DENTAL AGE IN KELANTANESE MALAY POPULATION BASED ON DEMIRJIAN'S METHOD

By

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DEDICATION

To

My beloved family

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Dr. Saifeddin Hamed Abu Asab

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CA	Chronological age
DA	Dental age
MS	Maturity score
OPG	Orthopantomogram or Panoramic radiographs
M_2	Second mandibular permanent molar
M_1	First mandibular permanent molar
PM_2	Second mandibular permanent premolar
PM_1	First mandibular permanent premolar
C	Permanent mandibular Canine
I_2	Permanent mandibular lateral incisor
I_1	Permanent mandibular central incisor
SPSS	Statistical Package for the Social Sciences
HUSM	Hospital Universiti Sains Malaysia
HKB	Hospital Kota Bharu

ABSTRAK

Umur gigi amat penting untuk menganggarkan umur seseorang individu yang belum dewasa, membantu pakar pergigian pediatrik dan pakar ortodontik merancang pelan rawatan yang betul dan membuat penilaian perkembangan tahap gigi untuk sesuatu masalah perubatan. Demirjian et al. (1973) telah memperkenalkan kaedah radiografi umur gigi iaitu peringkat kalsifikasi tujuh gigi kekal rahang bawah telah dinilai. Kaedah ini kemudiannya telah diubah sedikit pada tahun 1976 dan dua kaedah lagi telah di cadangkan iaitu empat batang gigi telah digunakan sebagai ganti tujuh batang gigi (Demirjian dan Goldstein, 1976). Kaedah Demirjian masih lagi digunakan secara meluas dan telah digunakan dalam pelbagai kajian mengenai radiografi umur gigi. Tujuan kajian ini ialah : 1) untuk memeriksa ketepaten kaedah Demirjian (1973, 1976) dalam menganggarkan umur kronologi kanak-kanak Melayu di Kelantan, lelaki dan perempuan yang berumur 5 hingga 16 tahun, 2) untuk mewujudkan standard baru umur gigi untuk populasi Melayu di Kelantan; jika kaedah Demirjian tidak sesuai untuk digunakan ke atas populasi Melayu di Kelantan, 3) untuk membandingkan kelok umur gigi di antara kanak-kanak Melayu di Kelantan dengan kanak-kanak Perancis-Kanada, 4) untuk menentukan dwimorfisme seksual pada penilaian umur gigi kanak-kanak Melayu di Kelantan 5) untuk mengesan perbezaen umur gigi dan skor kematengan di antara gigi kakal bahagian bawah kiri dan kanan dan 6) untuk mengesan median umur pencapaian pada setiap peringkat perkembangan, mengikut peringkat Demirjian untuk tujuh batang gigi bahagian bawah kiri. Sejumlah 905 radiograf panoramik (OPG) untuk kanak-kanak Melayu di Kelantan yang berumur 5 hingga 16 tahun telah dikumpul dari unit radiografi

di Hospital Universiti Sains Malaysia (HUSM) dan klinik pakar ortodontik di Hospital Raja. Perempuan Zainab II (HRPZ II). Umur gigi telah dinilai secara keratan lintang dengan menggunakan kaedah Demirjian (1973 dan 1976). Kanak-kanak yang mempunyai sebarang penyakjt yang diketahui telah menjejaskan perkembangan gigi, atau mempunyai agenesis di bahagian lengkung bawah telah diketepikan, begitu juga dengan imej-imej OPG yang bekualiti rendah. Kebolehpercayaan pemeriksa intra dan inter menunjukkan nilai yang tinggi untuk skor kematangan dan umur gigi. Diskrepensi pada peringkat gigi adalah tidak lebih atau kurang dari satu peringkat. Nilai kappa untuk persetujuan antara para pemeriksa adalah dalam korelasi yang baik. Keputusan kajian menunjukkan bahawa kaedah Demirjian (1973) telah menganggar lebih umur kronologi sebanyak dengan 1.24 tahun bagi kanak-kanak lelaki dan 1.27 tahun untuk kanak-kanak perempuan. 4 gigi kaedah: M_2, M_1, PM_2, PM_1 telah menganggar lebih umur dengan 1.23 tahun untuk kanak-kanak lelaki dan 1.20 tahun untuk kanak-kanak perempuan. Manakala 4 gigi kaedah: M_2, PM_2, PM_1, I_1 telah menganggar lebih umur dengan 0.64 tahun untuk kanak-kanak lelaki dan 0.71 tahun untuk kanak-kanak perempuan. Oleh sebab kaedah Demirjian (1973) adalah tidak tepat digunakan kepada kanak-kanak Melayu di Kelantan, standard umur gigi janita spesifik yang baru dinbahsuai telah dihasilkan dengan mengubahsuai kaedah Demirjian (1973). Empat puluh tujuh sampel dari luar yang terdiri dari kanak-kanak Melayu di Kelantan (23 lelaki dan 24 perempuan) telah di pilih secara rawak dari Hospital Universiti Sains Malaysia (HUSM) bertujuan utuk menguji ketepatan kaedah Demirjian yang telah diubahsuai untuk populasi Melayu di Kelantan dan keputusan menunjukkan bahawa perbezaan min di antara umur kronologi dan umur gigi hanyalah 2 minggu untuk kedua-dua jantina. Untuk perbandingan perkembangan gigi antara kanak-kanak Melayu di Kelantan dengan kanak-kanak Perancis-Kanada, keputusan menunjukkan bahawa umur gigi kanak-kanak lelaki yang lebih muda (7.0-9.99 tahun) adalah tidak berbeza secara statistik daripada kanak-kanak Perancis-Kanada ($P > 0.05$). Walau bagaimanapun Selepas umur 10 tahun, secara statistik, perbezaan menjadi signifikan untuk kanak-kanak lelaki ($P < 0.001$). manakala kanak-kanak perempuan lelaki maju pada umur gigi berbanding kanak-kanak perempuan kanada, pada semua kumpulan umur dan perbezaan adalah signifikan secara statistik ($P <0.05$) Kedua-dua kanak-kanak lelaki dan perempuan matang pada umur yang sama iaitu 6 tahun dan tiada perbezaan yang signifikan secara statistic anatara kedua duanye. Selepas umur 6 tahun, kanak-kanak perempuan adalah lebiah maju pada umur gigi berbanding kanak-kanak lelaki untuk semua kumpulan umur (7.00 – 10.99 tahun) tiada perbezaan yang signifikan yang didepati pada perkembangan gigi bahagian bawah kiri jika dibandingkan dengan bahagian gigi bawah kenen ($P > 0.05$). Median pencapaian umur untuk setiap peringkat perkembangan, mengikut peringkat Demirjian untuk 7 gigi kekal bahagian kiri bawah untuk kedua-dua janita, telah dihasilkan dan keputusan menunjukkan bahawa kanak-kanak perempuan adalah lebih maju pada umur gigi jika dibandingkan dengan kanak-kanak lelaki, untuk semua gigi kecuali molar yang pertama. Kesimpulannya, kaedah Demirjian (1973, 1976) menunjukkan bahawa ia tidak tepat untuk digunakan ketika menganggar umur kronologi pada sampel kanak-kanak Melayu di Kelantan. Pengubahsuaian pada sistem ini menghasilkan satu sistem umur gigi baru yang mempunyai ketepatan yang lebih baik untuk kanak-kanak Melayu di Kelantan. Umur gigi kanak-kanak lelaki dan perempuan Melayu di Kelantan adalah lebih maju berbanding kanak-kanak Perancis-Kanada pada kesemua kumpulan umur untuk kanak-kanak perempuan dan kumpulan kanak-kanak lelaki yang lebih tua (10 hingga 15.99 tahun). Umur gigi kanak-kanak perempuan di Kelantan adalah lebih maju jika dibandingkan dengan kanak-kanak lelaki pada semua kumpulan umur dan setiap peringkat perkembangan gigi. Perkembangan gigi adalah selaras pada kedua-dua belah rahang bawah.

