DENTAL DEVELOPMENT IN A SOUTHERN AFRICAN SUB ADULT POPULATION: DETERMINATION OF REFERENCE VALUES FOR PERMANENT TOOTH FORMATION AND EMERGENCE

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Declaration

I, Temitope Ayodeji Esan (Student number: 812615) am a student registered for the degree of doctor of philosophy in the academic year 2017.

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Abstract

Background: Population-based knowledge of human biological growth and development processes is fundamental for assessing the health status of a community. This requires an understanding of the growth pattern for the children as well as the environmental stresses that disrupt or impede their growth. These stresses are usually easy to identify, but data on normal development and growth variation in most populations is surprisingly lacking. Instead, researchers typically compare growth in the population of interest to references formulated for European or US children. The problems associated with using non-population specific references are complex, and their application can lead to misrepresentations of the health status. In addition, the influence of environmental factors on dental development is still debated and the relationship of dental development with life history events, such as sexual maturity, is unclear.

Aim: The aim of this study is to develop population-specific reference for permanent tooth formation and emergence among Black Southern Africans, to compare this reference with other population references, and to investigate the influence of sex and nutritional status on dental development.

Method:

Study design and population

This is a cross sectional study. A total of 642 children comprising of 270 males and 372 females from primary and secondary schools were recruited over one and half years. Only participants whose parents and grandparents are indigenous Southern Africans were included. Participants were screened in a mobile dental truck fitted with digital panoramic x ray.

Systematic Review

A literature search of PubMed, Scopus, Ovid, Database of Open Access Journals and Google Scholar was undertaken. All eligible studies published before December 28, 2016 were reviewed and analyzed. Meta-analysis was performed on 28 published articles using the Demirjian and/or Willems methods to estimate chronological age. The weighted mean difference at 95% confidence interval was used to assess accuracies of the two methods in predicting the chronological age of children**.**

Tooth formation in Southern Africa

To investigate tooth formation, all the 642 Black Southern African children comprising of 270 males and 372 females were recruited. The panoramic radiograph of each child was analysed and the dental maturity score of the left mandibular permanent teeth was obtained according to the Demirjian et al. (1973) method. The dental maturity score of each child was converted to dental age using standard tables and percentiles curves for both sexes by Demirjian et al. (1973). The ages of attainment of specific maturity stages were calculated with pr obit analysis and compared by sex and population.

Comparisons of age estimation methods

For comparison of the common methods used in estimation of age, 540 children (233 males and 307 females out of the 642 children were recruited. This is because all the children aged 16 years and above have reached 100% maturity and hence excluded from the study. Panoramic radiographs of the children were analyzed and the dental maturity scores of the left mandibular permanent teeth were calculated according to the Demirjian et al. (1973), Demirjian and Goldstein (1976) and Willems et al (2001) methods. The dental maturity scores were converted to dental ages using standard tables and percentiles curves for males and females (Demirjian et al. 1973; Demirjian and Goldstein 1976; Willems et al. 2001). The dental ages obtained were compared to the chronological ages of the children and the mean differences obtained by the three methods compared.

Nutrition and tooth formation

Effect of nutrition on tooth formation was investigated on all the 642 Black Southern African children comprising of 270 males and 372 females were recruited. The Panoramic radiograph of each child was analysed using the Demirjian et al. (1973) method. The dental maturity score of each child was converted to dental age using standard tables and percentiles curves for both males and females by Demirjian et al. (1973). In addition, measures of nutritional status such as, height, weight, mid upper arm circumference and head circumference were obtained from the children.

The timing, sequence of emergence and the effect of nutrition on tooth emergence

To investigate tooth emergence and the influence of nutritional status on emergence, information on type of teeth and number of teeth emerged were collected from 639 (266 males and 373 females) Black Southern African children aged 5-20 years out of the total 642 children because the emergence data for 3 children were found to be incomplete. An emerged tooth was defined as a tooth with any part of its crown penetrating the gingiva and visible in the oral cavity. Height, weight, mid upper arm circumference and head circumference of the participants were measured. Children with any form of tooth impaction and agenesis were excluded from the study.

Life history events and dental development

To determine the association between tooth development and life history variables, mean ages of attainment of sexual maturity stages were adapted from Lundeen et al. (2015) and Norris and Richter (2005) to identify if any stage of dental development co-occured with life history events.

Southern Africa specific reference values

The WITS Atlas was developed using the tooth formation stage with the highest frequency for each tooth. This stage was considered the developmental reference for an age cohort. Southern African tables of conversion of maturity scores were generated separately for males and females using polynomial regression functions (3rd degree). Maturity curves were plotted to determine the dental maturity curves for each sex. The Southern African specific tables of conversion of maturity score were tested on 540 participants aged 5 to 15.99 years and the results compared to the Willems and Demirjian methods of age estimation.

