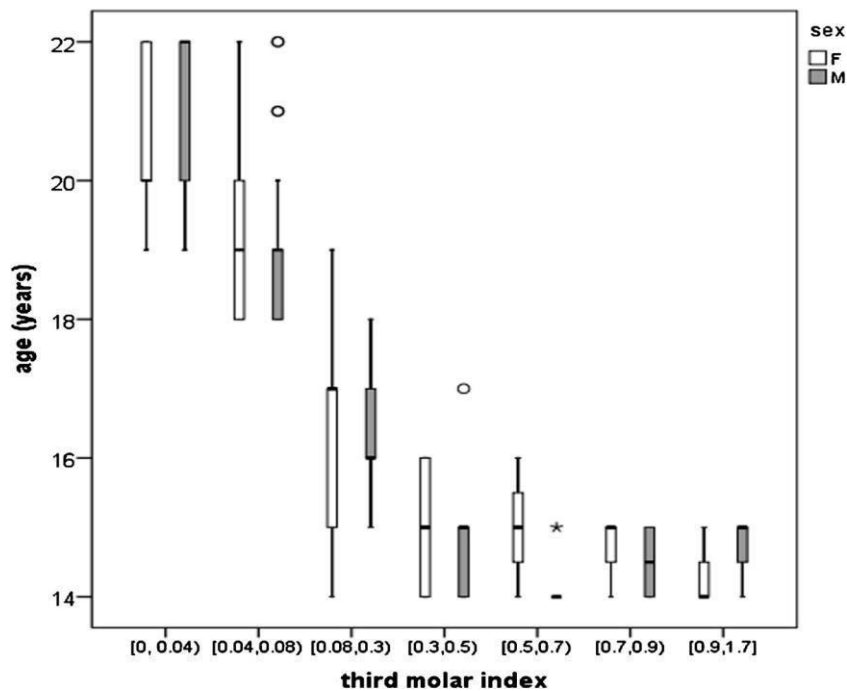


Figure 28. Relationship between age and Cameriere's third molar maturity index of open apices of the mandibular right third molar in Libyan females (white) and males (grey)

Table 14. chronological age statistics based on I_{M3} , number of individuals (N), average (AVG), mean standard deviation (SD), minimum (MIN), median (MED), maximum (MAX)

	N	AVG	SD	MIN	MED	MAX
Females						
(0.0, 0.04)	47	20.7	1.1	19	20.5	22
(0.04, 0.08)	40	18.9	1.0	18	19.0	22
(0.08, 0.3)	53	16.4	1.2	14	17.0	19
(0.3, 0.5)	14	15.0	0.8	14	15.0	16
(0.5, 0.7)	3	15.0	1.0	14	15.0	16
(0.7, 0.9)	3	14.7	0.6	14	15.0	15
(0.9, 1.7)	3	14.3	0.6	14	14.0	15
Males						
(0.0, 0.04)	42	21.1	1.0	19	22.0	22
(0.04, 0.08)	28	19.0	1.1	18	19.0	22
(0.08, 0.3)	45	16.4	1.0	15	16.0	18
(0.3, 0.5)	10	14.9	0.8	14	15.0	17
(0.5, 0.7)	12	14.1	0.3	14	14.0	15
(0.7, 0.9)	4	14.5	0.6	14	14.5	15
(0.9, 1.7)	3	14.7	0.6	14	15.0	15

The mean ages of the groups in each of I_{M3} differed between sexes, although the variations were not significant ($\chi^2 = 0.573$).

The results of the I_{M3} effectiveness are presented in 2-by-2 contingency tables (Table 15 and Table 16), listing the number of subjects with an I_{M3} of greater than or equal to 0.08 (who are younger than 18 years), I_{M3} of greater than or equal to 0.08 (who are more than 18 years of age), I_{M3} of less than 0.08 (who are less than 18 years of age), and I_{M3} of less than 0.08 (who are older than 18 years). The other two 2-by-2 contingency tables present the threshold value of 0.09 (Table 17 and Table 18).

Table 15. Discrimination performance indicating the test for males (when cut-off value is 0.08)

Test	>18	<18	Total
<0.08	70	0	70
>0.08	7	67	74
Total	77	67	144

Table 16. Discrimination performance indicating the test for females (when cut-off value is 0.08)

Test	>18	<18	Total
<0.08	87	0	87
>0.08	9	67	76
Total	96	67	163

Table 17. Discrimination performance indicating the test for males (when cut-off value is 0.09)

Test	>18	<18	Total
<0.09	74	0	74
>0.09	3	67	70
Total	77	67	144

Table 18. Discrimination performance indicating the test for females (when cut-off value is 0.09)

Test	>18	<18	Total
<0.09	94	0	94
>0.09	2	67	69
Total	96	67	163

4.3 Validation of Age Estimation in Libyan Population

In the Tripoli study, digital panoramic radiographs of 758 healthy subjects were recorded, with a minimum of 37 (23 years) and a maximum of 98 (19 years) individuals per age and sex, as shown in Table 19. As can be seen in Table 20 and Table 21, the effectiveness of I_{M3} is presented in two 2x2 contingency tables, which include the results for those who have $I_{M3} \geq 0.08$ and are below 18 years of age, those with $I_{M3} \geq 0.08$ who are over the age of 18, those

with $I_{M3} < 0.08$ who are under 18, and those with $I_{M3} < 0.08$ who are over 18. Similarly, Table 17 and Table 18 present the results for an I_{M3} cut-off of 0.09.

In Table 20, a close relationship between adult age and test positivity in the female group can be observed, where 203 out of 230 individuals were accurately classified. Accordingly, the sensitivity and specificity of the test for females was calculated at 88.2% and 96.5%, respectively, yielding a positive likelihood ratio of $LR = 25.7$ for the females. The results are displayed in Table 20. Similarly, Table 21 shows a trending relationship between adult age and the positivity of the test in males, when using an I_{M3} cut-off value of 0.8 (i.e. $I_{M3} < 0.08$). In this case, 148 out of 165 individuals were accurately classified. For these results, the sensitivity – proportion of individuals of 18 years of age or older whose test was positive – and specificity – proportion of individuals younger than 18 years whose test was negative – were 89.6% and 96.2%, respectively, leading to a positive likelihood ratio of $LR = 23.7$ for the males.

When a cut off of 0.09 is applied, it can be seen to improve the sensitivity for both, boys and girls, 89.6% to 93.3% for boys, as well as 88.2% to 96.9% for girls. Otherwise, for the specificity, no effects can be observed for the results for both girls and boys; it was 96.2 for girls and 96.5% for boys. The 0.09 cut-off improves the sensitivity more than specificity. Positive likelihood ratio is calculated for boys as $LR = 24.7$ and for girls as $LR = 28.2$, as shown in Table 22.

Table 19. Sample of panoramic radiographs from Tripoli (Libya) according to sex and age categories

Age (years)	F	M	Total
13	22	22	44
14	40	30	70
15	32	20	52
16	31	39	70
17	52	30	82
18	56	38	94
19	59	39	98
20	52	33	85
21	43	29	72
22	31	23	54
23-24	16	21	37

Table 20. Contingency table describing discrimination performance of the test for females in Tripoli (cut-off 0.08 and 0.09)

I_{M3}	>18 years	<18 years	I_{M3}	>18 years	<18 years	
<0.08	203	7	<0.09	223	7	
>0.08	27	197	>0.09	7	197	
	230	204	434	230	204	434

Table 21. Contingency table describing discrimination performance of the test for males in Tripoli (cut-off 0.08 and 0.09)

I_{M3}	>18 years	<18 years	I_{M3}	>18 years	<18 years	
<0.08	148	6	<0.09	154	6	
>0.08	17	153	>0.09	11	153	
	165	160	324	165	159	324

Table 22. Sensitivity, specificity and LR for boys and girls (Tripoli)

I_{M3} = 0.08	Sensitivity	Specificity	LR
Boys	0.89	0.96	23.76
girls	0.88	0.96	25.72
0.09			
Boys	0.93	0.96	24.73
girls	0.96	0.96	28.25

Table 23. Summary statistics of chronological age according to I_{M3} : number of individuals (N), average (AVG), mean standard deviation (SD), minimum value (MIN), median (MED), maximum value (MAX) of (Tripoli)

Male						
	N	AVERAGE	STDEV	MIN	MEDIAN	MAX
0.0,0.04	80	21.6	1.1	19	21.6	23
0.04,0.08	46	19.5	0.8	18	19.5	21
0.08,0.3	125	17.1	1.4	13	17.2	19
0.3,0.5	24	14.7	1.2	13	14.8	17
0.5,0.7	24	14.5	1.9	13	13.9	21
0.7,0.9	13	14.2	0.9	13	14.4	15
0.9,1.7	12	14.8	1.6	13	14.3	17
Female						
0.0,0.04	119	21.2	1.2	17	20.9	23
0.04,0.08	96	19.2	0.9	16	19.0	22
0.08,0.3	139	16.6	1.4	13	17.2	21
0.3,0.5	33	14.8	1.4	13	15.1	17
0.5,0.7	27	14.6	1.1	13	14.4	17
0.7,0.9	11	13.9	0.6	13	13.9	15
0.9,1.7	9	14.6	1.7	13	14.1	17

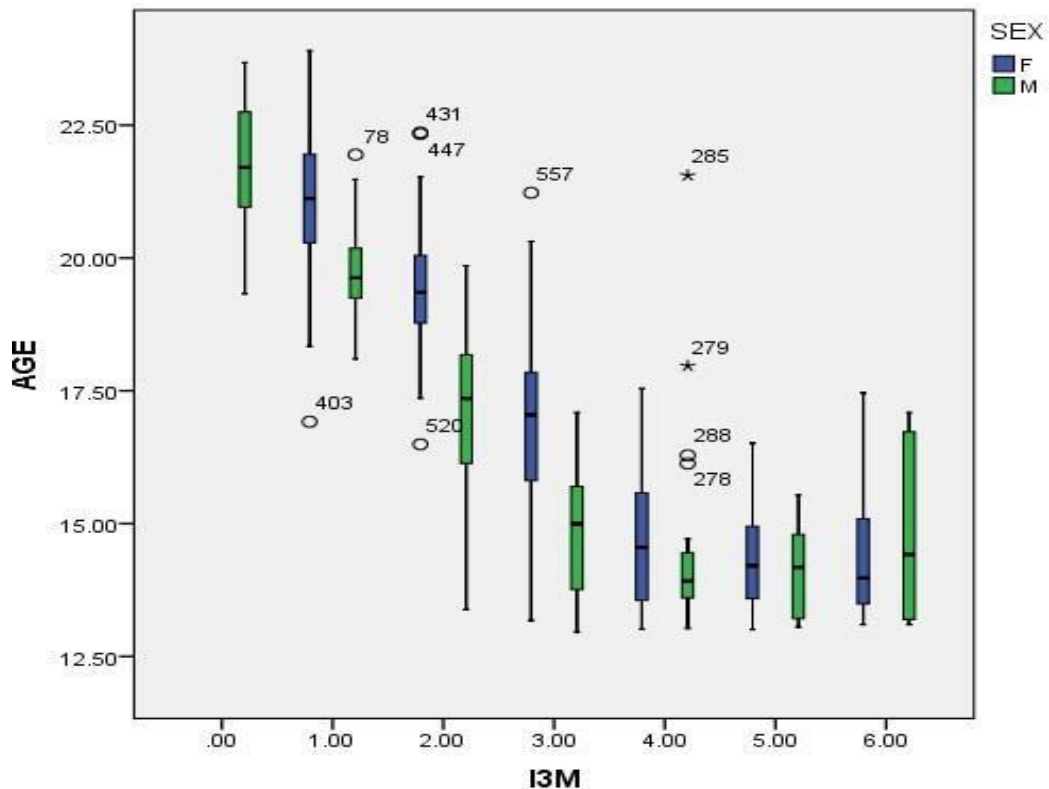


Figure 29. Relationship between age and Cameriere’ s third molar maturity index of open apices of the mandibular right third molar in Libyan females (Tripoli), (Blue) and males (Green)

In this part of study for Benghazi, digital panoramic radiographs of 160 Libyan healthy subjects were recorded, with a minimum of 6 (13 years) and a maximum of 22 (16, 18 years) individuals per age and sex, as shown in Table 24.

Similar to Tripoli sample, the results for the Benghazi sample are presented in two 2-by-2 contingency tables, as per Table 25, showing the numbers of those who have $I_{M3} \geq 0.08$ and are younger than 18, those with $I_{M3} \geq 0.08$ and over 18 years of age, those who have $I_{M3} < 0.08$ and are under 18, and those with $I_{M3} < 0.08$ who are over 18. The results for the I_{M3} cut-off of 0.09 are presented in Table 26.

In Table 25, a close relationship between adult age and the positivity of the test using the $I_{M3} < 0.08$ criterion can be found in males. Where 30 out of 33 individuals were accurately classified. The analysis results were calculated using a sensitivity and specificity of 90% and 100%, respectively, yielding a positive likelihood ratio of $LR = \infty$ for the males.

For completeness, the results for the female group are presented in Table 26, showing a close relationship between adult age and test positivity (i.e. $I_{M3} < 0.08$), where 37 out of 49 individuals were accurately classified. For the female group, the sensitivity and specificity measures were 75% and 100%, yielding a positive likelihood ratio of $LR = 8.91$ for the females.

The results for the I_{M3} cut-off of 0.09 of all individuals were accurately classified for males and for females, where 46 out of 49 individuals were accurately classified, leaving only 3 unclassified out of the range.

Table 24. Sample of panoramic radiographs from Libya (Benghazi) according to sex and age categories

Age (years)	F	M	Total
13	4	2	6
14	7	7	14
15	9	8	17
16	12	10	22
17	6	13	19
18	13	9	22
19	12	4	16
20	8	6	14
21	7	4	11
22	7	5	12
23-24	2	5	7
Total	87	73	160

Table 25. Contingency table describing discrimination performance of the test for males in Benghazi (cut-off 0.08 and 0.09)

I_{M3}	>18 years	<18 years	I_{M3}	>18 years	<18 years
<0.08	30	0	<0.09	33	4
>0.08	3	40	>0.09	0	36
	33	40		33	40
		73			73

Table 26. Contingency table describing discrimination performance of the test for females in Benghazi (cut-off 0.08 and 0.09)

I_{M3}	>18 years	<18 years	I_{M3}	>18 years	<18 years
<0.08	37	0	<0.09	46	4
>0.08	12	38	>0.09	3	34
	49	38	87	49	38
					87

Table 27. Sensitivity, specificity and LR for boys and girls (Benghazi)

I_{M3} = 0.08	Sensitivity	Specificity	LR
Boys	0.90	1	
girls	0.75	1	8.91
0.09			
Boys	1	0.9	10
girls	0.93	0.89	8.91

Table 28. Summary statistics of chronological age according to I_{M3}: number of individuals (N), average (AVG), mean standard deviation (SD), minimum value (MIN), median (MED), maximum value (MAX) of (Benghazi)

Female						
	N	AVERAGE	STDEV	MIN	MEDIAN	MAX
0.0,0.04	21	21.8	1.3	19	21.6	23
0.04,0.08	9	18.7	0.6	18	18.5	19
0.08,0.3	27	17.1	0.7	16	17.3	18
0.3,0.5	6	15.0	0.3	14	15.9	15
0.5,0.7	5	14.9	0.5	14	14.9	15
0.7,0.9	5	14.3	0.8	13	14.4	15
0.9,1.7	0	0	0	0	0	0
Male						
0.0,0.04	23	21.6	1.1	19.4	21.9	23
0.04,0.08	19	19.3	0.7	18.2	19.3	20
0.08,0.3	29	17.1	0.9	15.2	16.9	19
0.3,0.5	8	14.9	0.9	13.4	14.6	17

0.5,0.7	4	14.9	0.5	14.5	14.9	15
0.7,0.9	3	13.8	0.6	13.3	13.6	14
0.9,1.7	1	13.9	-	13.9	13.9	14