ABSTRACT

Dental age has a great importance in age estimation for non-adult individuals, helping the paediatric dentists and orthodontists to produce proper treatment plan, and assessment of the dental developmental level of certain medical conditions. Demirjian *et al.* (1973) introduced a method of radiographic dental age in which the calcification stages of the seven permanent mandibular teeth were assessed. The method was revised in 1976 and two methods based four permanent teeth instead of seven teeth were proposed (Demirjian and Goldstein, 1976). Demirjian's method is still widely accepted and used in studies of radiographic dental age in different geographic regions. The purposes of this study were 1) to examine the applicability of Demirjian's methods (1973, and 1976) for estimating the chronological age of male and female Kelantanese Malay children aged 5 to 16 years old, 2) to establish a new dental age standard; if Demirjian's methods were not applicable on the Kelantanese Malay population, 3) to compare the dental age curves between Malay children and French-Canadian children, 4) to determine the sexual dimorphism in the dental age assessment of Kelantanese Malay children, 5) to detect the differences in 'dental ages' and 'maturity scores' between the lower left permanent teeth and the lower right and 6) to detect the median ages of attainment of each stage of dental development according to Demirjian stages for the lower left seven teeth. A total number of 905 panoramic radiographs (OPG) of Kelantanese Malay children aged 5 to 16 years old have been collected from radiographic unit in the Universiti Sains Malaysia (HUSM), and Orthodontic Dental Specialist Clinic – Hospital Raja.

Perempuan Zainab II (HRPZ II). The dental age has been assessed cross-sectionally by using Demirjian's methods (1973, and 1976). Children who had any disease that was known to affect the dental development, or have agenesis in the lower arch were excluded, as well as, those poor quality OPG images. The intra and inter-examiner reliability showed high values for 'maturity scores' and 'dental ages'. Discrepancies in staging the teeth did not exceed one stage more or less. The kappa values for the agreement between the examiners were in good correlation. The results showed that Demirjian's method (1973) overestimated the chronological age by 1.24 year for boys and 1.27 years for girls, respectively. The 4 teeth: M_2, M_1, PM_2, PM_1 method overestimated the age by 1.23 years for boys and 1.20 years for girls, respectively. While 4 teeth: M_2, PM_2, PM_1, I_1 method overestimated the age by 0.64 year for boys and 0.71 years for girls, respectively. As Demirjian's method (1973) was not accurate on Kelantanese Malay children, new modified sex-specific dental age standards for Kelantanese Malay were produced by modifying Demirjian's method (1973). An external sample of 47 Kelantanese Malay children (23 boys and 24 girls) was randomly selected from the Universiti Sains Malaysia Hospital (HUSM) in order to test the accuracy of the modified Demirjian's method on Kelantanese Malay population and results showed that the mean difference between the chronological age and dental age is about 2 weeks for both sexes. In comparison between the dental development between Kelantanese Malay and French-Canadian children, results showed that the 'dental age' for younger age groups of boys (7.0 - 9.99 years) was not statistically different from French-Canadian children ($P > 0.05$). However, after the age 10 years, the difference became statistically significant in boys ($P < 0.001$). On the other hand, girls were more advanced in dental age as compared to

Canadian girls in all age groups as the difference was statistically significant (P < 0.05). Both boys and girls have similar maturation at 6 years old with no statistically significant difference between them. After the age group 6 years, the girls were more advanced in dental age as compared to boys for all age groups (7.00 – 10.99 years). No significant difference was found in the dental development of the lower left teeth when compared with the right lower teeth (P > 0.05). The median ages of attainment of each developmental stage according to Demirjians' stages for the lower permanent left seven teeth for both sexes have been produced and showed that girls are more advancement in dental age as compared to boys in all teeth except the first molar. In conclusion, Demirjians' methods (1973, 1976) showed to be not accurate to estimate the chronological age in Kelantanese Malay children samples. The modification of the system had resulted in a new dental age system that is more precise and more accurate for the Kelantanese Malay children. Dental age is more advanced in Kelantanese Malay boys and girls as compared to French-Canadian children in all age groups for girls and older age groups for boys (10 to 15.99 years). Dental age is more advanced in Kelantanese Malay girls as compared to boys in all age groups and in all stages of dental development. Dental development is in harmony in both sides of the lower jaw.