Data were analysed with Stata 12 for Windows. The analysis included frequencies and cross-tabulations. Associations between categorical variables were tested with chi square while those between continuous variables were tested with Student's *t*-tests. The mean ages of emergence and standard deviation were computed using probit analysis. Sex and population comparisons were done using Student's *t*-tests. The height and BMI were converted to z-scores using WHO z-scores for age tables (WHO 1995). A cut-off z-score of ≤−2 for BMI/height was used to place children into underweight/short for age, ≥-2 to 2.0 for normal, and ≥2 for overweight/obese/tall for age categories. Mean age of emergence and mean age of attainment of maturity stages were calculated for each tooth using these BMI subdivisions. Analysis of variance (ANOVA) and Games-Howell were used to determine the differences between the BMI/height subdivisions. A Student's *t*-test was used to compare any two means whenever one of the three subdivisions of BMI did not yield a mean age of emergence. Spearman's rho correlations between total number of teeth, dental maturity scores and anthropometric variables were done. A Shapiro-Wilk W test showed that the dependent variables (total number of teeth emerged and dental maturity) and the predictor variables were not normally distributed. Therefore, a generalized linear model (negative binomial) was used with the number of emerged teeth/dental maturity modelled as the dependent variable and anthropometric variables and age as predictors. Adequacy of fit was checked using the deviance residuals as recommended by McCullagh and Nelder (1989). The deviance residuals showed that it was normally distributed and the plot of the residuals against each of the covariates also showed model fit. As expected, the collinearity test showed that BMI, height and weight were significantly collinear. When these variables were excluded from the model, there was no difference in the values of the output. Hence, the variables were included in the final model for generalized linear regression analysis. The model was built using forward selection. Statistical significance was inferred at $p<0.05$.

Results:

Systematic review

Meta-analysis revealed that the Willems method has better accuracy globally compared with the Demirjian method.

Dental maturity in Black Southern Africans

The females show advanced dental maturity and dental ages compared to males ($p<0.05$). Cross-population comparison shows the Southern African females are advanced in dental maturity compared to European and Asian children.

Comparison of methods for estimating dental age

The Original Demirjian method significantly overestimated the age of the males by 0.85 years and the females by 1.0 years ($p<0.05$) with the same mean absolute error of 1.1 years for both sexes. Similarly, the Modified Demirjian method significantly overestimated chronological ages of males (0.90 years) and females (1.21 years) with the highest mean absolute error of 1.1 years and 1.4 years for males and females respectively. The Willems method had the lowest, but still significant mean differences (0.2 years for males and 0.3 years for females) between the dental age and chronological age. It also demonstrated the least mean absolute errors for males (0.70 years) and females (0.68 years).

Nutrition and tooth formation

Significant advancements were found in the age of attainment of H stage for all the permanent teeth in the overweight group compared to the underweight group ($p<0.05$). Negative binomial regression analysis indicates that age, height, and BMI are significant predictors of the dental maturity score for males ($p<0.05$), while age, height, weight, BMI and head circumference are significant predictors of the dental maturity score for females.

Tooth emergence

Females have all the permanent teeth emerged earlier than males except for the third molars ($p<0.05$). Generally, Black Southern African children have similar ages and sequence of emergence as children from other sub-Saharan Africa countries. Black Southern African children have earlier mean ages of emergence of permanent teeth compared to children from the USA, Europe, Australia and Asia. Sexual dimorphism was noted in the sequence of emergence of I1/M1 in the mandible with the females having the M_1I_1 sequence as opposed to I_1M_I in males. The sequence of emergence of Southern African males is similar in both jaws to males from the USA and Europe but differs from Iranians and Pakistanis. Females show similar patterns of sequence with sub-Saharan African, Australian and US females in the maxilla. They display M_I/I_1 variation in the mandible.

Nutrition and tooth emergence

Overweight/obese children generally show significantly earlier emergence times compared to normal weight/severely underweight children ($p<0.05$). Females and tall children have more emerged teeth than shorter children when corrected for age and sex ($p<0.05$). The generalized linear regression model (negative binomial) shows that height, weight and BMI have significant associations with the number of emerged teeth ($p<0.05$).

Dental development and life history variables

The number of teeth emerged in males correlate strongly with chronological age ($r=0.91$, $p=0.00$) and height ($r=0.89$, $p=0.00$), moderately with mid-upper arm circumference ($r=0.61$, $p=0.00$) and weakly with head circumference ($r=0.16$, $p=0.00$). In females, the number of teeth emerged correlates strongly with chronological age ($r=0.88$, $p=0.00$) and height ($r=0.83$, $p=0.00$), moderately with mid-upper arm circumference ($r=0.59$, $p=0.00$), and weakly with head circumference ($r=0.38$, $p=0.00$). Similar patterns of correlation are found for dental maturity.

The emergence of the maxillary and mandibular M2s co-occurs with the G2 stage of gonad development and the PH2 stage of pubic hair development in males. The M2s emerge coincident with the attainment of Tanner’s B2 breast stage and the PH2 pubic hair stage in females. The age of menarche does not coincide with any of the determined ages for emergence of teeth.

Attainment of the H stage of development in the C1 co-occurs with the G2 stage of gonad development and shortly after the pubic hair stage PH2 in the males. In females, the attainment of the H stage of C1 formation occurs shortly before the attainment of the B2 stage of breast development. Furthermore, the H stage of P1 formation coincides with the PH2 stage of pubic hair development, shortly after the attainment of the stage B2 of breast development. The attainment of the H stage in P2 formation coincides with the age of menarche at approximately 13 years.