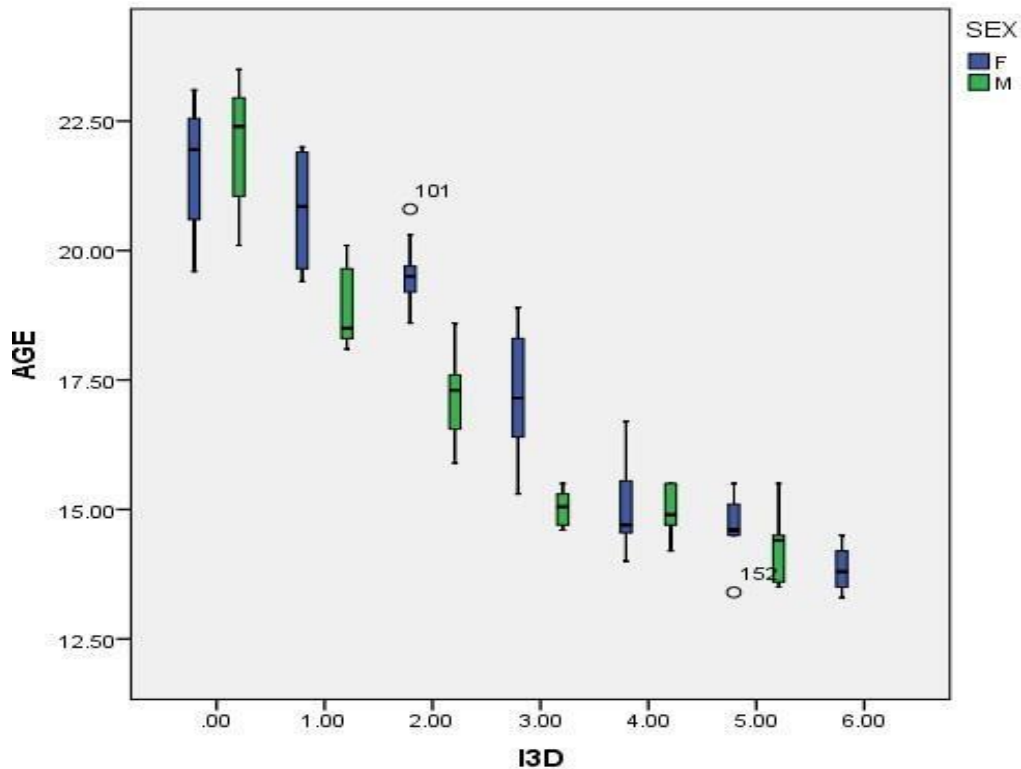


Figure 30. Relationship between age and Cameriere’s third molar maturity index of open apices of the mandibular right third molar in Libyan females (Benghazi), (Blue) and males (Green)

In order to form a comparative study amongst the 918 healthy Libyan subjects, a minimum of 44 (23 years) and a maximum of 116 (18 years) individuals were examined and the results, as per age and sex, are presented in Table 29. The combined results for both Tripoli and Benghazi samples are presented in two 2-by-2 contingency tables, as shown in Table 30 for males and in Table 31 for females, which list the numbers of individuals who have $IM_3 \geq 0.08$ and are younger than 18, those with $IM_3 \geq 0.08$ who are over 18, those with $IM_3 < 0.08$ who are under 18, and those with $IM_3 < 0.08$ who are over 18. The results obtained by using an IM_3 cut-off of 0.09 for males and females are also presented in Table 30 and Table 31, respectively.

Table 30 demonstrates a close relationship between adult age and test positivity in males using an IM_3 cut-off of 0.08, where 177 out of 197 individuals were accurately classified. Subsequently, based on a sensitivity and specificity of 89% and 96%, respectively, a positive likelihood ratio of $LR = 25.6$ was calculated for the males. Similarly, Table 31 shows the

relationship between adult age and test positivity for the female group, where using the $I_{M3} < 0.08$ threshold, 240 out of 279 individuals were accurately classified. In this case, the sensitivity and specificity for the female group were 86% and 97%, respectively, resulting in a positive likelihood ratio of $LR = 29.7$.

If a cut-off value of 0.09 is applied, an improvement in the sensitivity for both boys and girls can be seen, i.e. from 86.0% to 96.4% for girls and 89.8% to 94.4% for boys. In case of specificity, the cut-off value of 0.09 as made no improvements for both girls and boys, i.e. 97.1% to 95.4% for girls and 96.5% to 94.9% for boys. According to these results, it can be said that the 0.09 cut-off improves the sensitivity more than specificity. This is proven by the positive likelihood ratio that is calculated for boys $LR = 18.7$ and for girls $LR = 21.21$, as shown in Table 32.

Table 29. Sample of panoramic radiographs from Libya (Tripoli and Benghazi) according to sex and age categories

Age (years)	F	M	Total
13	26	24	50
14	47	37	84
15	41	28	69
16	43	49	92
17	58	43	101
18	69	47	116
19	71	43	114
20	60	39	99
21	50	33	83
22	38	28	66
23-24	18	26	44
Total	521	397	918

Table 30. Contingency table describing discrimination performance of the test for males in Tripoli and Benghazi (cut-off 0.08 and 0.09)

I_{M3}	>18 years	<18 years	I_{M3}	>18 years	<18 years	
<0.08	177	7	<0.09	187	10	
>0.08	20	193	>0.09	11	189	
	197	200	397	198	199	397

Table 31. Contingency table describing discrimination performance of the test for females in Tripoli and Benghazi (cut-off 0.08 and 0.09)

I_{M3}	>18 years	<18 years	I_{M3}	>18 years	<18 years	
<0.08	240	7	<0.09	269	11	
>0.08	39	235	>0.09	10	231	
	279	242	521	279	242	521

Table 32. Sensitivity, specificity and LR for boys and girls (Tripoli and Benghazi)

I_{M3} = 0.08	Sensitivity	Specificity	LR
Boys	0.89	0.96	25.67
girls	0.86	0.97	29.73
0.09			
Boys	0.94	0.94	18.79
girls	0.96	0.95	21.21

Table 33. Summary statistics of chronological age according to I_{M3}: number of individuals (N), average (AVG), mean standard deviation (SD), minimum value (MIN), median (MED), maximum value (MAX) of (Tripoli and Benghazi)

Male						
	N	AVERAGE	STDEV	MIN	MEDIAN	MAX
0.0,0.04	101	21.6	1.1	19	21.6	23
0.04,0.08	84	19.0	1.0	16	19.0	21
0.08,0.3	123	16.7	1.2	13	16.9	19
0.3,0.5	31	14.7	1.1	12	14.9	17

0.5,0.7	28	14.6	1.7	13	14.0	21
0.7,0.9	18	14.2	0.8	13	14.4	15
0.9,1.7	12	14.7	1.6	13	14.3	17
Female						
0.0,0.04	142	21.1	1.2	16	21.0	23
0.04,0.08	115	19.2	0.9	16	19.1	22
0.08,0.3	168	16.7	1.4	13	16.9	21
0.3,0.5	41	14.8	1.3	13	14.7	17
0.5,0.7	31	14.6	1.0	13	14.6	17
0.7,0.9	14	13.9	0.6	13	13.7	15
0.9,1.7	10	14.5	1.6	13	13.9	17

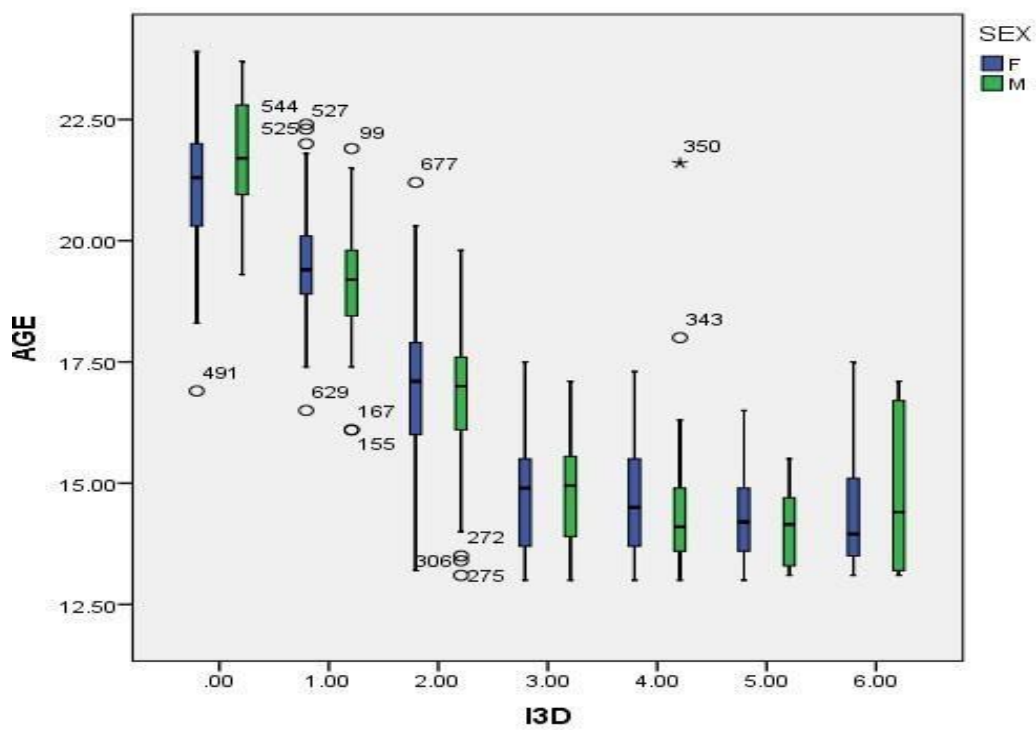


Figure 31. Relationship between age and Cameriere's third molar maturity index of open apices of the mandibular right third molar in Libyan females (Blue) and males (Green), (Tripoli and Benghazi)

Table 34. Shows a sensitivity, specificity and likelihood ratio (LR) in Libya (Tripoli and Benghazi) for both males and females

	Boys ($I_{M3} = 0.08$)			Girls ($I_{M3} = 0.08$)		
	Sensitivity	Specificity	LR	Sensitivity	Specificity	LR
LIBYA T	0.89	0.96	23.76	0.88	0.96	25.72
LIBYA B	0.90	1		0.75	1	
LIBYA T+B	0.89	0.96	25.67	0.86	0.97	28.66
	Boys ($I_{M3} = 0.09$)			Girls ($I_{M3} = 0.09$)		
LIBYA T	0.93	0.96	28.25	0.96	0.96	28.25
LIBYA B	1	0.9	10	0.93	0.89	8.91
LIBYA T+B	0.94	0.94	18.79	0.96	0.95	21.21

Table 34 present the sensitivities, specificities and likelihood ratios as obtained with $I_{M3} = 0.08$ and $I_{M3} = 0.09$ thresholds. It is apparent that the $I_{M3} = 0.09$ criterion has led to an increase in sensitivity and a partial decrease in specificity for both boys and girls.

Table 35 presents the I_{M3} measurement results as used in classification of a subject as an adult in different Mediterranean countries and one South American country (Brazil). The results present a sensitivity range of 0.89-1 for boys and 0.75-1 for girls, while the specificity for boys can be seen to have a range of 0.86-1 for both sexes. This is in line with the results found in both Libyan populations (i.e. Tripoli and Benghazi).

Table 35. Presents a sensitivity, specificity and likelihood ratio (LR) in different Mediterranean countries and one South American country (Brazil)

	Boys ($I_{M3} = 0.08$)			Girls ($I_{M3} = 0.08$)		
	Sensitivity	Specificity	LR	Sensitivity	Specificity	LR
LIBYA T	0.89	0.96	23.76	0.88	0.96	25.72
LIBYA B	0.90	1		0.75	1	
LIBYA T+B	0.89	0.96	25.67	0.86	0.97	28.66
ITALIAN	0.95	0.86	6.785714	0.95	0.86	6.785714
CROATIAN	0.91	0.91	10.111111	0.84	0.95	16.8
BRAZILIAN	0.87	0.86	6.214286	0.86	0.67	2.606061
ALBANIAN	0.9	0.94	15	0.84	0.75	3.36
TURKEY	1	0.86	7.142857	1	0.96	25

4.4 Age Estimation of Libyan Children by Teeth Open Apices

The aim of this chapter is to assess a method for estimating the age of children based on their teeth. To this end, a sample of living children from Tripoli, the capital of Libya with a diversity of ethnic groups, is tested and analysed.

In this study, carried out on 319 Libyan healthy subjects, a minimum of 16 (4-5 years) and a maximum of 94 (12-13 years) individuals were studied. The age and sex distribution of the sample studied for boys and girls are shown in Table 36 and Appendix 2.

Table 36. Age and sex distribution of the sample studied

Age (years)	Male	Female	Total
4 - 5	9	7	16
6 - 7	14	17	31
8 - 9	18	28	46
10 - 11	36	37	73
12 - 13	44	50	94
14 - 15	27	32	59
Total	148	171	319

The results show that sex (χ^2) and the variables χ^2 (second premolar), χ^2 , χ^2 and the first order interaction between χ^2 and χ^2 contributed significantly to the fit. Thus, only these variables were included in the regression model, yielding the following linear regression formula:

$$\text{Age} = 9.412 - 0.284\chi^2 + 0.996\chi^2 + 0.670\chi^2 - 0.942\chi^2 - 0.067\chi^2\chi^2$$

This best fitting formula can be used for Libyan population age estimation in the age range of 4-15. This formula has been produced for the Libyan population, however, one formula has already been produced by Cameriere for the Italian population and one for the European population. We compare the estimation performance of the proposed formula in this thesis with the Italian and European population as published by Cameriere's Formulae.

Repeated Measures ANOVA analysis has been used to compare the real age with the estimated ages obtained, applying two different formulae suggested by Cameriere for the Italian population as (Cameriere, Ferrante et al. 2006):

$$\text{Age} = 8.971 - 0.375\chi^2 + 1.631\chi^2 + 0.674\chi^2 - 1.034\chi^2 - 0.176\chi^2\chi^2$$

and for the European population as (Cameriere et al., 2007b):

$$\text{Age} = 8.387 - 0.282\chi^2 + 1.692\chi^2 + 0.835\chi^2 - 0.116\chi^2 - 0.0139\chi^2\chi^2$$

The obtained χ^2 value of 0.000 indicates a difference among the estimated and the real ages. In order to detect which estimation is different from the others, a paired samples T test has been repeated with the following results; the resulted χ^2 value for Libya formula was $\chi^2 = 0.994$, indicating no statistically difference between the real age and the estimated age. Also, the

resulted χ^2 value for the European formula $\chi^2 = 0.090$ indicates no statistically difference between the real age and the estimated age. However, the value obtained for the Italian formula provided a $\chi^2 = 0.000$, indicating a statistically significant difference between the real age and the estimated age.

In addition, the correlations value for Italian samples is 0.952, for the European sample it is 0.931 and for the Libya samples it came out as 0.963. The χ^2 for Libyan population is 0.927, which is higher than the χ^2 obtained applying the other formulae. This clearly indicates that it is the best approximation for the Libyan population (see Figure 32, Figure 33 and Figure 34).