Chapter 1

Introduction

1.1 Introduction

Dental age can be defined as a measure of how far the teeth have progressed towards maturity (Koch *et al.*, 1994), or a measure of childhood dental development (Corsini, 1999), and it corresponds to odontogenesis, development and emergence of teeth (Shimano. 1995).

Dental age can be assessed for deciduous or permanent teeth by determination of the chronology of emergence of teeth through the oral tissues (eruption time) (Nizam *et al.* 2004; Parner *et al.*, 2001), by counting the number of emerged teeth into the oral cavity (Foti *et al.*, 2003), by tracing the calcification progress of dental tissues using successive radiographic films (Demirjian *et al.*, 1973; Moorrees *et al.*, 1963; Nolla, 1960; Garn *et al.*, 1956). Dental age can also be assessed histologicaly by assessing the teeth developmental stage of dried mandible and maxilla bones that have been taken from dead children who their chronological ages are known at the time of death (Logan and Kronfeld, 1933).

The dental system is an integral part of the human body. Knowledge about dental age can be applied in estimation of chronological age at the time of death (Frucht *et al.*, 2000), assisting the paediatric dentists and orthodontists to plan for a proper treatment (Moorrees *et al.*, 1963), and can be used to assess the level of dental development in certain medical conditions (Ribeiro *et al.*, 2002; Kjellberg *et al.*, 2000).

Age estimation at death is one of the identification methods used by the forensic anthropologists and odontologists to narrow down the potential identities of the deceased individuals (Byers, 2001). Age estimation of sub-adult individuals is based on a physiological assessment of dental and/ or skeletal age (Lewis and Flavel, 2006). Sub-adult stage can be defined as the period in which the skeletal and dental tissues are in growing status (Byers, 2001).

Dental age seems to be better than skeletal age for age estimation at death as dental age is less affected by variation in nutritional and endocrine status when compared to other physiological age methods (Garn *et al.,* 1965 a, and b), and is correlated better with the chronological age than skeletal age (Demirjian *et al.,* 1985).

It is important for every dentist treating children to have a good understanding of development of the dentition. In interceptive orthodontic, knowing the time of each stage of tooth development can give a general idea to dental clinicians in proposing proper treatment plans and times. By way of example, prediction the emergence time of permanent teeth based on root developmental stage can help in planning for serial extraction (Moorrees *et al.*, 1963). Moreover, the time of apical part closure can help in anticipation the proper time for apexification.

Beside this, dental age can be used as a measurement tool to assess the degree of effect of some diseases (e.g. Turner's syndrome or cleft lip and palate) (Ribeiro *et al.*, 2002; Hass *et al.*, 2001), or drugs (e.g. chemotherapy or Cortisone) (Lehtinen *et al.*, 2000; Nasman *et al.,* 1997) on the dental development, this in turn will give a general knowledge to dental clinicians about planning a proper dental treatment for such cases.

1.2 Justification of the study

Variations in dental development exist between different populations (Reid and Dean, 2006; Frucht *et al.*, 2000; Davis and Hagg, 1994). Thus, foreign dental developmental standards and data might not be accurate for the local people, and each population should establish their own reference charts and standards. Therefore, a valid standard of dental age in Malay population should be constructed. Dental age based on time of permanent teeth eruption on Malay population has been conducted on more than 2000 school children aged 5- 17 years (Nizam *et al.*, 2003). Dental age, however, based on radiographic standard of permanent teeth need to be investigated since there is just one study available in this area (Mani *et al.*, 2008).

1.3 General objective

To determine the radiographic dental age standard for Kelantanese Malay boys and girls aged 5 to 16 years old.

1.4 Specific objectives

1) To evaluate the accuracy of Demirjians' methods (1973, 1976) in estimating the chronological age of Kelantanese Malay male and female aged 5 to 16 years old.

2) To establish a new dental age standards for the Kelantanese Malay population by modifying the Demirjian's method (1973); if Demirjian methods were not accurate on the Kelantanese Malay population.

3) To compare the dental age standards of Kelantanese Malay with that of French-Canadian children.

4) To determine the sexual dimorphism in the dental age assessment of all patients.

5) To assess the side differences of the dental age between the lower right and left quadrants within the same patient.

6) To determine the median age of attainment of each developmental stage according to Demirjian stages for the lower permanent left seven teeth for both sexes.

1.5 Hypothesis

1) The dental age which is estimated by Demirjians' methods (1973, 1976) are not in accordance to the chronological age of Kelantanese Malay children.

2) Kelantanese Malay Children are advanced in dental age as compared to French-Canadians.

3) Girls dental age is more advanced than boys' dental age.

4) Maturity scores and dental ages are equal in both sides of the lower jaw.

Chapter 2

Literature Review

2.1 Tooth formation

Many descriptions of the process of tooth formation have been written over the past several decades. Teeth development from two types of cells: oral epithelium cells form the enamel organ and mesenchymal cells form the dental papilla. Enamel develops from the enamel organ, and dentin forms from the dental papilla. The interaction of these epithelial and mesenchymal cells is vital to the initiation and formation of the teeth. In addition to these cells, the neural crest cells contribute to tooth development. The neural crest cells arise from the neural cells at an early stage of development and migrate into the jaws, intermingling with mesenchymal cells. They function by integrating with the dental papillae and epithelial cells of the early enamel organ, which aids in the development of the teeth. The cells also function in the development of the salivary glands, bone, cartilage, nerves, and muscles of the face (Chandra *et al.* 2004; Avery and Chiego, 2006).