Southern African specific reference

A new dental atlas (WITS Atlas) was developed due to the significantly earlier ages of emergence and formation among Black Southern Africans. When compared to the London atlas, the canines, premolars and second molars are at least a year ahead in the WITS Atlas. Third molar formation and emergence occurs three years earlier in the WITS Atlas. Polynomial regression formulae were generated and Southern African specific conversion tables were generated for the males and females. The new tables of maturity scores show no overestimation of the chronological ages of males (0.045, $p>0.05$) and females (0.08, $p>0.05$). Compared to the Willems and Demirjian methods, the Southern African specific maturity tables showed the least mean absolute error for both sexes.

Conclusion: There is sexual dimorphism in the timing of tooth emergence with females having earlier emergence times. Black Southern Africans show similarities in the ages and sequence of emergence of the permanent teeth with children from other sub-Saharan African countries but, they are advanced relative to children from the USA, Europe, Australia and Asia. Similarly, the Black Southern African children show advanced tooth formation compared to children from Europe, Asia and Australia.

The Willems method is more accurate at estimating chronological age for forensic and anthropological purposes compared to the Demirjian methods that significantly overestimate the chronological age of children. Of the three methods tested on Black Southern African children, the Willems method is the most accurate in estimating chronological age. However; it significantly overestimated the chronological age of Black Southern African children. Hence, there is a need for population-specific reference values for use in the age estimation of Black Southern African children

The WITS Atlas and new population-specific maturity tables for Black Southern African males and females were developed. The WITS Atlas differs significantly from the London atlas with earlier ages of tooth formation and emergence. The Southern African specific age estimation method shows good accuracy in the estimation of dental ages. By inference, this method could be used in other sub-Saharan African countries because of similarities in tooth formation and emergence times.

Contrary to some studies, nutrition was found to have a significant influence on the number of teeth emerged and the timing of emergence. Obese/overweight/tall children tend to have earlier timing of emergence and more emerged teeth compared to their underweight peers. Similarly, obese/overweight/tall individuals attained the H stage of tooth formation of most teeth earlier than their underweight and normal weight age-mates.

Emergence of second molars and the H stage of canine and first premolar formation co-occur with the onset of puberty in males and females. Menarche appears to coincide with the attainment of H stage of the mandibular second premolar.

Dedication

To all the children of Southern Africa

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Table of contents **Page**

List of Figures

List of Tables

Publications and conference presentation emanating from this thesis

Temitope Esan, Lynne Schepartz, Elizabeth Oziegbe. Do the modified Demirjian and Willems methods of dental age estimation accurately capture sex or population-level differences in tooth formation? A systematic review protocol. PROSPERO 2016:CRD42016029995 Available from http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016029995

Temitope A Esan, Veerasamy Yengopal, Lynne A Schepartz. The Demirjian versus the Willems method for dental age estimation in different populations: A meta-analysis of published studies (Accepted for publication by PLOS ONE October 2017)

Chapter 1. General Introduction, Aims and Objectives

Chapter 1

1.1 General introduction

Population-based knowledge of human biological growth and development processes is fundamental for assessing the health status of a community. This requires an understanding of the growth pattern for the children as well as the environmental stresses that disrupt or impede their growth. These stresses are usually easy to identify, but data on normal development and growth variation in most populations is surprisingly lacking. Instead, researchers typically compare growth in the population of interest to references formulated for European or US children. The problems associated with using non-population-specific references are complex, and their application can lead to misrepresentations of the health status with consequences for policy formation and basic research.

The importance of population-specific growth references extends beyond their utility in biological anthropology and health research. For many populations in rural Africa, including South Africa, birth registry and eliciting the date of birth is still a challenge. Features such as occlusal tooth wear and non-metric variation details can be very useful for identification and aging (Kim et al. 2000; Yun et al. 2007). Data on the timing of tooth formation, emergence and morphometrics are also needed for forensic purposes, especially with the increasing global incidences of mass deaths and disasters (Kieser et al. 2005; Perrier et al. 2006). Tables of emergence chronology are useful when birth records are unreliable or lost, where people seek asylum or where individuals seek favourable outcomes in civil or criminal cases, where specific aging is needed to prevent cheating in age-graded sports competitions (Schulze et al. 2006; Schmeling et al. 2007; Meijerman et al. 2007; Ríos et al. 2008; Baumann et al. 2009; Ríos and Cardoso 2009). The age at death is usually the only biological parameter that can be determined for unidentified juvenile remains with any degree of accuracy (Scheuer and Black 2000). Beyond this, information from dental development (tooth formation and emergence) may play a major role in determining many clinical decisions, including choices about treatment options and sequence (Suri et al. 2004). In the absence of population-specific data, information from other regions and populations are the only available reference.

With increasing globalization, there have been observable changes in the demographic features of many human populations as well as changes in their biological profiles (Kearney 1995). Dental features are also evolving. This may be in response to observable alterations in nutritional status, the assumption of “Western diets”, socioeconomic status and gene flow. Trends of tooth size reduction, agenesis and malocclusion are thought to be increasing globally, although the pattern of change is not uniform across populations (Esan and Schepartz 2017). With these transformations, it is expected that dental growth and development references will also modify with time. New studies need to be conducted to keep up with the expected evolution.