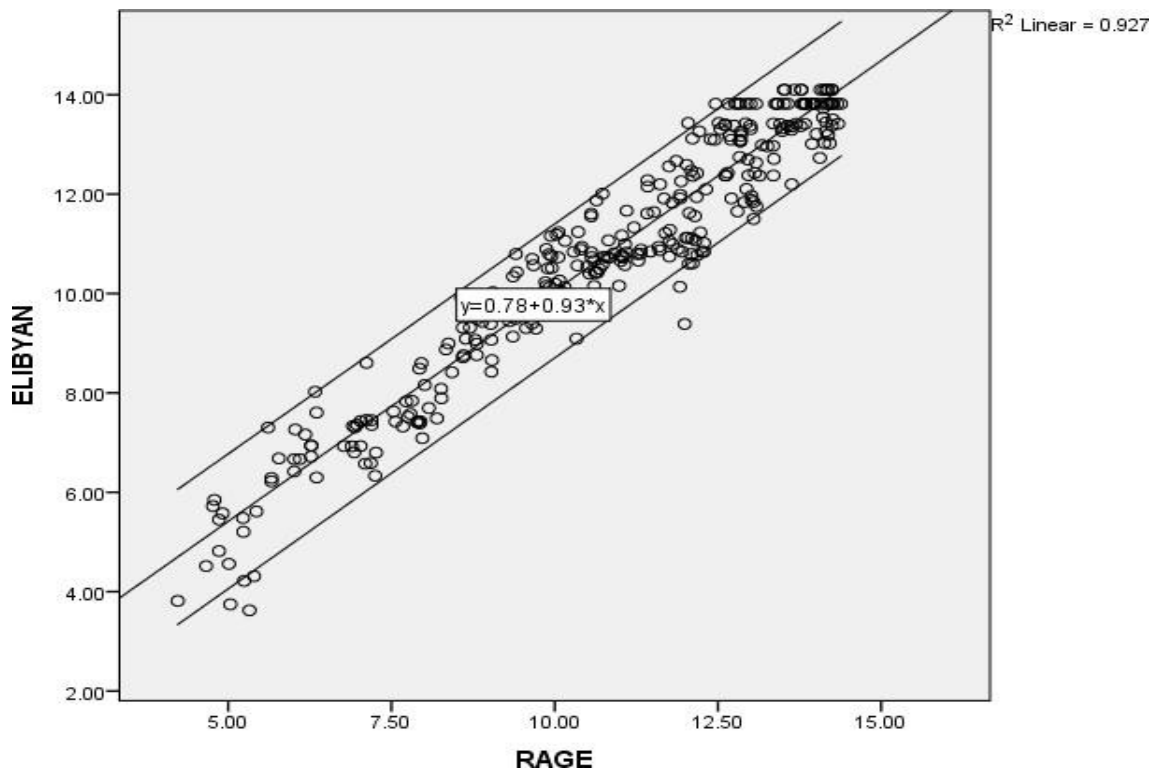


Figure 32. Plots of real age (RAGE, years) vs estimated age (ELIBYA, years) for the Libyan sample

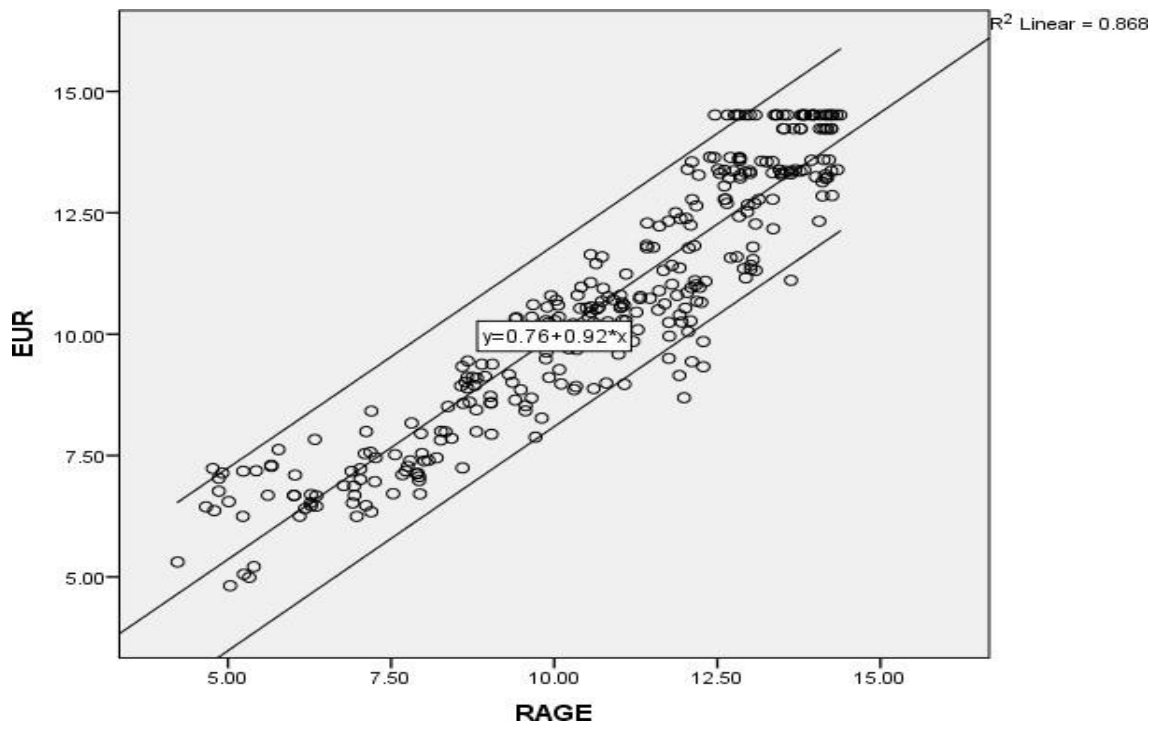


Figure 33. Plots of real age (RAGE, years) vs age based European formula (EUR, years)

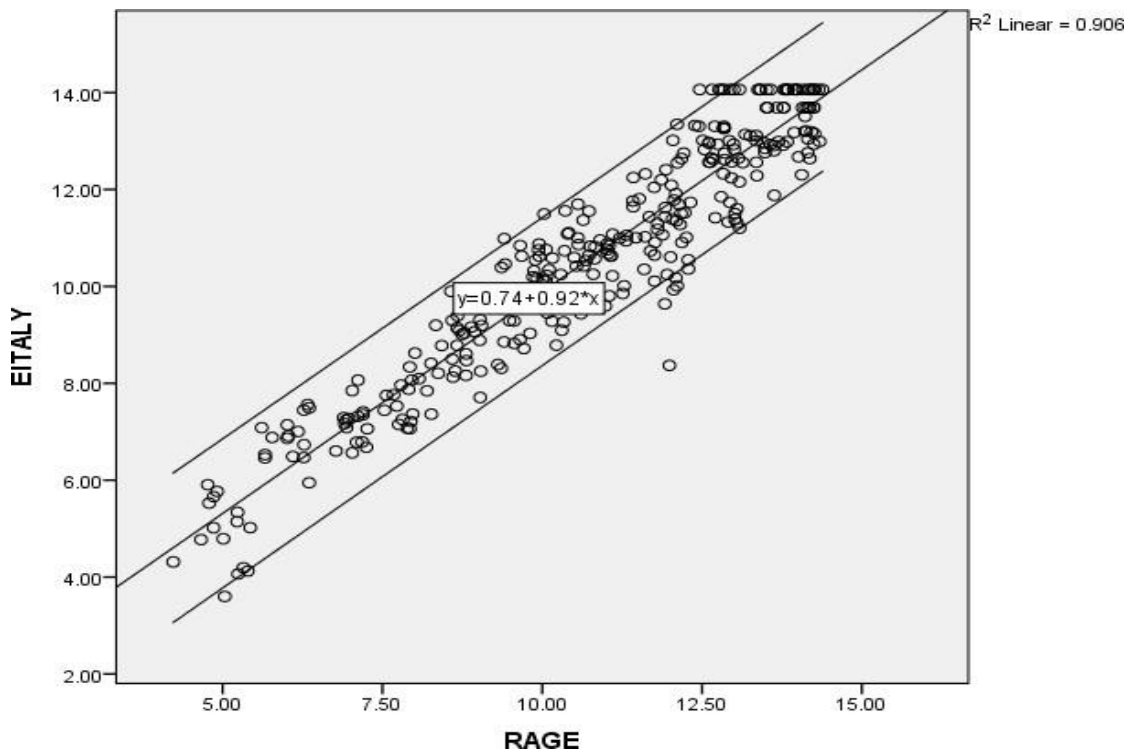


Figure 34. Plots of real age (RAGE, years) vs Italian estimation age (EITALY, years)

In Table 37 it can be seen that the mean willingness score for participants in the perceived relationship condition is 0.91 for males, and 0.96 for females. In addition, it can be seen from the standard deviations that the variation in data is wider for males (SD = 0.068) than females (SD = 0.054), and the number of participants in each group is seven for both males and females.

In the Table 38 variables, the F value for the test is 0.895 with a Sig. (p) value of .363 ($p < .001$). Because the Sig. value is less than our alpha of .05 ($p < .05$), we reject the null hypothesis, concluding that there is not a significant difference between the two groups' variances (males and females).

Table 37: Group Statistics

sex		N	Mean	Std. Deviation	Std. Error Mean
R	male	7	0.9136	0.06850	0.02589
	female	7	0.9573	0.05451	0.02060

Table 38: Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
R	Equal variances assumed	0.895	0.363	-1.320	12	0.211	-0.04368	0.03309	-0.11578	0.02841
	Equal variances not assumed									

The TEM, VAV, rTEM and R value results of the third molar measurements are presented in Table 39, showing higher or equal values for males than females: TEM, VAV, rTEM and R for males are 0.042, 0.027, 15.25 and 0.95; for females, they are 0.039, 0.027, 14.2 and 0.94, respectively.

Table 39: The Third Molar measurements, TEM,VAV, rTEM and R and values for Males and Females.

Third Molar	Male	Famle
TEM	0.043392	0.038821
VAV	0.284385	0.271049
rTEM	15.25817	14.32264
R	0.961858	0.937563

The TEM, VAV, rTEM and R value results for the seven mandibular teeth measured in children are presented in Table 40 for females and Table 41 for males. Our results suggest that the parameters evaluated are sufficiently precise. However, periodical training is necessary to control and minimize the anthropometric measurement error.

Table 40 : The seven mandibular teeth measurements, TEM,VAV, rTEM and R and values for Females

Female	Central incisor	Lateral incisor	Canine	First Premolar	Second Premolar	First Molar	Second Molar
TEM	0.011295	0.010447	0.031358	0.033636	0.031565	0.017779	0.131125
VAV	0.046552	0.087154	0.220733	0.23654	0.351171	0.082882	0.710419
rTEM	24.2639	11.98673	14.20647	14.22018	8.98858	21.45155	18.45739
R	0.801757	0.91912	0.970506	0.96824	0.985702	0.850319	0.899847

Table 41 : The seven mandibular teeth Molar measurements, TEM,VAV, rTEM and R and values for Males.

Male	Central incisor	Lateral incisor	Canine	First Premolar	Second Premolar	First Molar	Second Molar
TEM	0.002029	0.012282	0.0146	0.021564	0.014289	0.014169	0.123233
VAV	0.013993	0.046355	0.10296	0.127337	0.191115	0.063632	0.494127
rTEM	14.49798	26.49479	14.17993	16.93449	7.476647	22.26694	24.93946
R	0.989731	0.954337	0.968763	0.96912	0.994176	0.987188	0.837947

5 Discussion

5.1 Age Estimation by Dental Wear and Shading

Ajmal et al. (2001) reiterated that forensic odontology makes the use of age estimation derived from teeth an essential part in the identification field. Tooth wear is an ageing physiological procedure where certain changes can be seen directly while others can only be seen through a microscope. Ajmal et al. (2001) further added that compared to other body tissues, there is a closer relation between teeth and age. Researchers utilised a number of methods in estimating the age of individuals. The level of tooth wear investigation can be added as one of them. Besides, its advantage is the fact that it is considered to be an uncomplicated method.

The author, in the study, utilised two methods; the use of scores in dental wear and the use of shading. Scoring is actually a tooth wear index modification derived from (Smith and Knight, 1984). It comprises six dental wear levels, and it makes it possible for the researcher to obtain more accurate information. This method is more reliable and effective when compared to the use of the shading method. A few factors were taken into consideration during the examination process. Such factors include mainly the individual's sex and geographical location, together with environmental conditions and eating and chewing habits.

This researcher conducted investigation on two different ethnic groups of Europe and Africa. These two ethnic groups hail from two environmental conditions that are completely different,

as well as having different eating and drinking habits (Bartlett et al., 2011). These factors are not insignificant in affecting the level of tooth wear. The possibility of such difference influencing the developed results and system is quite high. Thus, carrying out comparable research, in this case, for these ethnic groups for the purpose of establishing separate shading for age estimation of African individuals, as well as European individuals separately would be quite fascinating.

The fact of the enamel on the front teeth being thicker compared to that of the back teeth is included in the factors that can affect accuracy. There is, in other words, a decrease in enamel from front to back (Brothwell, 1981). Thus, when an individual is asleep on his/her side with saliva and other chemicals coming from the diet, there is the possibility of him/her having an effect on the molars' surface wear during sleep, thereby making the possibility of age estimation, based on the results of wear, less precise (Brothwell, 1981).

The ages of some people, as mentioned above, were estimated to be between 20-25, 30-35, and 45-50 years old, while their real ages were later revealed to be 26, 36, 37 and 51 years. Although there was no clear coverage of them in the estimated age range they were, however, quite close. Besides, this is not a suggestion that there was inaccuracy in the method utilised, since human error could be the reason behind the inaccurate estimation obtained while undergoing analysis.

This study's results suggest that with people's increasing age, tooth wear in each and every tooth level rises as well. This result is applicable to males and females. Scores of tooth wear in females, with regards to this current study, are lower compared to males. This result is in line with what is stated by Pöllmann et al. (1987), who explain that it is a consequence of better developed chewing muscles in males, and therefore, they can generate a stronger bite force in comparison to females. Bartlett et al. (2011) further states that such is the result of better development in the chewing muscles of males, that men have the ability to generate a stronger bite force compared to that of females. Bartlett et al. (2011) reiterated that there is an influence of eating and drinking habits on the degree of tooth wear. It is worth mentioning that the results of Pöllmann et al. (1987)'s work will only provide a certain age range with some intervals, and not an accurate estimate for the individual's age. In this thesis, the researcher's aim is to achieve an age range estimate as narrow as possible, in order to have the greatest accordance accomplished.

5.2 Age Estimation by Measurement of Open Apices in Libyan Population

Since 2013, the Central and Eastern Mediterranean has remained the main path for illegal immigrants when moving to Europe. Uncontrolled immigration involves even minors from South Asian and Saharan African countries who may not have been registered after birth. In fact, in most of the developing nations, only 50% of the children who are below five years are registered after birth (Cavrić et al., 2016). It is reported that in South Asia 65% of births go unregistered while in sub-Saharan Africa about 64% are not registered (Cavrić et al., 2016).

In addition, the civil war in Libya has significantly increased the number of migrants and asylum-seekers crossing the Mediterranean from Libya (Toaldo, 2015). The impacts of having unaccompanied children moving alone can be at times monumental. Their invisibility makes them more vulnerable and having children moving without legal documents makes it difficult to establish their age and they are at times treated as adults (Cavrić et al., 2016), especially in the legal processes. If a child is treated as an adult during the legal process, it places them at increased risk of abuse. In addition, they can be recruited into fighting forces (Mohammed et al., 2015), be a victim of hazardous work or abused or forced into early marriage. The EU, in reference to ensuring that the rights of children are respected, is working towards establishing an accurate means of estimating the age of unaccompanied children moving to Europe. Rights of the child entail universally agreed obligations and standards providing the minimum freedoms and entitlements, which should be respected by all governments. They are restricted to be applicable to individuals who are under 18 years unless (Black et al., 2010). This implies that failing to identify a minor as a child will hinder that individual's opportunity to enjoy children's rights set in the convention. This will affect their development, care and protection. Following this, it is important for the state parties to treat age assessment subjects with sensitivity and due diligence.

Realistic age estimation is crucial in ensuring that juveniles and children are identified and treated properly. The approaches to estimating age can be weakened by errors (ethically and technically unacceptable errors) (Garamendi et al., 2005). Following this, it is vital to reduce these errors including classifying adults as minors or classifying minors as adults. The errors are evident in the criminal responsibility of the minors. (see Table 1)

Cameriere et al. (2008) developed a practical approach to estimating age using age and third molar index correlation, by measuring the third molar open apices (Cameriere et al., 2008b). A cut-off value of $I_{M3} = 0.008$ was proposed. The specificity and sensitivity were 98% and 70%

respectively. The fact that 83% of the people were correctly classified (Deitos et al.,1976), it was demonstrated that the approach is appropriate for estimating adulthood for forensic purposes in Brazil, even though it needed to be applied judiciously and carefully. 444 panoramic radiographs were analysed, which showed a specificity of 85% and sensitivity of 78.3%. The correct classification was 87% (Deitos et al., 2015). Currently, the validity of the cut-off value in the identification of children and adults has been tried in several populations. Despite the existence of other approaches, which are based on bone or teeth developments or the combination of the two, the cut of 0.08 has shown consistency in different populations (Cameriere et al., 2008b).

In the present research, third molar development of a sample of 307 individuals (144 boys and 163 female) with their ages ranging from 14 to 22 years were studied through measuring the third molar maturity index. The outcomes highlighted the significance of the conducted analysis. 94.5% was a correct classification for girls and 95.1% for boys. The test sensitivity was 90.9 percent for boys and 9.06% for girls while the specificity was 100% for both sexes. Specificity entails the chance that the test will give a true negative result. In this study, specificity of 100% was noted implying absence of false negative in the diagnostic test in all individuals.

Garamendi et al. (2005) discussed the aspect of combination of results scientifically and ethical dilemmas caused by the statistical variability when applying medical approaches in age assessment. They claimed that in forensic age diagnosis, even though it is critical to minimize technically unacceptable errors, the most vital thing is to ensure that there are no unethically unacceptable errors. Following this, a combination of dental and skeletal approaches should be applied in ruling out the appearance of false positive results, as it will be on the expense of raising false negative results. In this case, the reported error for females was 5.5% and 4.9% for males.

5.3 Validation of Age Estimation in Libyan Population

With reference to a number of recent published studies and guidelines, a multidisciplinary evaluation is deemed the most appropriate approach to human identification and ageing, where at the same time, a physical (e.g. height and weight growth) and dental assessment of puberty and growth are undertaken, including sexual (e.g. pubic hair or breast development), cognitive, behavioural and emotional assessments (Aynsley-Green et al., 2012, Whaites and Drage, 2013). However, this technique lacks of published evidence to prove its validity.