The first sign of tooth formation is the development of dental lamina rising from the oral epithelium. Dental lamina develops into a sheet of epithelial cells that pushes into the underlying mesenchyme around the perimeter of both the maxillary and mandibular jaws. At the leading edge of the lamina, 20 areas of enlargement appear which form tooth buds for the 20 primary teeth. At this early stage, the tooth buds have already determined tooth morphological class either an incisor or molar. This is the result of gene expression (Avery and Chiego, 2006). After primary teeth develop from the buds, the leading edge of the lamina continues to grow to develop the permanent teeth, which succeed the 20 primary teeth. This part of lamina is thus called successional lamina. The lamina continuous posteriorly into the elongating jaw and from it come the posterior teeth, which form behind the primary teeth. In this manner, 20 of the permanent teeth replace the 20 primary teeth, and 12 posterior permanent molars develop behind the primary dentition. The last teeth to develop are the third molars, which develop about 15 years after birth (Avery and Chiego, 2006). Because the molars do not succeed the primary teeth, they do not form from the successional lamina but from the general lamina. The initiating dental lamina that forms both the successional and general lamina begins to function in the sixth prenatal week and continuous to function until the fifteenth year, producing all 52 teeth (Avery and Chiego, 2006; Nanci and Ten Cate, 2003).

Although tooth formation is a continuous process, it is characterized by a series of easily distinguishable stages known as the bud, cap, and the bell stages (Figure 2.1). Each stage is defined according to the shape of the epithelial enamel organ, which is a part of the developing tooth (Avery and Chiego, 2006).

At this stage, the inner enamel epithelial cells are characterized by the shape of the tooth they form. The cells of the enamel organ have differentiated into the outer enamel epithelial cells which cover the enamel organ, and inner enamel epithelial cells, which become the ameloblasts that form the enamel of the tooth crown.

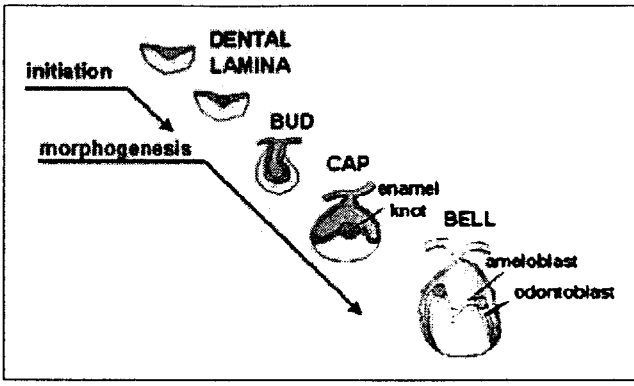

Figure 2.1: Stages of tooth formation (Bud, Cap, and Bell stages) (Theslef, 2004)

During the bell stage, cells in the periphery of the dental papilla become odontoblasts which form the dentin (Nanci and Ten Cate, 2003). These cells differentiate from mesenchymal cells. When several increments of dentin have formed, the differentiated ameloblasts deposit an enamel matrix. Dentinogenesis always precedes amelogensis (Avery and Chiego, 2006; Chandra *et al.* 2004; Nanci and Ten Cate. 2003).

2.1.1 Dentinogenesis

Increments of dentin are formed along the dentinoenamel junction. The dentinal matrix is first a meshwork of collagen fibers, but within 24 hours it becomes calcified. It is called predentin before calcification and dentin after calcification. As each daily increment of predentin forms along the pulpal boundary, the adjacent peripheral increment of predentin formed the previous day calcifies and becomes dentin (Avery and Chiego, 2006; Nanci and Ten Cate, 2003).

2.1.2 Amelogenesis

Ameloblasts begin enamel deposition after a few micrometers of dentin have been deposited at the dentoenamel junction. At the bell stage, cells of the inner enamel epithelium differentiate. They elongate and are ready to become active secretory ameloblasts.

Only a few ameloblasts at the tip of the crown cusp begin to function initially. As the process proceeds, more ameloblasts become active. and the increments of enamel matrix become more prominent (Avery and Chiego, 2006).

Growth of individual cusps by incremental deposition continues until tooth eruption. This occurs in posterior multicuspid teeth as the ameloblasts continue to differentiate from the inner enamel epithelium and from enamel. Cusps then coalesce in the intercuspal region of the crown (Nanci and Ten Cate, 2003).

2.1.3 Crown formation

As the process of amelogensis continues, the enamel matrix begins to mineralize. As soon as the small crystals of mineral are deposited, they begin to grow in length and diameter. The pattern of mineralization closely follows the pattern matrix deposition. The first matrix deposited is the first enamel mineralized, occurring along the dentinoenamel junction. Matrix formation and mineralization continue peripherally to the tips of the cusps then laterally on the sides of the crown, following the enamel incremental deposition pattern. Finally, the cervical region of the crown mineralizes (Nanci and Ten Cate, 2003). Human enamel is known to form at a rate of approximately 4 – um per day. Ground section of enamel reveal what appear to be periodic bands or cross striation at 4-um intervals across (Nanci and Ten Cate, 2003).

With the mineralization of enamel complete and its thickness established, the crown of the tooth is formed (Avery and Chiego. 2006).

2.1.4 Development of the tooth root

As the crown develops, cell proliferation continues at the cervical region or base of the enamel organ, where the inner and outer enamel epithelial cells join to form a root sheath. When the crown is completed, the cells in the region of the enamel organ continue to grow forming a double layer of cells termed the epithelial root sheath or Hertwig's root sheath. The inner cell layer of the root sheath forms from the inner enamel epithelium or ameloblasts in the crown, and enamel is produced. In the root, these cells induce odontoblasts of the dental papilla to differentiate and form dentin. The root sheath originates at the point that the enamel deposits end (Nanci and Ten Cate, 2003).

As the odontoblasts differentiate along the pulpal boundary, root Dentinogenesis proceeds and the root lengthens. Dentin formation continues from the crown into the root. The dentin tapers from the crown into the root to the apical epithelial diaphragm. Dentinogenesis continues until the appropriate root length is developed. The root then thickens until the apical opening is restricted to approximately 1 to 3 mm, which is insufficient to allow neural and vascular communication between the pulp and the peridontium (Avery and Chiego, 2006).

With the increase in root length, the root begins eruptive movements, which provides space for further lengthening of the root. The root lengthens at the same rate as the tooth eruptive movements occur (Avery and Chiego, 2006).