There are currently few or no reference values for many groups and populations in Africa. Where these exist, the data are often limited to tooth emergence or morphometrics; there are fewer studies on tooth formation. This gap in information on the timing of dental maturation in Africa, as well as other regions, is of specific anthropological importance as the information contributes to the elucidation of historical lineages of human groups and also helps to identify environmental factors that have effects on tooth development. With increasing interest in genomic research, these kinds of data would provide baseline information on the biological characteristics that can inform the design and implementation of genomic studies.

Dental development encompasses two distinct processes of calcification and emergence (Garn et al. 1958; Smith 1991; Liversidge 2003). Tooth formation is specifically the formation of an organic matrix and its subsequent mineralization (Smith 1991). It consists of a regular sequence of stages from crown formation to the completion of root formation   Eruption has been defined as the tooth piercing through the alveolar bone and oral mucosa until it reaches an opposing tooth (El-Nofely and İşcan 1989). Mani et al. (2008) believed that this term is vague since eruption is a continuous process including the period in life when no tooth erupts into the oral cavity. Moreover, the broad nature of the concept of eruption makes comparisons between different studies difficult. As a result, Demirjian (1986) posited that the term '*clinical/gingival' emergence* should refer to the appearance in the oral cavity of any part of the crown during the course of eruption.

Tooth formation and emergence have long been considered to show the least variability with chronological age when compared with other growth events such as skeletal, somatic and sexual maturity (Lewis and Garn 1960; Demirjian 1986; Liversidge et al. 2006). In particular, tooth formation is considered to be less variable and produce better accuracy when used to estimate chronological age compared to tooth emergence. Dental diseases, inadequate oral health care facilities and early loss of deciduous teeth have considerable influence on the timing of emergence of the permanent dentition.

The interrelationship between dental development and other growth events such as skeletal and sexual development has been well researched. While it is generally agreed that a strong relationship exists between skeletal and dental development, there are varying results regarding the relationship between dental and sexual maturity. Lewis and Garn (1960) and Nanda (1960) reported high correlations between dental and sexual maturity; while Demirjian et al. (1985) concluded that there is no relationship between them and thus inferred that the two growth processes are under different controlling influences. The inconsistency of results may be due to the different methods of data collection and analysis (Demirjian et al. 1985). Hence this study further examines the interrelationship between dental development and sexual maturity.

Variations in dental development within and among different populations have been reported in the literature (Pahkala et al. 1991; Liversidge 2003; Liversidge et al. 2006) and it is not clear to what extent they are influenced by genetic and/or environmental factors such as nutrition. Many studies have attempted to look at the relationship between nutrition and dental development. Jellife and Jellife (1973) concluded that moderate undernutrition does not delay dental development but severe undernutrition does. More recent work also shows that nutritional stress significantly affects dental development (Hilgers et al. 2006; Mani et al. 2008). However, other studies did not find a significant influence of nutrition on dental development (Eid et al. 2002; Cameriere et al. 2007; Elamin and Liversidge 2013).

While it is generally agreed that genetic factors influence the timing of tooth emergence and formation, the extent and nature of the genetic control is not well understood. Saleemi et al. (1993) inferred that delayed deciduous tooth emergence in Pakistani children is due solely to genetic factors. Similarly, Holman and Jones (1998) reported large differences in timing of deciduous tooth emergence in different populations and concluded that the variation is due to genetic diversity. It is not clear, however, that Holman and Jones (1998) considered environmental factors. Similar controversies over the roles of genetics and the environment have been raised with regards to tooth formation. To further examine these issues, this study explored the influence of nutrition on intra-population variation in dental development by examining both tooth formation and tooth emergence.

Age estimation methods based on tooth formation have been developed for several populations. The most popular method was developed by Demirjian et al. (1973). The Demirjian method is based on the panoramic radiograph records of 21328 French-Canadian children. Stages of development (A to H) of the seven left mandibular teeth were identified and weighted scores assigned to each stage. The summed scores of all seven teeth correspond to the maturity score, which can be converted to dental age using separate tables of conversions for males and females. (Demirjian et al. 1973).

Population variation in the pattern of dental maturation is documented in modern humans. For example, French, Finnish, and Swedish children were found to be advanced in dental maturity compared to the French-Canadian reference population used by Demirjian et al. (1973) (NyströmNyström et al. 1986; Mörnstad et al. 1995; Willems et al. 2001). This led many authors to advocate for population-specific reference values. Willems et al. (2001) modified the Demirjian method and found that they estimated age more accurately in a Belgium population. Similarly, other researchers found better accuracy with the Willems method (Djukic et al. 2013; Altalie et al. 2014; Kumaresan et al. 2016) while others did not (Zhai et al. 2016). A systematic review of published studies is needed to evaluate the accuracies of the different methods. Furthermore, the accuracies of the Demirjian Original and revised (Modified) methods and the Willems methods have not been tested on Southern African children. Therefore, this study investigates the validity and accuracies of all these age estimation methods in a Black South African population.

1.1 Aim of the study

Despite the growing number of studies in other world regions, there are no known reference values for age estimation based on tooth development and emergence in African children. The aim of this study is to develop a population-specific reference for permanent tooth formation and emergence among black Southern Africans, to compare this reference with other population references, and to investigate the influence of sex and nutritional status on the dental development of the children.