On the other hand, the third molar develops from mid-teens to early adulthood and the complete closure of the apices of the third molar teeth can be used as an accurate indication of the subject's age (i.e. ≥ 18 years) and, thus, prove whether they are an adult by definition or not. In order to identify a Libyan subject as a minor or adult, in this study, the third molar maturity index (I_{M3}) has been calculated and the accuracy of the estimated age was statistically verified. The criteria for the assessment was set as $I_{M3} = 0.08$, where an individual with an I_{M3} of 0.08 or less was considered to be at least 18 years of age and classified as a minor. Since the timing of the third molar development is sex-dependent, a separate analysis has been conducted on male and female groups. Although third molar root development is highly variable in both sexes and entails issues inherent to using a single indicator as an age estimation. The work of Bassed et al. (2011) has shown that, still, the development of the third molar could serve as a useful indicator for identifying whether an individual is aged under or over 18 years (Bassed et al., 2011).

In their study, Cameriere et al. determined that the cut-off value of I_{M3} for adult age identification of 0.08, has sensitivity and a specificity of 70% and 98%, respectively. Using these boundaries, the proportion of individuals correctly classified was 83%. This approach was also utilised with an I_{M3} cut-off of 0.08 to test samples from Albania and Croatia (Cameriere et al., 2014, Galić et al., 2015), Serbia (Zelic et al., 2016), Botswana (Cavrić et al., 2016) and Colombia (De Luca et al., 2016). These studies proven that the I_{M3} value can be a useful age indicator in countries outside Europe.

Presently, the I_{M3} cut-off limit of 0.08 for adult age estimation has been verified on samples from several populations, mainly Caucasian and South American. Now, despite the availability of other age estimation methods, which may involve investigation of the development of teeth, bones or perhaps both parameters considered (Cameriere et al., 2012b), it has been apparent that an I_{M3} cut-off value of 0.08 can produce consistent results amongst different populations, given a global criterion for adult age evaluation is used (i.e. under or over 18 years). In regard to dental age estimation on a Libyan population, Putul and Azza (2013) have authored the only known study in the field. In their work, Putul and Azza performed an analysis on the subjects' chronological ages based on their third molar eruption and compared the results with Egyptians (Mahanta and Mohamed, 2013). It was revealed that the earliest third molar eruption was in the females group at 16 years of age, and the eruption completed at 23 years of age in both sexes.

In this study, a sample of 918 healthy living Libyan subjects (521 females and 397 males), aged between 14 and 23, was used to analyse the third molar development by assessment of I_{M3} . The obtained results highlighted the significance of the I_{M3} -based approach to adult age estimation, as 86.4% of the females and 89% of the males were correctly classified. It was also shown that, by using an I_{M3} cut-off value of 0.09 instead of 0.08, an increase of around 3% was experienced in the numbers of individuals correctly identified in. The sensitivity of the test was 96% for the females and 94% for the males, while for both sexes a partial decrease in specificity was observed.

Thus far, the sensitivity, specificity and likelihood ratio of the results for two samples of the Libyan population (i.e. Tripoli and Benghazi) have been evaluated both separately and combined together.

5.4 Age Estimation of Libyan Children by Teeth Open Apices

In the last years, a large number of illegal immigrants have used the Central Mediterranean countries such as Libya, Italy, Malta and Tunisia and also the countries from the Eastern Mediterranean area such as Greece, Turkey and Egypt, as the main path towards their destination, Europe (Frontex, the European Agency for the Management of Operational Cooperation at the External Borders of the Member States of the European Union, www.frontex.europa.eu). An issue related to the immigration problem concerns to non-registered children. As data suggests, only half of the children under five years old in the developing countries have their births registered (UNICEF, 2010:44). Moreover, the civil war in Libya has increasingly accelerated the number of asylum seekers during recent years crossing the Mediterranean on makeshift boats organised by traffickers.

The official “invisibility” of unaccompanied children may, therefore, negatively impact their vulnerability. Children with no documents proving their real age may be treated as adults in legal processes, which increases the risk of abuse in a system (Singh et al., 2004). Reliable age estimation is, therefore, crucial to ensure that children are properly identified and treated. In this study, to obtain an estimated age in Libyan children aged between 4 and 15 years old, the measurements of the open apices of the seven left permanent mandibular teeth have been used.

Several methods and body parts, especially the teeth, are commonly used to indicate the age in both biological and forensic issues (Olze et al., 2005). In 2006, Cameriere and collaborators (Cameriere et al., 2006) investigated a new method for age estimation in children using

measurements of open apices in teeth. A new formula has been produced in this study for the Libyan population. However, age estimations based on dental methods have shortcomings; the biological variation is great and differences exist between populations.

Present results show that both Libyan and European formulae pose no statistical difference between the real age and the estimated age, however, the value obtained applying the Italian formula indicates a statistically significant difference between the real age and the estimated age. According to the result, it was found that the Libyan formula is the most accurate method compared with two methods tested in the present study, i.e. the Italian and European formulae for the Libyan population.

6 Conclusion

Age estimation has been defined as the process of using biometric, social and psychological features to determine the age of an individual. There are several methods that are used to estimate the age of a person, and admittedly, they are characterised by numerous problems. For example, age estimation performed by Hochschild's biological age method encounters problems in MLR. The biological age method for age estimation of a person poses challenges to the personnel because it is very complicated and is deemed to be substandard; hence, it cannot yield good results as expected. Aging biomarkers provided by the multiple linear regression mostly contradict the chronological age, thus, adversely affecting the age estimation (Guo and Mu, 2011). Nevertheless, the structural equation modelling as a method of age estimation is problematic in establishing the age of individuals because it does not give a comparison of biomarkers and also hides the main concept of biological age.

Moreover, there is the problem of qualified personnel and officers in the psychological and social evaluation because this approach calls for a clinician and a practitioner in social work with the needed training to carry out such evaluation. A lack of qualified officers makes it difficult to use the age estimation process because an underqualified officer would make the processes slow and costly. Klemra and Doubal's method poses problems in age estimation because it involves complicated and difficult calculations, which require highly qualified personnel who may not be available in most cases, thus, delaying the exercise or misinterpreting the results from the field.

The development of teeth commences during the sixth week of embryonic life. The development of teeth occurs 6 to 9 months after the birth of a child. It begins from the anterior

to the posterior area of the mouth. Permanent dentition begins at the age of 6 years, when the development of four molars starts. Between the age of 6 and 12 years, a person begins the development of permanent anterior teeth. The development of third molars that are often referred to as wisdom teeth begins between the age of 18 and 26. It is easy to estimate the age of an individual using the teeth since tooth development depends on the age of an individual mainly on a genetic basis. Dental wear is also used to estimate the age of individuals. This is because mechanisms such as abrasion and attrition make it easy to estimate the age of an individual (Black, 2011).

Therefore, the existence of teeth in an individual helps researchers of age to establish the estimated age of a person by considering the teeth sizes (Franklin 2010). Moreover, if a person is found to have all the teeth a normal person should have, then, that individual is considered to be an adult; hence, his or her age can be effectively estimated. The process of aging in human beings is defined by the incessant changes that occur in morphology and size and, therefore, it is logical to connect the changes of the skeleton and the age of a person. Development of the skeleton begins from mesenchyme and undergoes changes in the process of maturation, right from the connection of tissues in the embryo up to the adulthood. Creation of ossification centres, changes in sizes and morphology, as well as fusion of the ossification centres, are some of the fundamental methods of establishing and studying the age of a skeleton. Arguably, the ossification centres are visible from when an individual is born and during the first 10 years of life; therefore, it can be used to study the age of a skeleton. Commonly, the size of the skeleton of human beings is used in the estimation of the age of individuals in that the bones tend to change size as an individual continues to grow (Franklin, 2010). For instance, the bone structures are mostly used to mark the age of a young person. Moreover, the stages of development of bones in human beings are very critical in estimating the age of a skeleton. The cervical approach as a tool to estimate age is very important in determining the age of an individual. Radiological analysis of joints found in the knee helps to establish the estimated age of that particular skeleton.

There are different approaches that are used to estimate age using this technique. The hand (wrist) approach is one of the techniques that use radiographs to determine the development stages of critical bones. The knee approach is also used to focus on the maturation of the knee growth plate using magnetic resonance imaging. This method is precisely used to estimate the age of individuals between 10 and 30 years. Other methods include the rib and clavicle, which are used to estimate age through the analysis of ossification for the rib.

Ideally, dental age estimation is a very reliable technique to establish an estimated age for an individual, especially, those who do not know their date of birth or who want to conceal it. Dental development is very important in estimating the age of a person because advanced dental development shows that a person is of adult age, which differentiates him or her from the age of a child. In fact, tooth resilience and durability, being very special in parts of the body, correlates with the age of an individual (Liversidge, 2015). Teeth size and morphology is a clear indication of an individual's age and, therefore, it is logical to estimate the age of a person by considering the dental formulae of that person. For instance, to determine the age of a juvenile, an individual ought to consider the existence of deciduous teeth and permanent teeth.

Age estimation using dental wear involves two mechanisms, those of abrasion and attrition. Abrasion of teeth is caused by the contact of the tooth and the solid exogenous materials that occurs when forced over occlusal surfaces. Attrition, on the other hand, refers to the contact that occurs between tooth and tooth. Dental attrition is used as a means of age estimation, especially among a prehistoric population, where lifestyle could cause irregularities in teeth attrition. Dental wear shows in molar 1, molar 2 and molar 3 at 6, 12 and 18 years, respectively. Although different teeth have different rates of wear, the subjective analysis shows that the molars wear at the ratio of 6:5:7 and, thereby, can be used in age estimation.

If the juvenile has their permanent teeth, which means that the deciduous teeth have already withered, then that person is considered to be slightly older than other juveniles. Therefore, the above phenomenon in age estimation is reliable since it determines whether the juvenile is in their adolescent or childhood stages. Dental wear involves abrasion and attrition mechanisms. Attrition is caused by contact between teeth while abrasion is by contact of a tooth and a solid material, which is squeezed over surfaces. Actually, dental wear is a clear indication that an individual is aging. Erosion entails superficial loss of a tooth that results from chemical substances that affect it. Moreover, tooth extraction is culturally carried out to satisfy religious and ethical reasons (Liversidge, 2015). It is convenient to establish age estimation by looking into dental wear of individuals. Dental wear rates in individuals vary from one age to another according to research conducted on exhumed bodies.

Another common technique that is used to estimate the age of a person is a microstructural analysis of deciduous teeth where the enamel and dentine are analysed to establish their histological marker that is used to determine the dental development. This is an efficient technique that can be used on people who have not completed dental maturity. Dental shading

refers to wear of the surfaces of incisors, canines and premolars. As a person ages, the condition of the teeth and the enamel changes from being polished and in good condition and gradually wears. The dental wear and shading is a comprehensive way of estimating the age of an individual since it provides a means whereby the teeth can be analysed to provide the age estimate of an individual. Using various teeth, the surfaces can be analysed to enable age estimation by using age tooth attrition.

In Libya, there has been a growing need to develop an accurate means of estimating the age of individuals due to an increased rate of criminal activities. A lack of means to establish the age of criminals has become a major issue in the country and, therefore, there is a need to have a validated approach to age estimation. Dental age estimation techniques have not been thoroughly adopted in Africa. The third molar index is the approach that Libya wishes to uptake, in order to evaluate the age of an individual. A sample of 420 individuals taken from different ethnic groups in Tripoli was taken to determine whether the method could be relied upon or not.

The technique proposed by Cameriere states that the adult positively correlated with the third molar maturity index. The approach to the estimation of the age of individuals needed only to measure the width of the apical pulp and the tool length as seen in the orthopantomograph. The approach is very important and reliable since it collected all the measurements, therefore, making it possible to determine the invariant index scale. According to some populations in Libya, the techniques have yielded good results that can be used in the age estimation of a person.

Moreover, the population aged between 14 and 22 were the population sample that was selected to show the significance of the third molar maturity index in the estimation of the age of individuals. The results showed that it is advisable to use the technique of the third molar maturity index with correlation to age because the results obtained after analysis showed correct classification of people according to their various ages. The third molar maturity index shows that if the development of the third molar is complete to an extent that the apical pulp and the roots are closed, which means the third molar maturity index is equal to zero. Research shows that the third molar maturity index is calculated as the sum of the lengths between the inner sides of the couple of apices then divided by the length of the tooth. Finally, after analysis in the evaluation of the third molar maturity index, a person who lies from 0.08 and lower is

deemed to be eighteen years and above. The third molar maturity index significantly estimates the age of an individual.

The experiment on whether the third molar index using cut off 0.08, that Cameriere used, can be used for age estimation in Libya shows that there was 95% confidence level with the method. The approach correctly identified 86.4% of the females and 89% of the males. In addition, a cut-off value of 0.09 in I_{M3} was more accurate as compared to 0.08. Sensitivity of 96% for females and 94% for males was found. Regardless of sex, the method did not deviate much from the actual ages of individuals and, therefore, can be relied upon. The third molar index has no chance of giving true negative results and, therefore, can be relied upon to estimate the age of individuals.

Generally, the age of children can be estimated by examining various sizes and the morphology of their teeth. Durability and resilience of teeth are the two indicators of establishing that an individual is mature or not. Therefore, development of dental formulae is an important indicator, which helps in establishing the estimated age of children. According to Cameriere, the teeth of an individual are most critical in determining the age of a person because they form a crucial part of the body. Moreover, in juveniles, development of deciduous and permanent teeth helps people to estimate the age of a youth. Dental age, as opined by many scholars, happens as the main indicator of a maturing person (Liversidge, 2015). Ideally, by examining the teeth of a child, one will be able to determine whether he or she has reached the adolescent stage or is still a child. According to Demirjan, Goldstein and Tanner, teeth are the common part of the body prevalently used in estimating the age of children.

Nevertheless, Cameriere developed a concept of estimating the age of children by measuring the teeth open apices. His method of age determination in children was tested in Kosovo and Slovenia, where the teeth apices of several children were measured and analysed. The analysis held that the different origins of Europeans did not affect the estimation of ages of children and the teeth showed the desired results of determining their ages. The difference in length of tooth apices, as seen in the magnification and angulation X-ray machines, translated to a difference in age estimation of children.

The method is reliable when it comes to age estimation of children aged between 4 and 15 years. The teeth are the best method that is used to estimate the age of children in that case. This method has commonly been used to estimate the age of children in Europe and has proven

to be reliable, though, the estimation of age in different populations has shown significant variations.

In this method, a total of 319 OPTs (171 girls and 148 boys) were analysed and the seven left permanent mandibular teeth were valued. The number of teeth with root development complete, apical ends of the roots completely closed (N0), was calculated and a new formula was developed for the Libyan population:

$$\text{Age} = 9.412 - 0.284x + 0.996y + 0.670z - 0.942w - 0.067v$$

This best fitting formula can be used for Libyan population age estimation in the age range of 4-15. This formula has been developed in thesis for the Libyan population. Previously, Cameriere developed a similar formula for the Italian and European populations. The formula proposed in this thesis for the Libyan population has also been validated on the Italian and European populations by means of comparison against Cameriere's formulae.

Repeated Measures ANOVA analysis has been used to compare the real and estimated ages obtained by applying two different formulae suggested by Roberto Cameriere for the Italian and European populations. The obtained p value of 0.000 indicated a difference amongst the estimated and real ages.

In order to detect which estimation is different from the others, a paired samples T test has been repeated yielding the following results; the resulted p value for the Libya formula was $p = 0.994$, indicating no statistical difference between the real age and the estimated age. Also the resulted p value for the European formula $p = 0.090$ indicates no statistical difference between the real age and the estimated age, but the value obtained applying the Italian formula provided a $p = 0.000$, indicating a statistically significant difference between the real age and the estimated age.

In addition, the correlations value for the Italian samples is 0.952, 0.931 for the European sample, and 0.963 for Libya samples. The r^2 for the Libyan population is 0.927 higher than the r^2 obtained applying the other formulas, clearly indicating that it is the best approximation for the Libyan population.

This thesis offered the first valuable approach for evaluating the age in Libyan people applying a dental method. Outcomes were encouraging and the study indicated that I_{M3} was a highly accurate approach to age estimation.

7 Future Work

Assessing dental age is important to establish whether children and youths are growing properly and is particularly useful in orthodontics, forensic, podiatry, dentistry and anthropology. For orthodontists, knowing a child's developmental status is especially important in diagnosis and treatment arrangements. In the forensic field, dental age is mostly used in resolving issues with regards to immigration and prosecution in the criminal and civil courts.