2.2 Age estimation at death for sub-adults

Methods of estimating age at the time of death are many and vary. It is a parameter that once known, can be extremely useful in identifying an individual and it can narrow down the potential identification identity (Byers, 2001).

There are various definitions for adult and sub-adult stage in relation to age estimation at death that has been used by many researchers. McDonough and Stedman (1994) defined adult stage as when the person is fully grown and physically mature. Byers (2001) defined sub-adult stage as the period in which the skeletal and dental tissues are in growing status; as permanent teeth growing to reach the apical closure in the dental tissues and the secondary ossification centres of the bones continue to growth until they union their associated primary centres. When the growth ceases, the estimation of age will be based on deteriorating tissues as several structures in the body undergo changes over time; for example, the attrition of teeth or apposition of layers of cementum after the tooth is completely formed and (Lewis and Flavel, 2006; Byers, 2001). Byers (2001) definition is being used in this literature review to describe the sub-adult stage.

Age estimation of deceased sub-adult individuals is based on a physiological assessment of dental or skeletal maturation as they continue to develop until late into the second decade of life or the beginning of the third one (Byers, 2001). Thus, accurate age estimation for sub-adult relies on the accurate conversion of these two biological ages (dental and skeletal) into chronological age (Lewis and Flavel, 2006).

2.2.1 Skeletal tissues methods for sub-adults

The growth of the human skeleton is a complex process that is not fully understood. and the available information indicates that bones form within cartilaginous precursors by the deposition of calcium salts (Byers. 2001).

The deposition of calcium occurs initially in multiple areas, called primary and secondary areas of ossification (Byers, 2001). Primary centres of ossification or primary point of ossification can be defined as the first site where bone begins to form in the shaft of a long bone, or in the body of an irregular bone, while secondary centres of ossification are the centres of bone formation appearing later than the first centres and usually in epiphysis (McDonough and Stedman. 1994). Both centres eventually grow together forming larger segments such as diaphysis and epiphysis (Figure 2.2). When these areas of ossification unite, bone growth proceeds in earnest. Long bones grow to their ends under the epiphysial caps. At a mostly genetically predetermined time, the epiphyses fuse to the diaphyses (at the metaphyses), causing growth to cease (Bayer, 2001).

Other bones grow by apposition over all surfaces i.e. growth accomplished by the addition of new layers on those previously formed one (e.g. those of the wrist and ankles), while the skull grows along the edges of its bones at the suture lines (Byers, 2001).

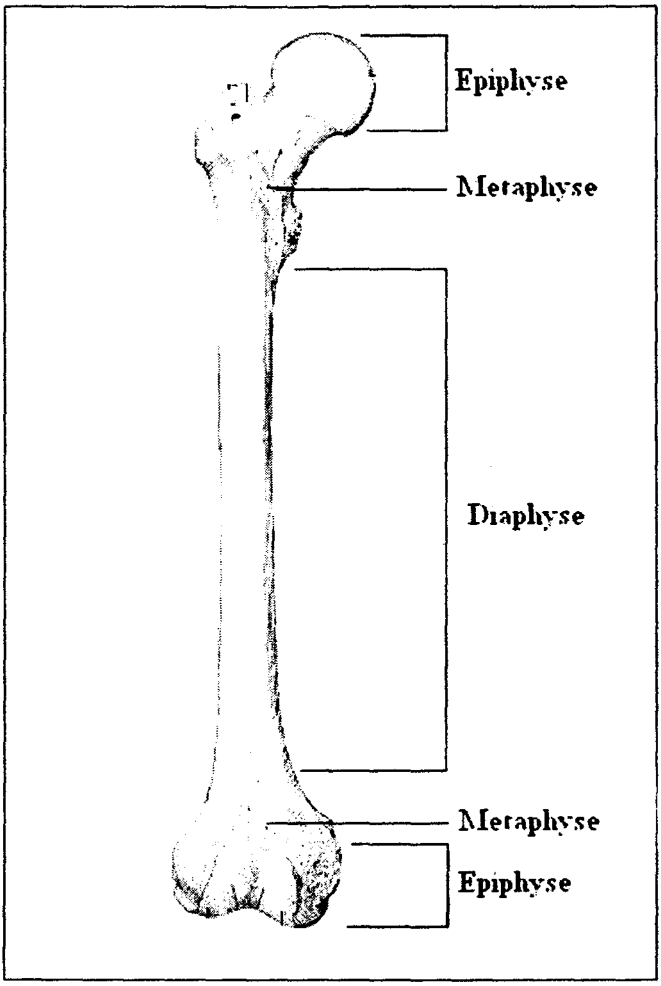

Figure 2.2: Schematic drawing of Epiphyse, Metaphyse, and Diaphyse (Adapted from Feneis & Dauber, 2000. p. 47)

2.2.1.1 Union of primary ossification centres

The time of appearance of primary and secondary centres of ossification is potentially valuable in age estimation (Stewart, 1979). Bone is deposited in these centres according to a rough schedule, so the amount of their development in a skeleton indicates an approximate age at death (Byers, 2001). Additionally, the union of secondary centres to their associated primary centres also follows a rough schedule that can be utilized in a similar manner, unfortunately the small size and fragility of these bony structures make their recovery difficult, and thus they have little utility in age estimation (Byers, 2001). However, the union of primary centres to each others can be used occasionally because these structures are more likely to be recovered (Stewart, 1979).