The study hypotheses are as follows:

a. The pattern and tempo of tooth formation and emergence in Black Southern African children is not significantly different from other populations.
b. There are no differences in dental development between males and females of Black Southern African origin.
c. Nutritional status does not influence tooth formation and emergence in Southern African Black children.
d. There is no relationship between tooth formation and emergence and measures of skeletal and sexual maturity in Black Southern African children.
e. There is no need for population-specific reference values for tooth formation and emergence.

1.2 Study objectives

The following objectives are designed to address the development of dental aging reference values for Black Southern Africa populations:

1. Conduct a meta-analysis of published articles to determine the accuracies of the Demirjian and Willems methods of age estimation. (Chapter 2)
2. Develop dental maturity scores for Southern African Black children using Demirjian’s method and compare the findings with other populations. (Chapter 3)
3. Investigate the accuracy of the Original Demirjian, Modified Demirjian and Willems methods of age estimation in Southern African Black children. (Chapter 4)
4. Determine the influence of nutrition (as measured by height, weight, mid-upper arm circumference and head circumference) on tooth formation. (Chapter 5)
5. Determine the reference values for tooth emergence in Black Southern African children and compare the results with other populations. (Chapter 6)

6. Determine the influence of sex and nutrition (measured by the anthropometric variables height, weight, mid-upper arm circumference and head circumference) on tooth emergence. (Chapter 7)
7. Examine the association among life history variables (sexual maturity, skeletal growth, brain development) and dental development (tooth formation and emergence). (Chapter 8)
8. Develop a population-specific reference for tooth formation and emergence (the WITS Atlas). (Chapter 9)

1.3 Methodology

The methods used in this study are presented in each of the research chapters.

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Contents

Chapter 2

The Demirjian versus the Willems method for dental age estimation in different populations: A meta-analysis of published studies

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Abstract

Background: The accuracy of radiographic methods for dental age estimation is important for biological growth research and forensic applications. Accuracy of the two most commonly used systems (Demirjian and Willems) has been evaluated with conflicting results. This study investigates the accuracies of these methods for dental age estimation in different populations**.**

Methods: A search of PubMed, Scopus, Ovid, Database of Open Access Journals and Google Scholar was undertaken. Eligible studies published before December 28, 2016 were reviewed and analyzed. Meta-analysis was performed on 28 published articles using the Demirjian and/or Willems methods to estimate chronological age in 14,109 children (6,581 males, 7,528 females) age 3-18 years in studies using Demirjian's method and 10,832 children (5,176 males, 5,656 females) age 4-18 years in studies using Willems' method. The weighted mean difference at 95% confidence interval was used to assess accuracies of the two methods in predicting the chronological age**.**

Results: The Demirjian method significantly overestimated chronological age ($p<0.05$) in males age 3-15 and females age 4-16 when studies were pooled by age cohorts and sex. The majority of studies using Willems' method did not report significant overestimation of ages in either sex. Overall, Demirjian's method significantly overestimated chronological age compared to the Willems method ($p<0.05$). The weighted mean difference for the Demirjian method was 0.62 for males and 0.72 for females, while that of the Willems method was 0.26 for males and 0.29 for females.

Conclusion: The Willems method provides more accurate estimation of chronological age in different populations, while Demirjian's method has a broad application in terms of determining maturity scores. However, accuracy of Demirjian age estimations is confounded

by population variation when converting maturity scores to dental ages. For highest accuracy of age estimation, population-specific standards, rather than a universal standard or methods developed on other populations, need to be employed.