Especially, worrying at this time is that Europe is facing growing numbers of immigrants arriving with uncertain birth data or without acceptable identification papers. Some of these immigrants come from North Africa, namely from Libya, Tunisia and Egypt. Still ongoing is the conflict in Libya that began with the Arab Spring protests in 2011 and led to the first Libyan civil war that erupted into instability and violence across the whole country, which is one of the largest countries in terms of area and the fourth country in size in the entire African continent.

Cameriere's method for age estimation based on tooth analysis is very effective and is applicable in various countries around the world. In order to improve the quality of the method and its diagnostic validity, it would be useful if it was applied to the Mediterranean area, while increasing the number of samples for both adults and children. Furthermore, a comparison between teeth and other skeletal parts would be necessary in order to evaluate the accuracy of different skeletal and dental techniques in those populations.

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Appendix

Appendix 1

Table 1: Standard deviation and average of North African and British.

	NAM	BM	NAF	BF
AVG	33.74242	38.39535	25.85714	30.28571
STD	11.48822	16.3564	8.945793	10.98979

Table 2: Age distribution of overall sample as well as standard deviation and average (the age classes are closed on the left)

AV	N	SCORE
0-5	9	5,5,4,5,5,5,4,4,
5-10	38	7,7,7,7,7,7,7,7,7,7,8,8,8,8,8,8,8,8,8,8,8,9,9,9,6,6,6,6,6,7,7,8,8,8,9,9
10 - 15	35	11,11,11,11,11,12,12,13,13,14,14,14,14,14,14,14,14,14,15,15,15,15,12,12,12,13,13,13,13,14,15,15,15,,15,15
15-20	41	16,16,16,16,17,17,18,18,18,18,18,19,19,19,19,19,19,19,19,19,19,19,19,19,20,20,20,20,20,20,20,20,20,20,20,20,20
20-25	38	21,21,21,21,21,21,21,21,21,22,22,23,23,23,23,23,23,23,24,24,24,24,24,25,25,25,25,25,25,25,25,,24,24,24,24,24,23,23,23,23,23,23,24,24,24
25-30	50	26,26,26,26,26,26,26,26,27,27,27,27,27,27,27,27,27,27,27,27,28,28,28,28,28,28,28,28,28,28,28,28,29,29,29,29,29,29,30
30-35	46	30,30,30,30,30,30,30,31,31,32,32,33,33,33,33,33,33,33,34,34,34,34,34,34,43,43,43,43,43,43,43,43,43,35,35,35,35,31,31,31,31,32,32,32,32,
35-40	45	35,35,35,35,35,35,35,35,36,36,36,36,36,37,38,38,38,38,38,38,38,38,38,38,38,38,38,38,38,38,38,38,38,39,39,39,39,39,39,39,39,39,39,39,39,40
40-45	39	41,41,41,41,41,41,41,41,41,41,41,41,41,42,42,42,42,42,43,43,43,43,43,43,43,43,53,53,53,53,53,53,53,53,53,53,44,44,45,45,45
45-50	28	45,45,46,46,46,46,46,46,46,46,46,46,46,46,46,46,46,46,46,46,47,48,47,47,48,48
50-55	21	50,50,52,52,53,53,53,53,53,53,53,53,53,53,53,53,53,54,54,54,55,54,55
55-60	10	56,56,57,57,57,57,58,58,58,59
60-65	8	60,60,60,60,62,63,63,64
65-70	4	65,66,68,70

	ST	AVG
	0,57735	4,666667
	0,752773	8,166667
	1,112697	13,28571
	1,536325	18,11765
	1,466355	22,17391
	1,367465	27,54839
	1,825742	32,75862
	1,74356	37,125
	1,813529	43,11111
	1,272	46,57
	1,669046	52,25
	1,30384	57,5
	1,516575	62,4
	2,217356	67,25

AV: A range, N: Number of sample, STD: Standard deviation, AVG: Average

Appendix 2

Age	Sex	I 3M		Age	Sex	I 3M
19	F	0		19	M	0
19	F	0		19	M	0
19	F	0		20	M	0
19	F	0		20	M	0
19	F	0		20	M	0
20	F	0		20	M	0
20	F	0		20	M	0
20	F	0		20	M	0
20	F	0		20	M	0
20	F	0		20	M	0
20	F	0		20	M	0
20	F	0		21	M	0
20	F	0		21	M	0
20	F	0		21	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0
22	F	0		22	M	0.019997

20	F	0.018123		22	M	0.020799
20	F	0.018211		21	M	0.023201
22	F	0.019877		21	M	0.024546
22	F	0.020892		22	M	0.025339
20	F	0.022026		22	M	0.02663
22	F	0.023413		22	M	0.027682
20	F	0.027244		20	M	0.028
22	F	0.027567		19	M	0.028946
19	F	0.028689		22	M	0.029974
20	F	0.028994		20	M	0.031148

20	F	0.0294		20	M	0.031362
20	F	0.032279		22	M	0.039363
20	F	0.033188		22	M	0.039959
20	F	0.035294		22	M	0.042682
20	F	0.035971		20	M	0.043611
20	F	0.038907		19	M	0.044693
21	F	0.039959		19	M	0.056656
20	F	0.042131		19	M	0.057143
19	F	0.044665		19	M	0.057194
22	F	0.045432		21	M	0.057994
19	F	0.045502		19	M	0.0625
20	F	0.045674		21	M	0.064767
19	F	0.046757		18	M	0.066868
20	F	0.049667		19	M	0.067852
20	F	0.050233		22	M	0.069736
19	F	0.052538		19	M	0.070154
18	F	0.057629		18	M	0.071967
20	F	0.057629		19	M	0.073957
20	F	0.061119		18	M	0.074317
18	F	0.063727		18	M	0.075263
20	F	0.067786		19	M	0.075505
19	F	0.06807		18	M	0.075889
19	F	0.072		18	M	0.076169
19	F	0.072361		19	M	0.07648

19	F	0.073223		19	M	0.076707
20	F	0.073223		18	M	0.077867
18	F	0.073526		20	M	0.078125
18	F	0.074034		18	M	0.078889
18	F	0.074034		18	M	0.078907
20	F	0.074627		18	M	0.079292
18	F	0.074799		18	M	0.079645
20	F	0.074799		18	M	0.080292
20	F	0.074852		18	M	0.081731
18	F	0.07561		18	M	0.082603
18	F	0.075666		18	M	0.084383
19	F	0.075737		18	M	0.093204
18	F	0.075949		18	M	0.095146
18	F	0.075949		17	M	0.097858
19	F	0.077093		16	M	0.098978
18	F	0.077904		17	M	0.098978
18	F	0.078099		17	M	0.101019
18	F	0.078108		17	M	0.105027
18	F	0.078154		17	M	0.106186
19	F	0.078336		17	M	0.106186

18	F	0.078578		16	M	0.110063
18	F	0.079822		16	M	0.111111
19	F	0.07996		16	M	0.111304
18	F	0.08		16	M	0.113191
18	F	0.080745		16	M	0.116279
18	F	0.08082		16	M	0.130185
18	F	0.081489		16	M	0.133998
18	F	0.08284		16	M	0.140504
18	F	0.084836		17	M	0.141972
19	F	0.089722		16	M	0.153191
18	F	0.091667		16	M	0.157687
18	F	0.093537		16	M	0.162514
17	F	0.095652		16	M	0.162609
17	F	0.097561		16	M	0.165237

17	F	0.097561		17	M	0.166468
17	F	0.102082		17	M	0.166777
17	F	0.107692		15	M	0.171233
17	F	0.1125		17	M	0.175
17	F	0.116468		15	M	0.183824
17	F	0.118182		16	M	0.195489
17	F	0.124863		16	M	0.199282
17	F	0.129388		16	M	0.199707
18	F	0.133215		16	M	0.208247
17	F	0.145513		15	M	0.213793
17	F	0.14819		16	M	0.218126
16	F	0.160701		15	M	0.23523
17	F	0.16092		18	M	0.240402
16	F	0.179215		16	M	0.254902
17	F	0.181356		17	M	0.2577
16	F	0.181612		15	M	0.271251
17	F	0.182004		15	M	0.284314
15	F	0.182745		16	M	0.287356
16	F	0.186947		17	M	0.331307
17	F	0.187852		15	M	0.36293
16	F	0.198915		14	M	0.40625
17	F	0.200387		14	M	0.434517
15	F	0.200515		15	M	0.439919
15	F	0.217435		15	M	0.464405
15	F	0.218297		15	M	0.472222
14	F	0.221239		15	M	0.475659
17	F	0.223104		14	M	0.478827
15	F	0.234023		15	M	0.497119
16	F	0.241408		14	M	0.556818
16	F	0.252261		14	M	0.558333
15	F	0.257714		14	M	0.579221
16	F	0.26183		14	M	0.629213
15	F	0.261961		14	M	0.629213
14	F	0.265306		14	M	0.632583
15	F	0.269306		14	M	0.639447

16	F	0.272366		14	M	0.671014
15	F	0.282745		14	M	0.671014
15	F	0.285714		14	M	0.684138
16	F	0.288934		14	M	0.693738
15	F	0.290021		15	M	0.693738
15	F	0.295455		15	M	0.714286
14	F	0.295477		15	M	0.722892
14	F	0.308571		14	M	0.779221
15	F	0.31236		14	M	0.833333
15	F	0.322892		14	M	0.972189
16	F	0.338488		15	M	0.972189
14	F	0.33945		15	M	1.1
16	F	0.34836				
16	F	0.355865				
14	F	0.36036				
14	F	0.380952				
15	F	0.39218				
15	F	0.397516				
15	F	0.408276				
16	F	0.4083				
15	F	0.480769				
16	F	0.519495				
15	F	0.66129				
14	F	0.698413				
14	F	0.821918				
15	F	0.836066				
15	F	0.875				
15	F	0.984375				
20	F	1.176				
14	F	1.208333				
14	F	1.347826				

Appendix 3

	Tripoli				Benghazi		
Sex	Age	IM3		Sex	Age	IM3	
F	18.33699	0		F	13.32329	0.864364	
F	18.75342	0		F	13.4274	0.497489	
F	18.99178	0		F	13.65753	0.803693	
F	19.40548	0		F	13.89863	1.037458	
F	19.40822	0		F	14.0411	0.423838	
F	19.52877	0		F	14.49041	0.648567	
F	19.56438	0		F	14.50411	0.3255	
F	19.59726	0		F	14.52877	0.787944	
F	19.70685	0		F	14.62466	0.376353	
F	19.76438	0		F	14.64658	0.678579	
F	19.81096	0		F	14.72877	0.313295	
F	19.87123	0		F	15.12329	0.592557	
F	19.87397	0		F	15.25205	0.226355	
F	20	0		F	15.52055	0.602342	
F	20.01918	0		F	15.52329	0.398376	
F	20.0274	0		F	15.57534	0.489374	
F	20.09863	0		F	15.64658	0.293746	
F	20.16986	0		F	15.73151	0.210838	
F	20.1726	0		F	15.7589	0.282956	
F	20.23836	0		F	15.81096	0.219735	

F	20.33151	0		F	16.06575	0.198365
F	20.34247	0		F	16.16712	0.101747
F	20.34521	0		F	16.16712	0.102847
F	20.34795	0		F	16.35068	0.138676
F	20.35068	0		F	16.4	0.262935
F	20.44384	0		F	16.49589	0.239279
F	20.47945	0		F	16.6411	0.092635
F	20.50685	0		F	16.72603	0.302649
F	20.67671	0		F	16.86027	0.190274
F	20.70137	0		F	16.88493	0.265938
F	20.79726	0		F	16.94521	0.097069
F	20.84932	0		F	16.95616	0.201735
F	20.88767	0		F	17.14247	0.093766
F	20.93151	0		F	17.18356	0.095649
F	21.05205	0		F	17.26027	0.089465
F	21.08767	0		F	17.43014	0.102658
F	21.11781	0		F	17.64932	0.089847
F	21.16438	0		F	17.74247	0.103655

F	21.22192	0		F	18.13425	0.082175
F	21.26849	0		F	18.1726	0.079372
F	21.30685	0		F	18.31507	0.079827
F	21.40274	0		F	18.33151	0.080227
F	21.4274	0		F	18.51233	0.078355
F	21.60274	0		F	18.51781	0.086285
F	21.67945	0		F	18.58356	0.091076
F	21.76712	0		F	18.60548	0.073783
F	21.77808	0		F	18.65205	0.090286
F	21.80548	0		F	18.65479	0.082466

F	21.82466	0		F	18.74521	0.0789
F	21.83836	0		F	18.76164	0.079565
F	21.84384	0		F	18.94795	0.084014
F	21.86301	0		F	19.01644	0.070897
F	21.86849	0		F	19.18356	0.069254
F	21.87671	0		F	19.23562	0.066328
F	21.91781	0		F	19.31507	0.066005
F	21.92603	0		F	19.34247	0.049783
F	21.93425	0		F	19.43014	0.036821
F	21.94247	0		F	19.48767	0.063179
F	21.94795	0		F	19.49589	0.063278
F	21.95616	0		F	19.5726	0.067319
F	21.9726	0		F	19.63562	0
F	22.00548	0		F	19.68219	0.047247
F	22.09589	0		F	19.85205	0.04288
F	22.17808	0		F	20.06849	0.031794
F	22.42466	0		F	20.12055	0.063828
F	22.53425	0		F	20.15616	0
F	22.64384	0		F	20.28767	0
F	22.70137	0		F	20.29315	0.050215
F	22.72877	0		F	20.48493	0.021093
F	22.7863	0		F	20.71233	0
F	22.90137	0		F	21.56712	0
F	23.10137	0		F	21.67945	0
F	23.18082	0		F	21.74795	0
F	23.22466	0		F	21.82466	0.035833
F	23.25479	0		F	21.93425	0.018999
F	23.27671	0		F	21.95068	0.032895
F	23.31233	0		F	21.98356	0.036842
F	23.89863	0		F	22.01096	0
F	16.91781	0.010289		F	22.06301	0
F	22.18356	0.010799		F	22.1589	0
F	19.49863	0.012894		F	22.34521	0
F	23.0274	0.018214		F	22.83288	0.019938

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F	20.40274	0.019983		F	22.92603	0
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F	21.00274	0.021361		F	23.12329	0
F	22.1726	0.022092				
F	21.30685	0.022783				
F	19.4	0.023218				
F	21.5726	0.02399				
F	20.42466	0.025734				
F	21.68493	0.026524				
F	20.47123	0.029683				
F	20.89863	0.029719				
F	20.92603	0.029947				
F	20.95342	0.029991				
F	20.17534	0.030129				
F	22.01644	0.030331				
F	19.61918	0.031794				
F	19.39726	0.031809				
F	22.04932	0.032786				
F	22.87397	0.033992				
F	21.0137	0.034568				
F	19.64384	0.035013				
F	20.76164	0.035123				
F	20.85479	0.036874				
F	22.33699	0.036922				
F	21.52603	0.03699				
F	19.16712	0.037803				
F	19.38904	0.038033				
F	20.43014	0.038239				
F	19.40822	0.03839				
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F	20.92603	0.038526				
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F	21.16438	0.047629				

F	19.93699	0.048039				
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F	20.40822	0.049731				
F	19.65753	0.05027				
F	20.53973	0.050827				
F	21.04658	0.051694				
F	20.77808	0.051982				
F	19.60274	0.052793				
F	19.78904	0.052809				
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F	21.06575	0.056769				
F	20.00274	0.057219				
F	21.0137	0.058974				
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F	20.86027	0.059217				
F	21.02192	0.060142				
F	19.25753	0.06269				
F	19.40822	0.062793				
F	18.78356	0.062968				
F	19.50685	0.063802				
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F	19.01644	0.064703				
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F	19.25205	0.068026				
F	19.31507	0.068251				
F	18.56712	0.068265				
F	19.9726	0.068281				
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F	18.93973	0.069317				
F	18.45479	0.069376				
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F	18.6	0.071693				
F	19.09315	0.071704				
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F	18.32603	0.091267				
F	18.97808	0.092693				
F	17.98082	0.093962				
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F	17.7726	0.095993				
F	17.46849	0.096185				
F	17.43014	0.096198				
F	17.35616	0.096245				
F	17.58904	0.096493				
F	17.90685	0.096498				
F	16.75616	0.0967				
F	17.91507	0.096827				
F	17.93699	0.096984				
F	17.92055	0.097241				
F	17.83014	0.097299				
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F	17.10959	0.097726				
F	15.85753	0.097732				
F	17.61644	0.097837				
F	17.46849	0.097947				