The main primary centres that can be used for age estimation are the skull. mandible. atlas, and axis. In the skull, two types of unions occur. First, new born babies exhibit gaps where three or more bones come together, called fontanels such as frontal, sphenoid, mastoid, and occipital fontanels. In addition to these gaps. the ossification centres of a number of cranial bones unite during growth as the right and left halves of the frontal bone and left and right halves of the mandible (Stewart, 1979). According to Terry (1942), the timing of the more reliable primary centres for age estimation was as follows: (Adapted from Stewart. 1979: p.139)

Bone	*Location*	*Period of closure/union*
Fontanels	Sphenoid and mastoid	Close soon after birth
	Occipital	Closes during first year
	Frontal	Closes during second year
Mandible	Symphysis	Union completed in second year
Frontal bone	Metopic suture	Said to remain open in 8 to 9 percent of whites. When closure takes place, it begins in second year
Atlas	Posteriorly	Union occurs in third year
	Anteriorly	Union occurs about the sixth year
Axis	Dens, body and two sides of arch	Union of all four parts occurs during third year
Occipital bone	Squamous with lateral parts	Union completed in fifth year
	Lateral parts with basilar parts	Union completed before seventh year

Development of the centres of ossification of the hand and foot are useful in assessment of biological age of a child (Malina *et al.*. 2004). One of the method which uses to assess the hand wrist is the Greulich and Pyle radiographic atlas of skeletal development (Malina *et al.*. 2004).

2.2.1.2 Epiphysial union

Bones grow by the deposition of osseous material on their ends. This process starts after the primary centres of ossification have fused with each other and the entire cartilaginous precursor has transformed into bone. At this point, their ends do not exhibit bony joint surfaces; instead they are covered by cartilaginous joint area. Later on, the epiphysial caps unite to their diaphyses leaving a temporarily visible line. Finally, this line becomes completely obliterated by the mechanisms of bone remodelling (Byers, 2001).

There are numerous epiphysial centres in the human skeleton. All long bones have at least two (one on each end) or more (e.g. the femur has four). Similarly, the first ten ribs have two, one on the head and the other on the auricular surface of the tubercle. By contrast, each metacarpal and metatarsal bone exhibits only one, which is located on their proximal ends. The bottom twenty two vertebrae have at least five epiphysial centres (Byers, 2001).

One of the schedules which are used to assess the epiphysial union was introduced by Buikstra and Ubelaker (1994) (Figure 2.3). the authors determined the range of age within which the epiphysial fusion occurs for several bones based on samples of Native American children. Generally. the bones from the early part of this range of age are recognisable by the presence of clearly visible lines that appear to cut deeply into the bones (same as in Figure 2.4b), whereas those that have less visible and/or almost obliterated lines are more often from the end of these ranges (same as in Figure 2.4c) (Byers, 2001). It seems that schedules which was made by Buikstra and Ubelker (1994) are accurate for estimating the age between 10 to 25 years of age, because very few epiphysial centres fuse before 10 years and the majority of epiphysial centres fused by the age of 25 years (Byers. 2001).

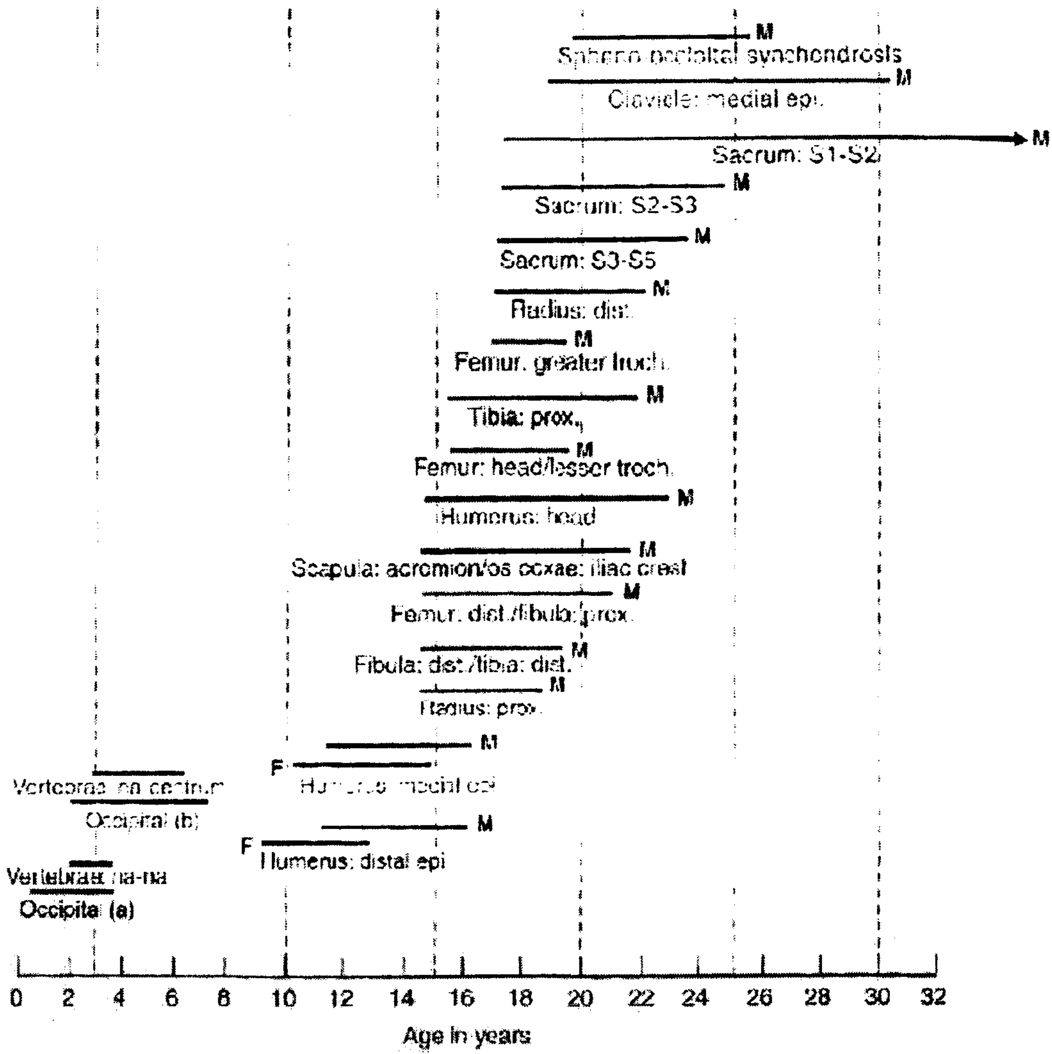

Figure 2.3: Buikstra and Ubelker's Schedule of epiphysial union (Adapted from Byers (2001), p.212).