Systematic review protocol registration number is: CRD42016029995

CHAPTER 2: SYSTEMATIC LITERATURE REVIEW OF DENTAL AGE ESTIMATION USING THE DEMIRIJIAN AND WILLEMS METHODS

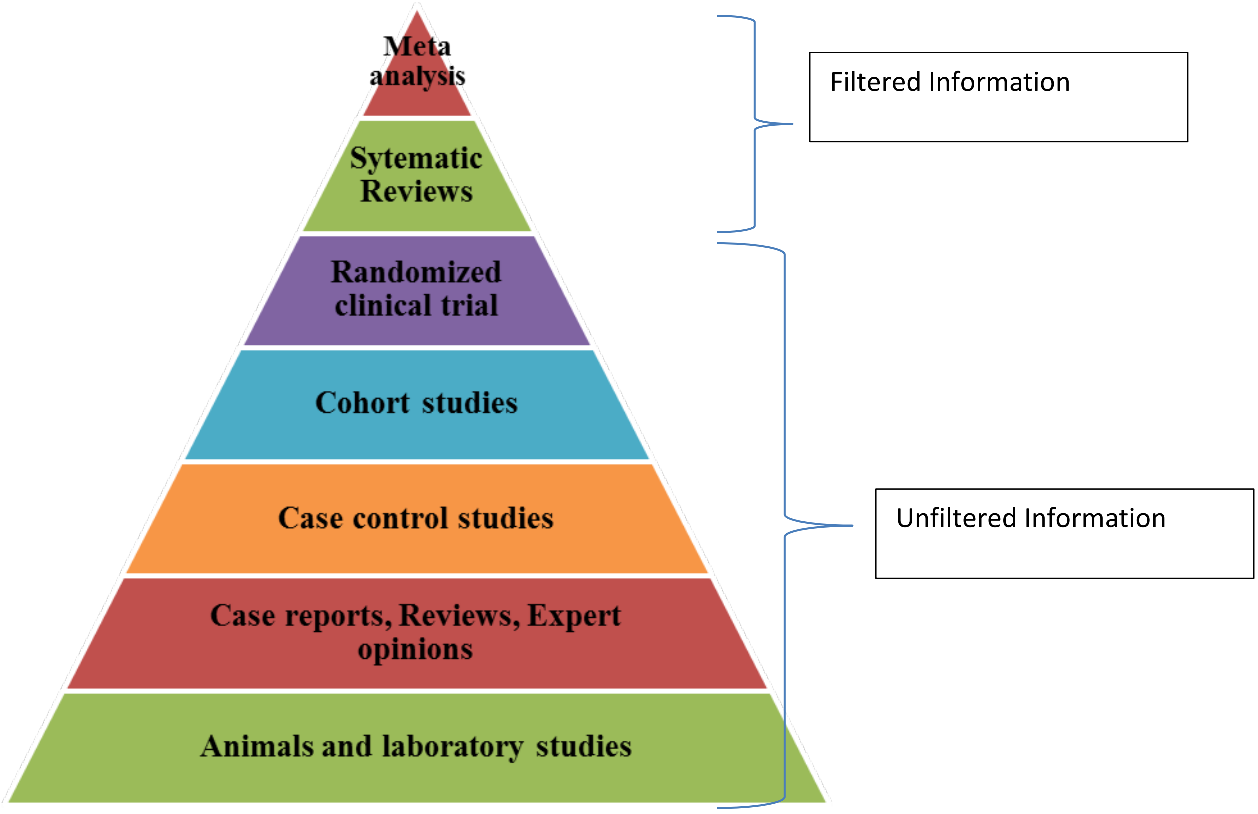
Systematic Review Title: The Demirjian versus the Willems methods for dental age estimation in different populations: A meta-analysis of published studies

Research Question: Does the Demirjian method for dental age estimation provide a more accurate estimate of chronological age when compared to the Willems method in dental age estimation of different populations?

2.1 Introduction and Context

It is believed that the higher up the hierarchy a study design is situated, the more stringent the method and hence the higher the probability that the study design can reduce the effect of bias on the study outcomes (Hoffman et al. 2013) (Figure 2.1). Well-designed systematic reviews and meta-analyses are usually at the top of the pyramid of evidence hierarchies, while laboratory studies, expert opinion and reviews are at the bottom. A systematic review synthesizes the results from all published studies on a specific topic, and provides a comprehensive analysis on the strengths, weaknesses and the research outcomes of the collected studies (Cook 1997). Systematic review is well regarded as the principal and foremost source of evidence to guide clinical and policy decisions regarding the effectiveness of therapies and methods for improved health (Yengopal and Mickenautsch 2009). Systematic reviews and meta-analyses provide the best evidence for all research question types because their outcomes are based on the findings of many researches that were identified by thorough methodical literature searches.

Figure 2.1. Hierarchy of evidence guiding clinical decision making (adapted from the NHMRC, 2009)



Systematic reviews are of great value if they include meta-analyses of clinical and methodologically homogeneous studies that are combined to provide a cumulative weight of evidence for or against a particular therapy or method. The advantages of meta-analysis over narrative or qualitative syntheses of the literature are that meta-analysis provides the opportunity to detect a treatment effect or outcome as statistically significant and to improve estimation of the effect by quantifying its outcome; thus making its estimation more precise (Higgins and Green 2011). Since the research question for the present study was highly focused, a systematic review of the evidence was undertaken rather than a traditional review because it provided the opportunity to employ a rigorous methodology to minimise the effect of bias when synthesizing the current information on the topic. Traditional narrative reviews (often called “Reviews”; in the case of Masters or PhD dissertations these are the “Literature review”) are based on individual opinions with selective references from the literature. They do not provide adequate evidence to answer research questions such as the question examined here. They only provide a synopsis of the research on a particular topic rather than answering a specific research question, may only be useful for background information, and are usually not publishable. Traditional reviews generally lack systematic search protocols or unambiguous criteria for selection of published studies and evaluating evidence and are therefore very prone to bias (Cook et al. 1997) (Table 2.1).

Table 2.1. Differences between systematic reviews and narrative reviews (adapted from Cook et al. 1997)

Feature	**Systematic Review**	**Narrative Review**
Question	A focused research question	Usually broad in scope
Sources and search	Comprehensive sources and explicit search strategy	Not usually specified, potentially biased
Selection	Criterion-based selection uniformly applied	Not usually specified, potentially biased
Appraisal	Rigorous critical appraisal	Variable
Synthesis	Qualitative summary that often includes statistical synthesis (meta-analysis)	Often a qualitative summary
Inferences	Evidence-based	Sometimes evidence-based

Based on the above considerations, it was felt that a systematic review would be a more rigorous exploration of the literature pertaining to the research question under investigation in this study.

2.2 Background

Population-based data on human biological growth and development processes are fundamental for assessing the health status of a community. This includes an understanding of the growth pattern for the children as well as the environmental stresses that disrupt or impede their growth. These stresses are often easy to identify, but data on uncompromised development and growth variation in most populations are surprisingly lacking. Instead, researchers typically compare growth in the population of interest to references formulated for European or US children. The problems associated with using non-population-specific references are complex, and their application can lead to misrepresentations of health status.