F	17.7863	0.098046				
F	17.18082	0.098225				
F	17.18904	0.098227				
F	17.07123	0.09833				

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F	17.20274	0.099366				
F	17.91781	0.099959				
F	15.31233	0.099983				
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F	14.21096	0.100211				
F	17.07671	0.100368				
F	17.93151	0.10213				
F	17.06301	0.102299				
F	15.30685	0.102319				
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F	17.01644	0.189476				
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F	17.53973	0.237849				
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F	15.50137	0.289636				
F	16.41096	0.289769				
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F	13.22466	0.299353				
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F	17.53973	0.331758				
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F	16.22192	0.375567				
F	17.33973	0.378265				
F	14.48493	0.38				
F	15.48767	0.38436				
F	15.47945	0.384589				
F	13.02192	0.387634				
F	13.18356	0.388864				
F	13.98082	0.393276				
F	16.7863	0.393401				
F	14.26849	0.397516				
F	16.67671	0.398354				
F	14.25205	0.402122				
F	13.18356	0.405479				
F	16.48767	0.406755				
F	15.46575	0.411259				
F	13.29589	0.412522				
F	15.09589	0.432159				
F	15.54521	0.432672				
F	15.1726	0.442787				
F	13.93973	0.445714				
F	13.06849	0.473417				
F	13.09315	0.478659				
F	13.2411	0.480769				
F	13.67945	0.492462				
F	15.30137	0.493423				
F	13.56164	0.49346				
F	15.66027	0.503268				
F	13.7726	0.517877				
F	15.72877	0.520856				

F	13.47397	0.521169				
F	15.29315	0.523468				
F	16.4	0.542838				
F	13.65205	0.579669				
F	17.27945	0.583922				
F	14.38082	0.597423				
F	14.02192	0.598229				
F	14.61644	0.60111				
F	13.90137	0.612714				
F	15.60822	0.614584				
F	13.50685	0.629213				
F	13.88767	0.632093				
F	13.05479	0.665785				
F	13.0137	0.66888				
F	14.61918	0.676813				
F	13.55068	0.678733				
F	15.74795	0.693462				
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F	13.26849	0.698593				
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F	14.94795	0.698931				
F	15.44658	0.698931				
F	14.23836	0.698945				
F	13.39178	0.701218				
F	15.01096	0.750201				
F	13.58904	0.778238				
F	14.17534	0.789319				
F	13.17534	0.798479				
F	13.88767	0.834622				
F	13.00822	0.836066				
F	14.44658	0.854649				
F	13.67945	0.8779				
F	14.25753	0.879369				
F	14.92877	0.894238				

F	14.0274	0.901231				
F	17.46027	0.967365				
F	13.97808	0.973215				
F	17.45479	0.981645				
F	15.09041	1.021589				
F	13.09863	1.074586				
F	13.87397	1.165333				
F	13.21096	1.245166				
F	13.48767	1.256528				

	Tripoli				Benghazi	
Sex	Age	IM3		Sex	Age	IM3
M	19.32877	0		M	13.49863	0.824897
M	19.6	0		M	13.5589	0.746824
M	19.60274	0		M	14.21096	0.518795
M	20.12603	0		M	14.44384	0.892169
M	20.18356	0		M	14.53151	0.794531
M	20.19178	0		M	14.63014	0.368923
M	20.25205	0		M	14.65753	0.578348
M	20.39452	0		M	14.72055	0.478357
M	20.8	0		M	14.90959	0.637944
M	20.85479	0		M	15.03562	0.414577
M	21.15068	0		M	15.12603	0.356767
M	21.25479	0		M	15.25753	0.307263
M	21.26301	0		M	15.45479	0.623548
M	21.27397	0		M	15.49041	0.745677

M	21.3726	0		M	15.49863	0.636789
M	21.6411	0		M	15.52877	0.402345
M	21.70685	0		M	15.89589	0.206997
M	21.76438	0		M	16.15616	0.146733
M	21.88219	0		M	16.17534	0.224578
M	21.89863	0		M	16.28219	0.134568
M	21.91507	0		M	16.36164	0.124568
M	22	0		M	16.36164	0.178977
M	22.01096	0		M	16.49041	0.234568
M	22.09041	0		M	16.55616	0.096789
M	22.09863	0		M	16.90959	0.099657
M	22.15342	0		M	16.98082	0.235678
M	22.23836	0		M	16.98904	0.145679
M	22.2411	0		M	17.08767	0.097878
M	22.28767	0		M	17.17808	0.089756
M	22.41918	0		M	17.27123	0.10235
M	22.64658	0		M	17.32055	0.123578
M	22.68493	0		M	17.4411	0.164657
M	22.81644	0		M	17.46849	0.097446
M	22.81918	0		M	17.51233	0.102548
M	22.8274	0		M	17.53699	0.091776
M	22.8274	0		M	17.56438	0.088255

M	22.84384	0		M	17.56712	0.098563
M	22.93151	0		M	17.68493	0.089553
M	23.0137	0		M	17.76986	0.095583
M	23.06575	0		M	17.82192	0.089357

M	23.09315	0		M	18.06849	0.074081
M	23.11781	0		M	18.09315	0.081084
M	23.1726	0		M	18.11781	0.072371
M	23.2274	0		M	18.19452	0.073967
M	23.48767	0		M	18.40274	0.083433
M	23.49589	0		M	18.43288	0.073268
M	23.65753	0		M	18.49315	0.07434
M	23.67671	0		M	18.52055	0.072762
M	21.35068	0.010218		M	18.59178	0.080455
M	21.18082	0.012314		M	19.26301	0.066735
M	20.07397	0.016577		M	19.48219	0.069836
M	22.83288	0.018296		M	19.77534	0.057527
M	20.54795	0.019267		M	19.84932	0.037846
M	21.85205	0.019826		M	20.10137	0.026528
M	21.4137	0.020012		M	20.12055	0.032386
M	20.63288	0.020019		M	20.15616	0
M	21.30685	0.020231		M	20.18082	0
M	21.37534	0.020232		M	20.93151	0.024367
M	22.9589	0.020242		M	20.95068	0.021234
M	21.93151	0.020576		M	21.06849	0
M	21.5726	0.020884		M	21.07123	0.018667
M	22.93151	0.023794		M	21.51233	0
M	21.50411	0.025834		M	21.67945	0.017544
M	21.0137	0.02853		M	22.42466	0
M	21.09863	0.028948		M	22.67397	0
M	20.12055	0.029321		M	22.84658	0.021202
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M	20.91507	0.030116		M	22.93151	0
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M	19.48767	0.030894		M	23.46301	0
M	21	0.031363		M	23.47397	0
M	20.6411	0.032093		M	23.50137	0
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M	21.94521	0.039121				
M	20.10137	0.039343				
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M	20.26849	0.043215				
M	19.89589	0.044794				
M	20.76438	0.045776				
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M	19.42466	0.055902				
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M	19.61644	0.05693				
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M	20.3726	0.059847				
M	20.01918	0.060217				
M	18.87123	0.060366				
M	19.64658	0.060372				
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M	19.60548	0.062415				
M	19.79726	0.06371				
M	19.47945	0.06388				
M	19.80548	0.064691				
M	19.26575	0.06475				
M	21.28493	0.065821				
M	18.60822	0.068265				
M	19.00274	0.068265				

M	19.59178	0.068366				
M	19.00822	0.069378				
M	20.60548	0.069522				
M	19.32329	0.069679				
M	21.23562	0.070112				
M	18.56164	0.070126				
M	21.0274	0.070182				
M	18.10137	0.070184				
M	18.59452	0.070258				
M	18.69041	0.07098				
M	18.75342	0.071029				
M	18.57808	0.071303				
M	18.51233	0.071365				
M	18.06575	0.071378				
M	18.1726	0.071463				

M	18.86027	0.071532				
M	18.75616	0.071794				
M	18.01918	0.071937				
M	17.91781	0.072058				
M	18.01096	0.072124				
M	19.13425	0.072173				
M	16.07123	0.072319				
M	18.06027	0.072474				
M	17.67397	0.072571				
M	18.4	0.07274				
M	19.58082	0.072795				
M	18.26301	0.072804				
M	19.00548	0.072985				
M	19.26301	0.073091				
M	17.40822	0.073267				
M	16.13425	0.073429				
M	18.39178	0.073592				
M	19.85479	0.073603				
M	18.8	0.074026				

M	18.86027	0.074091				
M	18.08767	0.074127				
M	18.93425	0.074318				
M	18.00274	0.074711				
M	18.32329	0.074719				
M	19.18356	0.074771				
M	18.99726	0.074792				
M	18.06575	0.0748				
M	19.16986	0.074984				
M	17.3589	0.077141				
M	19.16438	0.078978				
M	18.17808	0.079038				
M	18.42192	0.080358				
M	18.18904	0.081274				
M	18.67945	0.083254				
M	18.65479	0.083267				
M	17.60274	0.084811				
M	18.61918	0.087024				
M	18.49863	0.087599				
M	18.60548	0.088236				
M	17.99726	0.091364				
M	19.8	0.093603				
M	19.34795	0.094676				
M	17.36986	0.095613				
M	17.68767	0.096031				

M	17.23014	0.09615				
M	17.85479	0.096462				
M	17.89041	0.096774				
M	17.53699	0.096825				
M	17.64384	0.096981				
M	19.37808	0.097048				
M	17.56164	0.097198				
M	17.8411	0.097318				
M	17.40548	0.097474				

M	15.58082	0.097561				
M	18.51781	0.097583				
M	16.13699	0.097927				
M	17.83836	0.098094				
M	17.14247	0.098373				
M	16.97534	0.098438				
M	16.86849	0.098457				
M	16.57534	0.09898				
M	17.18356	0.099265				
M	17.38082	0.09928				
M	15.90959	0.099348				
M	17.75616	0.099364				
M	16.67671	0.099489				
M	16.16164	0.099546				
M	16.33151	0.100198				
M	17.35068	0.10211				
M	16.78082	0.102186				
M	17.76164	0.102499				
M	15.36712	0.106755				
M	17.76164	0.109268				
M	16.16712	0.109786				
M	17.7589	0.112085				
M	17.26027	0.117835				
M	17.27397	0.119875				
M	17.26301	0.120139				
M	16.4137	0.123178				
M	17.97808	0.128129				
M	17.01644	0.137492				
M	17.50685	0.138493				
M	17.63014	0.139578				
M	14.89863	0.156785				
M	17.15616	0.157927				
M	16.15616	0.158403				
M	15.83836	0.167533				
M	16.20274	0.172239				

M	16.90137	0.172766				
M	15.86027	0.180897				
M	16.79726	0.186275				
M	16.09863	0.187963				
M	15.86575	0.189673				
M	15.50685	0.191322				
M	13.51507	0.194435				
M	14.35068	0.196546				
M	16.58082	0.198097				
M	13.38082	0.199833				
M	16.86027	0.199911				
M	13.97534	0.200193				
M	16.09863	0.201198				
M	16.23836	0.201211				
M	15.6274	0.203265				
M	15.89589	0.203657				
M	15.65205	0.212347				
M	15.00548	0.21565				
M	15.8411	0.222733				
M	14.68493	0.232308				
M	15.64658	0.245381				
M	16.19178	0.251102				
M	16.99178	0.252312				
M	15.91233	0.252318				
M	14.15342	0.26358				
M	16.98082	0.271175				
M	15.93973	0.274568				
M	16.17534	0.275482				
M	14.16986	0.276372				
M	15.70411	0.281231				
M	14.18356	0.282433				
M	16.50959	0.285646				
M	14.38904	0.286422				
M	15.08493	0.287438				

M	16.1589	0.289938				
M	14.01096	0.293212				
M	13.06575	0.293449				
M	15.33699	0.293613				
M	15.70137	0.298565				
M	15.25753	0.307263				
M	17.0274	0.324829				
M	14.92877	0.36036				
M	13.7589	0.36523				
M	15.73151	0.365344				

M	13.33699	0.367849				
M	16.33973	0.36875				
M	14.08493	0.376501				
M	15.91233	0.376848				
M	14.13425	0.378453				
M	16.39726	0.379334				
M	15.06849	0.39218				
M	13.80822	0.399891				
M	14.63014	0.407689				
M	13.10137	0.408276				
M	17.08767	0.410124				
M	12.96438	0.411259				
M	15.1589	0.413854				
M	15.39452	0.42132				
M	13.33425	0.443219				
M	14.05205	0.453216				
M	13.06849	0.454362				
M	15.56438	0.47321				
M	14.03288	0.476569				
M	13.74521	0.486579				
M	13.62466	0.519495				
M	16.13151	0.519495				
M	17.96712	0.520195				
M	13.03014	0.537894				

M	14.71233	0.567805				
M	14.23836	0.567854				
M	13.92055	0.578677				
M	13.92055	0.589743				
M	21.55342	0.589743				
M	13.60548	0.595662				
M	13.13151	0.599227				
M	16.28493	0.618536				
M	13.13699	0.636864				
M	13.48493	0.66129				
M	13.98356	0.665745				
M	13.76438	0.667704				
M	13.42192	0.678545				
M	13.90959	0.687945				
M	14.45205	0.689613				
M	14.92877	0.689661				
M	13.21644	0.689703				
M	13.2	0.698321				
M	14.59452	0.698413				
M	14.66301	0.779453				
M	13.05479	0.7835				
M	14.53151	0.794531				
M	13.70137	0.801119				
M	13.83014	0.802147				
M	15.52877	0.802342				
M	15.53425	0.807844				
M	15.13699	0.817894				
M	13.20822	0.821918				
M	14.65753	0.832149				
M	13.16712	0.837547				
M	13.33151	0.837844				
M	14.44384	0.892169				
M	13.11507	0.975676				
M	14.20822	0.981213				
M	16.87123	0.984375				

M	17.08767	1.038927				
M	13.13973	1.084717				
M	14.41918	1.087965				
M	13.09863	1.208333				
M	16.7863	1.208333				
M	13.8411	1.252328				
M	15.00822	1.289453				
M	13.2	1.343289				
M	16.72603	1.347826				

Appendix 4

AGE (Y)	Estimated age					Real-Estimated age		
	E EU	E Italy	E Libya			E EU	E Italy	E Libya
4.227397	5.308557	4.313313	3.813929			1.168906	0.007382	0.170956
5.326027	4.990174	4.193016	3.624819			0.112797	1.283716	2.89411
4.860274	6.766936	5.018346	4.815954			3.635361	0.024987	0.001964
5.010959	6.550943	4.790767	4.558951			2.371551	0.048484	0.204312
4.663014	6.444049	4.772153	4.514658			3.172086	0.011911	0.022009
4.767123	7.233277	5.907056	5.721212			6.081912	1.299446	0.910285
4.860274	7.030343	5.660153	5.450575			4.709199	0.639807	0.348456
5.232877	7.177973	5.337752	5.204795			3.783401	0.010999	0.000789
4.915068	7.139928	5.767945	5.574203			4.950002	0.727398	0.434458
6.010959	6.676032	6.862858	6.418675			0.442323	0.725733	0.166233
5.663014	7.276601	6.458601	6.218845			2.603665	0.632959	0.308949
6.010959	6.682406	7.141947	6.666429			0.450842	1.279134	0.429641
5.663014	7.300644	6.528015	6.286322			2.681832	0.748227	0.388513
6.271233	6.695292	7.44588	6.937787			0.179826	1.379795	0.444294
5.775342	7.623274	6.880876	6.681606			3.414851	1.222204	0.821314
7.249315	6.963614	6.678647	6.331246			0.081625	0.325662	0.842851
6.931507	6.871851	7.239783	6.802105			0.003559	0.095034	0.016745