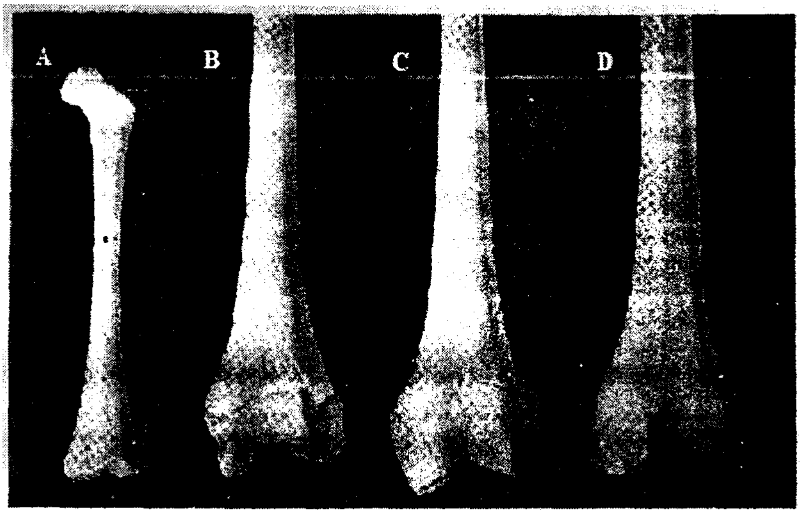

Figure 2.4: Stages of epiphysial union of the distal femur: a) no union (missing epiphysis); b) unfused; c) fused; d) obliterated (adapted from Byers (2001), p.211).

2.2.1.3 Long bones lengths

From the time of first appearance of the long bones up to birth. the relationship between age and long bone length appears linear; however, the method of age estimation for non-adult based on the measured length of specific long bones is one that has been used cautiously, it is a method that suffers from inaccuracy due to the wide variation seen in absolute growth in any sample population and quite wide interracial variation and as such, population specific tables should be used if available when utilizing this method (Byers, 2001). In addition, the method gives estimation interval which is broad for forensic purposes, being incremented in six-months to one year interval (Weaver, 1986).

2.2.2 Dental tissues methods for sub-adult

Unlike the union of ossification centres, most, if not all, of developing teeth are likely to remain in place during the process of skeletonization (Stewart, 1979). When compared with skeletal development, dental development also showed to be less affected by the diseases and nutrition (Weaver, 1986; Garn *et al.,* 1965a). Moreover, dental development showed to be a more reliable means of estimating the age of sub-adult (Stewart, 1963 and 1954).

2.2.2.1 Eruption time and teeth counts

"Eruption" or "clinical emergence" refers to the passage of a tooth through the alveolar process and perforation of the gums. It is the first choice for fast and simple visual evidence of age, Gron (1962) considered a tooth erupted if it had just pierced the gingiva, but was no more than 3mm from the gingival level. The first use of eruption as an indicator of development was in England in 1837 when the factory act stipulated that; a child without a second permanent molar would not be allowed to work in the factories. In the first quarter of the 20th century, dental criteria were used as an indicator for school entrance purposes (Demirjian, 1978).

The chronology of emergence for both deciduous and permanent teeth has been broadly studied as a method of age estimation, such studies differ with regard to selection of patients such as number of patients, and cross sectional or longitudinal study (Saleem and Hagg *et al.,* 1996; Pahkala *et al.*, 1991; Manji and Mwaniki, 1985; Hoffding *et al.*, 1984; Lee *et al.*, 1965). Nizam *et al.* (2003) conducted a cross-sectional study of eruption time of permanent teeth in Kelantan state, Malaysia. More than 2300 school children aged 5 to 17 years old were evaluated. Those children included into the study were children of Malay origin and had no history of extraction of deciduous teeth due to caries. Those with congenital anomalies, with history of any systemic disease and those undergoing orthodontic treatment were excluded from the study.

Counting of the emerged deciduous and permanent teeth was considered one of the dental age methods which showed good estimation of age. Foti *et al.* (2003) conducted a study in the aim of providing mathematical models for age calculation based on counting of emerged teeth. and. if possible. tooth germs. Eight hundred and ten panoramic radiographs of 397 boys and 413 girls of French origin from 6.10 to 21.08 years of age (average age of 12.63 years) were assessed. They defined the criterion of tooth eruption. from a radiological point of view; the line lying over the erupting tooth's cusps had to reach over the line joining the mesial and the distal cemento–enamel junctions of adjacent teeth. The number of germs was also noted. A germ was considered to be present at the stage of crown calcification, corresponding to Demirjian's stage (A) that is the start of calcification of the cusp tips. The authors came out with three regressive models that did not yield significant difference between the means of estimated age and real age, and they concluded that these models really provide reliable age estimations.

Hagg and Taranger (1985) conducted a study to establish and test the validity of a dental age based on counting the emerged deciduous and 29 permanent teeth. They examined more than 200 Swedish children longitudinally at intervals of 1, 3, 6, 12, 18 months then annually from 2 years to 18 years. The emergence of teeth was assessed clinically. The authors came out with values representing the number of teeth and the chronological age, and then these values were examined cross-sectionally on another 200 samples of Swedish children to test the validity. The results showed difference between the real age and estimated age to be around one month.

2.2.2.3 Radiographic methods

Age estimation for sub-adult can also be assessed by tracing the tooth calcification by radiographs; since it represents a series of recognizable events that occur in the same sequence from an initial event (beginning of calcification of the crown) to a constant end point (closure of the apex) (Moorrees *et al.*, 1963). It seems that the study of dental age based on criteria of calcification of the teeth seen in radiographic is superior to the teeth emergence time; as the emergence of teeth is a short period event, thus it is difficult to detect it exactly, while calcification of teeth is a continuous event; which starts form calcification of the crown to fully form of apical part and it can be traced by permanent records such as the radiograph films (Moorrees *et al.*. 1963). In addition. the emergence can be affected by a diverse range of factors as the local oral factors such as infection of the deciduous predecessor (Yawaka *et al.*, 2002; Brook and Winter, 1975), or influence of crowding that could delay the tooth emergence, pathology, trauma, and the presence of supernumerary teeth (Suri *et al.*, 2004). Also, emergence time can be affected by severe protein-calorie malnutrition (Demirjian, 1978). Moreover, the technique is limited to those periods when teeth are erupting (i.e. 0-3 years for the deciduous and 5-14 years for the permanent dentition), and the various techniques rely on different definitions of the term eruption.