The importance of population-specific growth references extends beyond their utility in biological anthropology and health research. For many populations in rural Africa birth registry and eliciting date of birth is still a challenge. Occlusal tooth wear and anthropological details can be very useful for identification and aging (Kim et al. 2000; Yun et al. 2007). Data on timing of tooth formation, tooth emergence and dental morphometrics are also needed for forensic purposes, especially with the increasing global incidences of mass deaths and disasters (Kieser et al. 2005; Perrier et al. 2006). Additionally, tables of tooth emergence chronology are useful when birth records are unreliable or lost, where people seek asylum (Schmeling et al. 2007), where specific aging is needed to prevent cheating in age-graded sports competitions, or where individuals seek favourable outcomes in civil or criminal cases (Schulze et al. 2006; Meijerman et al. 2007; Ríos et al. 2008; Baumann et al. 2009; Ríos and Cardoso 2009). The age at death is usually the only biological parameter that can be estimated for unidentified juvenile remains with any degree of accuracy (Scheuer and Black 2000). Beyond this, information from dental development may play a major role in determining many clinical decisions, including choices about treatment options and sequence (Suri et al. 2004). In the absence of population-specific reference values, data from other regions and populations are used as reference, often without considering whether they are appropriate for comparison.

Variation in dental development among populations is reported in the literature (Pahkala et al. 1991; Willems et al. 2001; Liversidge 2003; Liversidge et al. 2006; Tunc and Koyuturk 2008). The reason for the variation among groups is not fully understood, although several explanations involving the interplay of genetic and environmental factors have been proposed (Chaillet et al. 2005). With increasing globalisation, there have been observable changes in the demographic features of many populations as well as changes in their physical profiles (Kearney 1995). Dental parameters are also evolving, and may be related to observable alterations in nutritional status, socioeconomic status, and genetic admixture. With these transformations, it is expected that dental growth and development reference of populations will modify with time.

Another source of variation in the timing of dental development is biological sex. Universally, females in any given population are more advanced in tooth formation than their male counterparts (Demirjian 1973; Demirjian and Levesque 1976: Demirjian 1980; Oziegbe et al. 2014). Furthermore, Kochhar and Richardson (1998), Eskeli et al. (1999) and Moslemi (2004) found that girls are also ahead of boys in permanent tooth emergence in Northern Irish, Finnish and Iranian children respectively, and similar differences are found for most populations.

The effect of malnutrition on dental development remains controversial, with conflicting results from different studies. Malnutrition is thought to have a greater negative impact on skeletal development than on the forming dentition. A recent study by Elamin and Liversidge (2014) on severely undernourished children in South Sudan reported no significant impact of nutrition on tooth formation. However, studies of African Americans and European Americans (Garn et al. 1973; Clemens et al. 2009) found that children from high socioeconomic backgrounds had earlier tooth emergence, which was attributed to better nutritional status.

Age estimation

Different methods have been proposed to estimate dental age using permanent tooth formation. Among these is Demirjian's method of age assessment formulated on a sample of French Canadian children, which involves the assessment of eight specific stages of tooth formation of the seven left mandibular teeth. Biologic weights, which are numerical, and derived using the method described in research on skeletal maturity (Tanner and Whitehouse 1962) are assigned to each tooth stage. The weights are added together to give the dental maturity score. Separate tables of dental maturity for males and females are used to convert the maturity scores to dental age (Demirjian et al. 1973). The advantage of the Demirjian method is the objective criteria for describing the stages of tooth development. The methodology gained worldwide acceptability and became the most commonly used method for estimation of dental age (Demirjian et al. 1973; Demirjian and Goldstein 1976). Studies using the method on other populations documented patterns of comparatively advanced or delayed tooth formation (Haavikko 1974; Hägg and Matson 1985; Davis and Hägg 1994; Mörnstad et al. 1994; Liversidge et al. 1999; Chaillet et al. 2005; Baghdadi and Pani 2011). This led several authors to question the cross-populational validity of Demirjian’s method and to argue for population-specific references for age estimation (Chaillet et al. 2004; Chaillet et al. 2005; Baghdadi and Pani 2011; Lee et al. 2011)

Willems et al. (2001) modified the Demirjian technique by creating new tables from which a maturity score could be directly expressed in years. The cumbersome step of converting the maturity score to a dental age was omitted, making the new method simpler to use while retaining the advantages of Demirjian's method. There was also a reduction in the overestimation of dental age, which was not statistically different from zero in a Belgian population (Willems et al. 2001). This modification was evaluated for several populations and reported to be more accurate than Demirjian's method (Maber et al. 2006; Mani et al. 2008; El Bakary et al. 2009; Liversidge et al. 2010; Pinchi et al. 2012; Ramadan et al. 2012).

No systematic review has compared the accuracy of the Demirjian and Williams methods for dental age estimation versus chronological age in different populations. This review therefore posed the following research question: ***Does the Demirjian method for dental age estimation provide a more accurate estimate of chronological age when compared to the Willems method in dental age estimation of different populations?*** The null hypothesis tested was that there was no difference in the accuracy of the two methods for dental age estimation against chronological age.