6.893151	7.175527	7.288602	6.924351			0.079736	0.156381	0.000973
7.027397	7.226229	7.849688	7.432322			0.039534	0.676162	0.163964
7.183562	7.567505	6.789172	6.5862			0.147412	0.155543	0.356841
7.19726	8.415868	7.408867	7.353883			1.485005	0.044778	0.024531
7.09863	7.539774	6.784311	6.574682			0.194608	0.098796	0.274522
7.263014	7.456524	7.061529	6.797412			0.037446	0.040596	0.216785
8.073973	7.399494	8.096766	7.695375			0.454922	0.00052	0.143336
8.260274	7.819556	8.41044	8.081516			0.194232	0.02255	0.031954
8.427397	7.854859	8.778384	8.415164			0.3278	0.123192	0.00015
7.791781	7.391419	7.964065	7.576258			0.16029	0.029682	0.04645
7.673973	7.105676	7.763307	7.324713			0.322961	0.007981	0.121982
8.19726	7.45337	7.844412	7.486909			0.553372	0.124502	0.504599
8.008219	7.388073	8.623848	8.15716			0.384582	0.378999	0.022183
7.909589	7.131332	7.874946	7.429844			0.605683	0.0012	0.230155
7.561644	7.518053	7.752248	7.422511			0.0019	0.03633	0.019358
7.972603	7.542886	7.365691	7.088135			0.184656	0.368342	0.782283
8.334247	7.989704	9.198049	8.873277			0.11871	0.746154	0.290553
8.70411	8.600225	9.090948	9.314506			0.010792	0.149644	0.372584
8.572603	8.936634	9.898931	9.911064			0.132519	1.759146	1.791478
8.594521	7.2456	9.297541	8.714043			1.819586	0.494238	0.014286
9.361644	9.934587	10.39024	10.34325			0.328264	1.058005	0.963544
8.890411	9.380412	9.159505	9.430907			0.240101	0.072412	0.292136
8.783562	8.94225	9.042609	9.070052			0.025182	0.067106	0.082077
9.027397	8.582289	9.310037	9.068196			0.198122	0.079885	0.001665
10.10685	8.97577	10.34565	10.03953			1.27934	0.057024	0.004532

10.34795	9.684523	10.73553	10.56372			0.440129	0.150223	0.046558
10.00822	10.2781	10.14006	10.19771			0.072835	0.017381	0.035907
9.621918	9.84721	9.734874	9.875606			0.050757	0.012759	0.064358
10.07397	10.3509	10.22444	10.26331			0.076689	0.022641	0.035849
9.857534	10.14554	10.1986	10.2272			0.08295	0.116324	0.136652

10.33425	8.922679	9.263378	9.091783			1.992524	1.14676	1.543715
9.419178	10.34013	10.46181	10.42897			0.848159	1.087076	1.019687
9.893151	10.55727	10.5426	10.50468			0.441055	0.421778	0.37397
9.676712	10.60802	10.62675	10.56819			0.867336	0.902563	0.794727
9.882192	9.629445	9.86521	9.765336			0.063881	0.000288	0.013655
9.956164	10.02805	10.62053	10.51307			0.005168	0.441388	0.310144
9.654795	10.35087	10.84304	10.69749			0.484519	1.411922	1.087224
9.950685	10.79252	10.87309	10.75724			0.708692	0.850827	0.65053
9.405479	10.31633	10.98592	10.79476			0.829653	2.497782	1.930094
10.4137	10.9721	11.10784	10.93773			0.311817	0.481831	0.274605
11.41644	11.83227	11.76359	11.61004			0.172917	0.120514	0.037482
10.0274	10.69859	11.49522	11.18569			0.450505	2.154497	1.341633
10.3589	10.80333	11.55857	11.23932			0.197513	1.43919	0.775126
11.0137	10.54302	10.75752	10.65429			0.221535	0.065628	0.129177
10.39726	10.53276	11.08346	10.88216			0.018359	0.470872	0.235132
10.53425	10.34912	10.41771	10.39881			0.034272	0.013582	0.018344
10.49863	10.5406	10.59664	10.54116			0.001762	0.009606	0.001809
11.32055	10.77418	11.05434	10.88285			0.298518	0.070868	0.191578
10.64932	10.51337	10.42407	10.41764			0.018481	0.050737	0.053671
10.68219	10.55261	10.53348	10.49788			0.016792	0.022114	0.033971
11.07397	10.59007	10.63939	10.57549			0.234161	0.188861	0.248483
10.56712	10.56276	10.86414	10.73085			1.9E-05	0.088218	0.026807
10.8274	10.76803	10.81778	10.71628			0.003524	9.24E-05	0.012347
10.73151	10.67767	10.64229	10.58519			0.002898	0.00796	0.021408
10.75068	10.94266	10.8299	10.74006			0.036854	0.006274	0.000113
11.01644	10.80186	10.87911	10.76229			0.046046	0.018858	0.064593
11.04384	10.64072	10.83793	10.71928			0.162498	0.042396	0.105337
11.31233	10.73296	10.93981	10.79886			0.335663	0.138768	0.263649
10.90411	10.71247	10.95667	10.8089			0.036725	0.002763	0.009065
10.5589	11.63817	11.69684	11.55351			1.16481	1.294893	0.989238

12.23014	10.96486	11.52146	11.22741			1.60093	0.502224	1.005465
11.42466	12.28938	12.2469	12.15135			0.747743	0.676077	0.528083
11.6137	12.2286	12.32253	12.19825			0.3781	0.502446	0.341699
12.03836	10.86124	11.38685	11.12386			1.385594	0.424457	0.836302
12.14521	11.82085	11.68709	11.55786			0.105204	0.209874	0.34497
11.93699	12.37809	12.40944	12.26056			0.194571	0.223215	0.104701
12.18356	10.678	10.90696	10.77099			2.266706	1.629706	1.99535
12.10137	10.96059	11.35452	11.10986			1.30138	0.557781	0.983086
11.60822	10.89385	11.02118	10.87005			0.510328	0.344613	0.54489

11.51781	11.78832	11.80909	11.63804			0.073176	0.084844	0.014455
12.06027	11.77498	11.78298	11.61967			0.081395	0.076893	0.194136
12.11507	12.77428	12.55145	12.36821			0.434555	0.190427	0.064082
11.80548	11.02711	11.17566	10.99014			0.605863	0.396673	0.664774
11.88219	10.80044	11.0632	10.89137			1.170194	0.670755	0.981734
12.25479	10.6546	11.01086	10.84187			2.560621	1.547362	1.996347
11.46575	10.73754	11.00459	10.84473			0.530289	0.21267	0.385671
12.15616	11.09963	11.27609	11.06698			1.116267	0.774536	1.186326
13.37534	14.514	14.064	13.818			1.296541	0.474249	0.195946
13.41644	14.514	14.064	13.818			1.204642	0.419336	0.161252
12.93699	14.514	14.064	13.818			2.486972	1.27016	0.776185
12.99726	14.514	14.064	13.818			2.300499	1.137934	0.673614
12.65479	14.514	14.064	13.818			3.456645	1.98586	1.353047
13.04658	11.79648	11.60419	11.50061			1.562726	2.080489	2.390015
13.13699	12.77642	12.5565	12.37158			0.13001	0.336964	0.585842
13.34795	12.7783	12.56094	12.37455			0.324496	0.619369	0.9475
12.61096	12.77852	12.56145	12.37489			0.028075	0.002451	0.055729
12.62466	12.77902	12.56264	12.37568			0.023827	0.003846	0.06199
12.8	11.59351	11.84932	11.65347			1.455618	0.903789	1.314521
12.96712	12.66475	12.57237	12.37762			0.091431	0.155831	0.34751
12.8411	13.6186	13.25713	13.06255			0.604517	0.173081	0.049044

12.8411	13.62288	13.26653	13.0686			0.611183	0.180995	0.051759
12.10959	13.54815	13.34634	13.11754			2.06945	1.529553	1.015971
12.8411	13.63993	13.30404	13.09272			0.638134	0.214321	0.063317
12.45205	13.6408	13.30596	13.09395			1.413111	0.729148	0.412036
12.69863	13.64109	13.30659	13.09436			0.888227	0.369619	0.156606
12.8411	13.57028	13.26583	13.06703			0.531704	0.180401	0.051046
12.3863	13.6484	13.32267	13.1047			1.592881	0.876786	0.5161
12.17808	12.64395	12.6388	12.42006			0.217032	0.212257	0.058555
13.06575	12.69482	12.64466	12.42584			0.137593	0.177318	0.409495
12.64658	12.69435	12.65043	12.42957			0.002282	1.49E-05	0.04709
12.80274	14.514	14.064	13.818			2.928412	1.590777	1.030753
12.46301	14.514	14.064	13.818			4.206545	2.563157	1.835988
12.84384	14.514	14.064	13.818			2.789449	1.488801	0.948996
12.76712	14.514	14.064	13.818			3.051578	1.681889	1.104342
13.93425	14.514	14.064	13.818			0.336114	0.016836	0.013513
13.34521	13.55433	13.11573	12.97162			0.043733	0.052661	0.139563
13.25479	13.55031	13.10689	12.96594			0.08733	0.021877	0.083437
13.17534	13.56489	13.13896	12.98657			0.15175	0.001323	0.035636
13.94247	13.58283	13.17843	13.01195			0.129338	0.583756	0.865869
14.21096	13.58983	13.19382	13.02184			0.385804	1.034571	1.413993
14.13151	13.59334	13.20155	13.02682			0.289621	0.864813	1.220339
14.2411	14.514	14.064	13.818			0.074477	0.031363	0.17901
13.80822	14.514	14.064	13.818			0.498127	0.065424	9.57E-05

13.82192	14.514	14.064	13.818			0.478978	0.058604	1.53E-05
14.26849	14.514	14.064	13.818			0.060274	0.041817	0.202944
14.18356	14.514	14.064	13.818			0.10919	0.014295	0.133635
13.9589	14.514	14.064	13.818			0.308131	0.011045	0.019854
13.57534	14.514	14.064	13.818			0.881078	0.238786	0.058883
14.15342	14.514	14.064	13.818			0.130015	0.007997	0.11251
13.97808	14.514	14.064	13.818			0.287208	0.007382	0.025626

14.23014	14.514	14.064	13.818			0.080578	0.027601	0.169857
13.40548	14.514	14.064	13.818			1.228818	0.433649	0.170173
13.84658	14.514	14.064	13.818			0.445456	0.047273	0.000817
13.83562	14.514	14.064	13.818			0.460204	0.052159	0.00031
13.08493	14.514	14.064	13.818			2.042237	0.958575	0.537389
13.50959	14.514	14.064	13.818			1.008841	0.307372	0.095117
14.07397	14.514	14.064	13.818			0.193624	9.95E-05	0.065522
13.78082	14.514	14.064	13.818			0.53755	0.08019	0.001382
14.32603	14.514	14.064	13.818			0.035334	0.068658	0.258092
13.98356	14.514	14.064	13.818			0.281365	0.00647	0.027411
14.38356	14.514	14.064	13.818			0.017014	0.10212	0.31986
5.032877	4.818736	3.599792	3.745232			0.045856	2.053731	1.65803
5.243836	5.056563	4.064242	4.216794			0.035071	1.391442	1.054815
5.39726	5.212012	4.125449	4.311306			0.034317	1.617504	1.179297
4.789041	6.36514	5.527228	5.848088			2.484087	0.54492	1.121581
5.432877	7.187168	5.019443	5.614728			3.077539	0.170928	0.03307
5.227397	6.24729	5.145223	5.480513			1.04018	0.006753	0.064068
6.268493	6.529663	6.467204	6.719836			0.06821	0.039486	0.20371
6.353425	6.672211	5.946358	6.297748			0.101625	0.165704	0.0031
6.09863	6.252112	6.490142	6.667676			0.023557	0.153282	0.323813
5.610959	6.682258	7.084219	7.303696			1.147681	2.170496	2.865361
6.027397	7.099587	6.914835	7.26318			1.149591	0.787545	1.527159
6.273973	6.466735	6.732038	6.936946			0.037158	0.209824	0.439534
6.353425	6.456982	7.491403	7.603985			0.010724	1.294995	1.563901
6.180822	6.408654	7.004059	7.161657			0.051907	0.677719	0.962037
6.331507	7.8321	7.559996	8.023104			2.25178	1.509186	2.861501
6.912329	6.52287	7.167562	7.335616			0.151678	0.065144	0.179172
7.027397	7.007818	6.563311	6.929284			0.000383	0.215376	0.009626
6.972603	6.247997	7.276199	7.359721			0.525053	0.092171	0.149861
7.115068	6.469215	7.319126	7.455267			0.417126	0.04164	0.115735

6.939726	6.682258	7.084219	7.303696			0.06629	0.020878	0.132475
6.772603	6.881038	6.603066	6.931274			0.011758	0.028743	0.025177
7.194521	6.340438	7.352553	7.451157			0.729456	0.024974	0.065862
7.813699	8.169502	7.252925	7.840334			0.126596	0.314468	0.000709
7.942466	6.70946	7.212541	7.423941			1.520304	0.532791	0.268868
8.265753	7.999766	7.361564	7.891861			0.070749	0.817558	0.139795
7.958904	7.95414	8.066031	8.598456			2.27E-05	0.011476	0.409027

7.726027	7.183748	7.529981	7.827545			0.294067	0.038434	0.010306
7.531507	6.714931	7.441591	7.627336			0.666795	0.008085	0.009183
7.887671	7.125056	7.073289	7.409542			0.581582	0.663218	0.228607
7.928767	6.984335	8.337506	8.487586			0.891951	0.167067	0.312278
7.928767	7.065574	7.059701	7.382047			0.745102	0.755277	0.298903
7.756164	7.278682	7.149324	7.516654			0.227989	0.368255	0.057365
7.117808	7.99461	8.064891	8.60477			0.768781	0.896966	2.211056
8.372603	8.50921	8.205747	8.993024			0.018661	0.027841	0.384923
8.808219	7.993356	8.60638	8.987827			0.664003	0.040739	0.032259
9.038356	7.939607	8.248781	8.65849			1.207251	0.623428	0.144298
8.638356	9.011984	8.251286	9.089608			0.139598	0.149823	0.203628
8.668493	8.890117	9.144445	9.624196			0.049117	0.22653	0.913367
8.60274	8.573676	8.125942	8.756716			0.000845	0.227336	0.023709
9.030137	8.591654	7.706481	8.425917			0.192267	1.752066	0.365082
8.673973	9.446073	9.392392	9.989201			0.596139	0.516127	1.729827
8.591781	9.334812	8.499786	9.314051			0.552095	0.008463	0.521674
9.358904	9.008598	8.308771	9.131769			0.122715	1.10278	0.05159
9.306849	9.169629	8.387088	9.442197			0.018829	0.84596	0.018319
8.939726	9.130412	9.066498	9.70832			0.036361	0.016071	0.590737
8.813699	9.094897	8.46826	9.49263			0.079073	0.119328	0.460948
8.805479	8.442803	8.161088	8.761348			0.131534	0.415241	0.001948
9.052055	9.381398	9.193725	10.0269			0.108467	0.02007	0.950324
8.747945	9.110397	9.011555	9.665117			0.131371	0.06949	0.841204

8.668493	9.113517	8.783349	9.715417			0.198046	0.013192	1.096051
9.021918	8.720247	8.885505	9.387695			0.091006	0.018608	0.133793
9.558904	8.419967	9.284779	9.652043			1.297177	0.075145	0.008675
10.15068	9.826983	9.281515	10.12752			0.104783	0.755457	0.000537
9.915068	10.23639	10.1757	10.79096			0.103245	0.067927	0.767194
9.920548	9.107631	9.632573	10.12482			0.660834	0.08293	0.041727
10.29041	10.21247	10.24664	10.83866			0.006075	0.001916	0.300582
9.652055	8.684358	8.902687	9.394972			0.936437	0.561552	0.066091
10.1589	10.04328	10.58244	11.05955			0.013369	0.179381	0.811166
10.05479	10.05398	10.11249	10.73064			6.7E-07	0.003329	0.456762
10.07945	9.270457	9.449051	10.00922			0.654474	0.397406	0.004932
9.561644	8.52915	8.822583	9.303491			1.066044	0.546211	0.066643
9.487671	8.855563	9.294004	9.73712			0.399561	0.037507	0.062225
9.871233	10.27932	10.3198	10.89587			0.166534	0.201211	1.049872
10.30685	8.856624	9.094953	9.57881			2.103154	1.468692	0.530042
9.871233	9.492708	9.608913	10.15543			0.143281	0.068812	0.080768
10.06027	10.59756	10.76238	11.23436			0.288678	0.492948	1.378476
9.40274	8.643792	8.855043	9.490846			0.576001	0.299971	0.007763
9.808219	8.270573	9.030579	9.574229			2.364357	0.604725	0.054751
9.712329	7.879168	8.71748	9.293359			3.360479	0.989724	0.175535
10.22192	9.698909	8.786781	9.769059			0.273538	2.059618	0.205081

9.942466	9.762072	10.75631	11.15698			0.032542	0.662339	1.475042
10.73151	11.59425	11.5542	12.00814			0.74432	0.676832	1.629801
10.5589	11.06197	11.0027	11.60511			0.253073	0.196954	1.094554
10.9863	9.588336	9.593879	10.15625			1.954307	1.93884	0.688992
10.6411	9.886057	9.918581	10.434			0.570084	0.522028	0.042888
11.2137	9.85259	10.99469	11.33222			1.852617	0.047967	0.014046
10.60548	8.879058	9.434701	10.15208			2.98053	1.370723	0.205575
11.07671	8.967951	10.62002	10.99182			4.446873	0.208564	0.007206
10.61644	10.00383	9.680377	10.42295			0.375286	0.876211	0.037437