The types of radiograph films which are usually used for dental age included: intraoral radiographs (periapical film) (Demirjian and Goldstein, 1976) or extraoral radiographs such as panoramic (Demirjian *et al.*, 1973) or lateral oblique radiographs (Moorrees *et al.,* 1963; Nolla, 1960). There is no difference between these types of films as long as the tooth stage can be assessed clearly. However, periapical films seems to be easily obtained for children below five years old (Demirjian and Goldstein, 1976).

Gleiser and Hunt, in 1955, studied 25 boys and 25 girls longitudinally, from birth to age 10, and traced the development of the first permanent mandibular molar only by using lateral oblique radiograph. They described 15 different stages of maturity, from the calcification of the cusp tip to closure of the apex. The description of the stages is based on length criteria, as ½ of the crown or ¾ of the root completed (Demirjian, 1978).

Nolla (1960) described a method based on 10 length stages of tooth development in order to develop norms for American children that displayed the average development of teeth for males and females aged between 5 to 18 years, and to develop tables that could be used to estimate chronological age based on the degree of observed dental development. These developmental stages were studied longitudinally for both the maxillary and mandibular teeth in 25 boys and 25 girls giving the total number of lateral oblique radiographs of 1746 for the female sample, and 1656 for the male sample. She assessed the teeth from the right and left sides. A score of 1-10 was assigned to each stage, starting from the presence of the crypt without calcification, to the completion of the apical end. Nolla also suggested that if a tooth was located between two stages, the solution was to use the lower stage and to add 0.5 to the value assigned to that specific tooth. The description of the stages is based on length criteria. She found the sexual difference in the rate of dental development was not significant; the development, however, begins earlier in girls.

Similarly, few developmental differences were shown between right and left teeth of same kind.

In 1961. Fanning applied Gleiser and Hunt's 13 calcification stages of dental development to all mandibular and maxillary incisors. A total number of seven more stages were added for more precision (two initial stages, two for cleft formation in the molars, and three to describe the different degrees of apex closure). The description of the stages is based on length criteria (Demirjian, 1978). However these added stages proved to be too difficult to differentiate between successive stages (Demirjian, 1978).

Moorrees *et al.* (1963) studied the development pattern of eight mandibular and two maxillary incisors permanent teeth of 380 children from birth until 25 years of age. The sample radiographs were collected from Boston (Forsyth Dental Infirmary) and Ohio (Fels Research Institute, Yellow Springs). The aim was to obtain norms for the age of attainment for each of the eight mandibular and maxillary incisor teeth. The authors utilized 13 calcification stages of dental development of single rooted teeth and 14 stages for multi rooted teeth and the stages is based on length criteria (½ of the crown or ¾ of the root completed). The incisors were assessed from intraoral radiographs (Boston children); while uniradicular and multiradicular posterior teeth were assessed from lateral or oblique jaw radiographs (Ohio children). The authors derived a mean age of attainment for each stage of each tooth and graphically represented the results including ± 2 standard deviation age limits.

Moorrees' method has been applied on Sardinian population where Diaz *et al.* (1993) assessed cross-sectionally 382 healthy children (178 boys and 204 girls). A single OPG was examined for each patient. The authors evaluated the development of permanent mandibular canines. premolars. and molars only. The author used OPGs instead of lateral oblique which was used by Moorrees' method because as long as the stage of the tooth can be read so the any radiographic image can be used. The results showed differences in dental development between the North American children and Sardinian which accounting for a delayed dental maturation in Sardinian children.

2.2.2.3.1 Demirjian's Method (1973)

Demirjian. Goldstein and Tanner in 1973 have developed a method of estimating dental age based upon the stages of dental development seen in radiographs, where the authors found that radiographic dental age methods were more reliable indicator of dental age than tooth emergence; as tooth emergence can be affected by local oral factors such as infection, pathology, and trauma. Also, emergence time can be affected by severe protein-calorie malnutrition (Demirjian *et al.*, 1973).

Demirjian et al. used panoramic radiographs as the image of the mandibular region in the OPG was clear and only little distortion which is around 3 to 10% (Sapoka and Demirjian, 1971). Demirjian considered this as not a drawback because the rating system is based on shape criteria and relative values of the developing teeth rather than on absolute lengths (Demirjian, 1978).

The sample used by Demirjian in 1973 study, consisted of 1446 males and 1482 females of French-Canadian origin aged between 2 and 20 years. The authors divided the developmental stages of the tooth into 8 stages from A to H (4 for crown development and 4 for root development) starting from the beginning of calcification through to final mature form (with 0 indicating calcification not yet commenced) (Figure 2.5). The authors assigned developmental stages based on shapes of teeth which are easily recognisable and not on absolute length (Demirjian *et al.*, 1973).

Demirjian *et al.* (1973) have created their method based on the method described by Tanner *et al.* (1962) in relation to skeletal age, where each bone was given a score depending on its stage. The scores of all bones then added together to give a total maturity score which can be converted directly into a skeletal age using an appropriate table of standards. Thus, for dental age, each tooth is given a score depending on its stage. The scores of all the teeth are then added together to give a total 'maturity score' (MS) which can be converted directly into 'dental age' (DA) using an appropriate table of standards.

The final scores for each tooth, previously constrained each to be 100, are allowed to vary so that only their sum (or average) over all the teeth is 100. This makes allowance for the different ages at which different teeth reach maturity. Boys and girls are given different systems of scores. The stages of the tooth development are shown respectively below in Figure 2.5