10.79726	8.992885	10.25113	10.73508			3.255771	0.298253	0.003866
10.82192	10.2536	10.57014	11.06933			0.322989	0.063392	0.061211
11.04932	10.28622	9.805936	10.75321			0.582308	1.545992	0.087676
10.56164	10.45777	9.902764	10.82864			0.010789	0.434122	0.071288
11.09863	10.28049	10.21828	10.82471			0.669346	0.775013	0.07503
11.0274	10.56946	10.67526	11.17075			0.209703	0.123999	0.020551
10.60274	10.24079	9.671209	10.65981			0.131008	0.86775	0.003258
11.26027	10.45251	9.855012	10.79619			0.65249	1.974761	0.215378
11.28219	10.0928	10.01043	10.6624			1.414646	1.617382	0.384144
11.10137	11.2388	11.07738	11.66595			0.018886	0.000575	0.318752
10.6411	11.45348	11.36752	11.87407			0.659967	0.527686	1.520227
11.80274	11.4164	11.29912	11.82582			0.149262	0.253634	0.000533
11.42192	11.77933	11.64487	12.27882			0.127744	0.049707	0.734274
11.75068	12.32769	12.04396	12.55966			0.332935	0.086013	0.654433
12.02192	12.38612	12.088	12.59056			0.13264	0.004367	0.323357
12.09041	12.2462	11.91474	12.47241			0.024269	0.030859	0.145926
12.10959	9.432345	10.00964	10.60404			7.167634	4.409807	2.266681
11.98904	8.690608	8.369271	9.387768			10.87966	13.10274	6.766624
12.09041	10.27092	10.17464	10.79325			3.310547	3.670173	1.682637
11.91781	9.150535	9.63944	10.13527			7.657799	5.19096	3.177436
11.95342	10.25029	10.24548	10.84116			2.900663	2.917067	1.237125
11.75616	10.24045	10.10663	10.74285			2.297402	2.72095	1.026816
12.05479	10.05096	9.928978	10.60157			4.015353	4.519097	2.111871
12.28493	9.330019	10.36029	10.8412			8.731511	3.704263	2.084356
11.76438	9.957749	10.90954	11.28166			3.26393	0.730759	0.233026
12.28493	9.849492	10.54867	11.01889			5.931366	3.014593	1.602854
11.59452	10.49877	10.3525	10.93802			1.200661	1.542622	0.430989
11.75616	9.500079	10.64643	11.05693			5.089921	1.231516	0.488935
11.69315	10.62359	10.73277	11.21586			1.14396	0.922331	0.22781
12.00822	10.5359	10.60711	11.11998			2.167711	1.963099	0.78896

11.67397	11.31275	11.4383	11.9133			0.130481	0.055543	0.057278
11.86027	12.50235	12.20022	12.66807			0.41226	0.115564	0.65254
11.92055	11.36474	11.43416	11.91362			0.308922	0.236576	4.8E-05
12.1726	11.01519	11.50753	11.94211			1.339603	0.442323	0.053125
11.92329	10.39626	11.62786	11.98611			2.331806	0.087276	0.003947

12.31781	11.08712	11.73251	12.09785			1.51459	0.342576	0.048384
13.0137	11.41694	11.50223	11.96256			2.549632	2.284547	1.104894
13.0137	11.341	11.38681	11.88033			2.797927	2.646776	1.28452
13.04658	11.53361	11.31079	11.84068			2.289053	3.012944	1.454183
12.93699	11.15951	11.73373	12.103			3.159407	1.447821	0.69554
12.90685	11.34975	11.32844	11.84157			2.424563	2.491367	1.134819
12.9589	12.51928	12.24017	12.69473			0.193271	0.516574	0.069788
12.70685	11.57395	11.4151	11.9133			1.283472	1.668612	0.629719
13.08767	11.31249	11.20246	11.75454			3.151254	3.554037	1.777231
12.67945	13.21639	12.61767	13.17649			0.288306	0.003818	0.247046
13.00274	13.31133	12.82653	13.3108			0.095229	0.03105	0.094903
12.85479	13.27732	12.7517	13.26268			0.178526	0.010629	0.166371
12.74521	13.36014	12.93391	13.37985			0.378146	0.035609	0.402779
12.92603	13.33936	13.00496	13.42441			0.170841	0.006231	0.248383
12.04384	13.397	13.015	13.432			1.831054	0.94316	1.927
12.51233	13.397	13.015	13.432			0.782643	0.252678	0.845795
12.60822	13.37703	12.97106	13.40374			0.591063	0.131651	0.632856
12.83288	12.42294	12.32522	12.74639			0.168044	0.257713	0.00748
13.08493	12.26996	12.15852	12.632			0.664177	0.858236	0.205142
12.60822	13.05345	12.95518	13.38675			0.198232	0.120385	0.606108
13.35616	12.17122	12.28595	12.71116			1.404103	1.145351	0.416031
12.85205	13.21639	12.61767	13.17649			0.132743	0.054938	0.105258
12.53699	13.31133	12.82653	13.3108			0.599611	0.083836	0.598792
12.2137	13.27732	12.7517	13.26268			1.131285	0.289444	1.100363
12.99452	13.36014	12.93391	13.37985			0.133678	0.003674	0.148482

13.34795	13.32377	13.00225	13.42235			0.000585	0.119505	0.005537
14.33973	13.38621	12.99126	13.41673			0.909193	1.818357	0.851913
14.24384	14.232	13.689	14.102			0.00014	0.307843	0.020117
14.1589	14.232	13.689	14.102			0.005343	0.22081	0.003238
14.1863	14.232	13.689	14.102			0.002088	0.247309	0.007107
14.12055	14.232	13.689	14.102			0.012422	0.186234	0.000344
14.06027	12.32636	12.30356	12.72858			3.006449	3.086054	1.773417
13.63014	11.10967	11.87949	12.19812			6.352742	3.064781	2.050668
14.26027	12.85546	13.14921	13.50538			1.973516	1.234461	0.569865
14.10959	13.13642	13.20289	13.5454			0.947067	0.822105	0.318307
14.15616	13.20836	13.04365	13.4461			0.898324	1.237685	0.504194
14.11233	12.84624	13.50948	13.73335			1.602985	0.363421	0.143627
13.6274	13.34864	12.90862	13.36359			0.077703	0.516646	0.069595
13.76712	13.35278	12.91772	13.36945			0.171677	0.721479	0.158148
13.56438	13.35888	12.93115	13.37808			0.04223	0.400989	0.03471
13.6274	13.29988	12.80135	13.29461			0.107264	0.682359	0.110749
14.24658	13.35888	12.93115	13.37808			0.787995	1.730354	0.75429
13.69589	13.38637	12.9916	13.41696			0.095806	0.496019	0.077805
13.45479	13.38001	12.97763	13.40797			0.005592	0.227684	0.002193
14.1863	13.22558	12.63787	13.18948			0.922991	2.397639	0.993649
14.00822	13.24184	12.67365	13.21249			0.587336	1.781076	0.633184
13.48493	13.32228	12.85061	13.32629			0.026456	0.402361	0.025168
14.15616	13.28097	12.75973	13.26785			0.765966	1.950022	0.789107
13.48493	13.28184	12.76165	13.26908			0.041246	0.523135	0.046592
13.83288	13.38369	12.98572	13.41317			0.20177	0.717682	0.176155
13.77534	14.232	13.689	14.102			0.208536	0.007455	0.106705
13.77534	14.232	13.689	14.102			0.208536	0.007455	0.106705
14.07123	14.232	13.689	14.102			0.025846	0.146102	0.000947
14.24932	14.232	13.689	14.102			0.0003	0.313953	0.021702
13.66849	14.232	13.689	14.102			0.31754	0.000421	0.187928

13.52329	14.232	13.689	14.102			0.502273	0.027461	0.334908
13.50959	14.232	13.689	14.102			0.521878	0.032188	0.350951
						301.4258	215.1595	159.88
						0.944908	0.674481	0.501191
						0.944908	0.674481	0.501191
						1.449879	1.100984	0.700807

Appendix 5

	Females				Males	
Age	CA	Es		Age	CA	Es
12.78082	0.781897	15.25497		7.854795	0.542657	8.187964
8.019178	0.594255	10.33723		10.4	0.778037	14.3568
11.04932	0.754945	14.5486		13.51507	0.718	12.78334
13.98082	0.742959	14.23447		8.863014	0.621582	10.25641
14.27397	1.017986	21.44237		11.63014	0.757419	13.81643
12.70411	0.774194	15.05306		12.06301	0.738526	13.32128
5.416438	0.43472	6.156136		13.83014	0.866215	16.66776
12.2274	0.641278	11.5696		13.80548	0.65221	11.05913
14.13973	0.84493	16.90694		10.52877	0.755011	13.75333
11.72055	0.660014	12.06065		10.3863	0.676971	11.70805
14.16986	0.657883	12.0048		13.98082	0.756419	13.79024
13.26301	0.769006	14.91711		12.84658	0.719264	12.81648
11.39178	0.743342	14.24449		12.5863	0.615524	10.09765
11.18082	0.80807	15.9409		8.936986	0.684211	11.89779
11.09863	0.757576	14.61755		8.824658	0.637807	10.68164
8.158904	0.537536	8.850739		11.53425	0.675342	11.66538
7.827397	0.542281	8.975112		9.857534	0.725322	12.97524
10.69589	0.696117	13.00682		12.81096	0.762898	13.96003
13.2411	0.802553	15.7963		5.246575	0.486475	6.715548
5.928767	0.509513	8.116322		13.80274	0.757576	13.82055
11.98082	0.716284	13.53537		13.80274	0.737547	13.29562
9.194521	0.606533	10.65901		13.8411	0.729889	13.09494

13.49863	0.750795	14.43984		7.372603	0.541126	8.147818
12.70137	0.733803	13.9945		14.78904	0.857647	16.44321
10.65205	0.867044	17.48649		6.882192	0.510579	7.34725
5.534247	0.502076	7.921411		13.40822	0.784767	14.53318
6.980822	0.563826	9.539755		12.91507	0.829461	15.7045
11.47671	0.763858	14.78219		14.80274	0.861842	16.55316
8.652055	0.603281	10.5738		10.3589	0.732416	13.16116
14.47671	0.7006	13.12431		15.29315	0.865815	16.65727
10.47945	0.604478	10.60515		8.224658	0.727273	13.02636
11.32329	0.758025	14.62931		11.9726	0.633838	10.57764
12.2274	0.789474	15.45353		14.87671	0.633838	10.57764
7.235616	0.447872	6.500838		14.44384	0.865485	16.64863
13.30685	0.802105	15.78457		13.8137	0.895928	17.44647
13	0.733628	13.98993		8.671233	0.684706	11.91077
12.29589	0.859155	17.27973		13.71507	0.743226	13.44446
9.619178	0.681542	12.62484		13.29315	0.689571	12.03827
12.87671	0.810039	15.99251		12.72055	0.754762	13.74679
14.53151	0.790566	15.48215		7.750685	0.653479	11.09238

10.50137	0.604214	10.59824		8.791781	0.665347	11.4034
8.035616	0.733835	13.99534		12.96712	0.690169	12.05396
12.18904	0.580116	9.966676		8.4	0.703892	12.41361
12.40548	0.840353	16.78696		7.717808	0.622142	10.27109
22.91507	0.864951	17.43164		8.849315	0.607865	9.89693
12.07123	0.617512	10.94676		14.10411	0.753902	13.72425
12.01644	0.72627	13.79709		13.90959	0.741984	13.41192
19.38904	0.959147	19.90031		12.26575	0.736248	13.26159
16.03288	0.857459	17.23528		22.93151	0.845807	16.13291
16.49315	0.784726	15.3291		16.17534	0.805704	15.08189
12.4274	0.73924	14.13699		16.50959	0.794403	14.78571
15.70137	0.787837	15.41063		16.39726	0.757709	13.82402
8.328767	0.553644	9.272893		15.36712	0.695508	12.19386
7.512329	0.538375	8.872738		20.83836	0.989251	19.8923
12.59452	0.721961	13.68416		22.07123	1.03187	21.00926
16.74795	0.853382	17.12842		21.50411	0.941789	18.64839
16.66027	0.843423	16.86743		22.41918	1.063868	21.84785

15.06301	0.843707	16.87487		20.09315	0.945825	18.75419
13.17534	0.812529	16.05777		20.54795	0.894114	17.39894
11.2137	0.675931	12.47779		6.150685	0.514932	7.461333
21.11781	0.959878	19.91947		17.63014	0.776964	14.32867
26.34521	1.199938	26.21097		16.16164	0.78334	14.49578
19.89589	0.858952	17.27442		21.5726	0.950031	18.8644
19.25205	0.927097	19.06036		19.00274	0.877856	16.97285
13.21096	0.69423	12.95737		6.821918	0.531111	7.885351
19.93699	0.905205	18.48661		15.6274	0.737703	13.29971
20.09863	0.875464	17.70717		18.06027	0.833577	15.81238
18.10959	0.887029	18.01025		15.38082	0.796196	14.83271
16.06849	0.791228	15.4995		16.13151	0.766031	14.04213
15.60822	0.742201	14.2146		13.98356	0.639885	10.7361
16.62192	0.834613	16.63653		20.3726	0.950824	18.88519
19.60274	0.896442	18.25695		9.936986	0.566606	8.815608
18.60548	0.857329	17.23189		18.01918	0.826966	15.63914
17.75342	0.894393	18.20325		14.38904	0.757251	13.81204
22.09589	0.994926	20.83801		23.47671	0.959461	19.11156
17.7863	0.785927	15.36058		9.460274	0.591897	9.478427
16.68219	0.809218	15.97099		19.10411	0.969685	19.37952
17.20274	0.939171	19.3768		13.18356	0.668249	11.47948
15.72877	0.746495	14.32715		13.20274	0.692261	12.10878
14.92877	0.722594	13.70074		16.0411	0.773985	14.25061
23.0274	0.999144	20.94855		12.73151	0.657266	11.19163
18.56712	0.822634	16.32258		15.47671	0.678813	11.75633
17.61644	0.826098	16.41338		10.03288	0.531985	7.908254
17.51781	0.816154	16.15277		17.46849	0.825467	15.59984

23.22466	0.900249	18.35673		18.38904	0.852864	16.31785
22.7863	1.056769	22.45881		21.52603	0.966987	19.3088
20.1726	0.854154	17.14868				
18.94247	0.843344	16.86535				
19.31507	0.903242	18.43515				
18.2274	0.878922	17.7978				
19.76164	0.838277	16.73256				

21.02192	0.97901	20.4209				
16.80822	0.777947	15.15143				
14.25753	0.863131	17.38394				
18.76712	0.824251	16.36498				
16.22192	0.757928	14.62678				
15.30685	0.756137	14.57984				
21.16986	0.898984	18.32358				
21.93425	0.866999	17.48531				
20	0.901123	18.37963				
16.75616	0.746537	14.32823				
17.16164	0.804478	15.84675				
17.83014	0.856333	17.20577				
14.08493	0.604478	10.60515				
17.36438	0.758089	14.63099				
17.56712	0.784451	15.3219				
10.31781	0.590169	10.23016				
11.75068	0.604214	10.59824				
19.39452	0.846006	16.93512				
18.11781	0.882581	17.89368				
21.91781	0.951076	19.6888				
18.06027	0.82522	16.39035				
24.04658	0.9241	18.98181				
20.92603	0.988897	20.68002				
23.87945	1.035761	21.90822				
16.5726	0.672228	12.38074				
17.4137	0.789743	15.46058				
14.38082	0.74975	14.41246				
14.44658	0.793204	15.55128				
21.00274	0.931499	19.17574				
18.97808	0.804778	15.85461				
20.79726	0.932125	19.19213				
22.64384	0.934951	19.2662				
16.4	0.870401	17.57447				
15.37534	0.699793	13.10317				
15.29863	0.829715	16.50818				

8.964384	0.550018	9.177873				
15.71781	0.719557	13.62115				
14.61918	0.717771	13.57433				
17.93699	0.880912	17.84995				
15.30137	0.710801	13.39168				
19.49863	0.727263	13.82311				
22.04932	0.919031	18.84896				
21.04658	0.867171	17.48983				
9.69863	0.775977	15.09982				
12.97808	0.49853	7.828474				
12.97808	0.669704	12.3146				
10.97534	0.700264	13.11552				
7.046575	0.520285	8.39862				
9.526027	0.612697	10.82056				
20.70137	0.929973	19.13572				