2.3 Methodology

2.3.1 Systematic Literature Search

The literature search was designed to find both published and unpublished studies on the research question. A three-step search strategy was utilized. An initial limited search of MEDLINE and CINAHL was undertaken, followed by an analysis of the text words contained in the title and abstract, and of the index terms used to describe articles. A second search using all identified keywords and index terms was then conducted across all the included databases. Thirdly, the reference lists of all identified reports and articles were searched for additional studies. Studies published in English and only those published from

1973 onward were considered for inclusion. This systematic review is registered with PROSPERO International prospective register of systematic reviews with registration number CRD42016029995. The protocol can be accessed via the following website. http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42016029995

The databases searched included:

MEDLINE, accessed via PubMed: SCOPUS: OVID: Biomed Central: Database of Open Access Journals (DOAJ): Ended: OpenSIGLE and Google Scholar

The search for unpublished studies included:

Hand search: reports: Thesis

Search terms included the following adjusted for the search engine/database used:

- (“Age estimation”) AND (Demirjian OR Willems)
- (“Dental age”) AND (Demirjian OR Willems)
- (“Tooth formation” AND Demirjian)
- Willems AND (“Tooth formation”)

The search was limited up to 28 December 2016.

Studies were eligible for inclusion if they met the following criteria:

- Cross-sectional studies
- Non-cross-sectionalstudies
- Comparative studies of either method or both methods,
- Study focus relevant to the research question,
- Full reports (abstracts without full reports not included),
- Study participants ranging in age from 0-18 years.

Articles were further excluded according to the following criteria:

- No computable data reported

- For comparative studies, test and control groups not evaluated the same way
- Studies conducted on subjects who were physically or medically compromised and those with developmental anomalies
- Studies conducted exclusively on third molars
- Studies published in any language other than English.

Titles and abstracts of identified citations from data sources were scanned by two reviewers (Temitope Esan (TE) and Veerasamy Yengopal (VY)) in duplication, for possible inclusion according to the above criteria. Articles with a suitable title but without a listed abstract were retrieved in full copy. All included articles were judged separately by the authors for possible exclusion with reason or for acceptance, in line with the exclusion/inclusion criteria. Disagreements between authors were solved through discussion and consensus with the third reviewer (Lynne Schepartz (LS)).

2.3.2 Data collection from accepted trials and analysis

Two reviewers (TE, VY) extracted data from accepted studies independently without being blinded to authors, institutions, journal name or study results. Disagreements between authors concerning data extracted were solved through discussion and consensus. All data were entered in specifically designed data sheets and are reported in the Table of included studies (Table 2.2). The following data were extracted:

(i) General important information: First author; year of publication and full article reference; place of trial; age; trial participant characteristics; type of study design

(ii) Information per test and control group: details of method used, age of participants (dental and chronological age), sex, numbers included

There were three outcome measures assessed:

(1) The difference in the dental age versus chronological age for the Demirjian method

(2) The difference in the dental age versus chronological age for the Willems method

(3) The mean age difference using the Demirjian method versus the Willems method

The above outcomes were compared independently for age and sex in different populations as per the included studies.

Datasets were created to facilitate pooling of similar outcomes into a meta-analysis. A dataset was defined as any extracted set of N, mean and standard deviation (SD) for test and control groups. For comparisons of continuous variables (dental age and chronological age), the mean with the SD was used. If the mean was reported without an SD, then attempts were made to obtain an SD from either the standard error of the mean or the 95% confidence intervals. If the standard error (SE) was reported instead of the SD, then the following formula was used:

$\mathbf{SD = SE} \times \sqrt{\mathbf{N}}$ (Higgins and Green 2011)

When making this transformation, the standard errors were from means calculated from within a group and not standard errors of the difference in means computed between the groups.

If studies reported the 95% confidence intervals, then the following formula was used to calculate the SD:

$\mathbf{SD} = \sqrt{\mathbf{N}} \times \textbf{(upper limit-lower limit)/3.92}$

The above formula applies to larger sample sizes (>60). If the sample size was small or less than 60 in each group then the denominator (3.92) in the formula above was replaced by 4.128. Again, when making this transformation, the confidence intervals were from means calculated from within a group and not standard errors of the difference in means computed between groups (Higgins and Green 2011).

For each dataset, the Mean Difference (MD) for continuous data with 95% Confidence Intervals (CI) and p-values were computed using a fixed effects model that used the inverse variance for continuous data to include studies directly proportionate to their sample size. Statistical significance was set at $p<0.05$. For computation of all point estimates, the statistical software program Cochrane RevMan version 5.3 was used.

In order to fulfill the criteria of clinical and methodological homogeneity, which allow for pooling of data for meta-analyses, datasets from the accepted publications did not differ in the following minimum set of characteristics: similar characteristics of children, assessment criteria similar in both groups, data collection and measurements similar in both groups.

2.3.3 Pooling of datasets

The I^2 test with 95% CI was used to establish whether any statistical heterogeneity existed between datasets that were assumed to be methodologically homogenous. The thresholds for I^2 point estimates (in %) and upper confidence values were used in order to interpret the test results (Higgins and Green 2011): 0-40% = might not be important; 30-60% = may represent moderate heterogeneity; 50-90% = may represent substantial heterogeneity; 75-100% = considerable heterogeneity. Identified (clinically/methodologically/statistically) homogenous datasets were pooled using a fixed effects meta-analysis with the Cochrane RevMan 5.3 software.

2.3.4 Assessment of methodological quality

Quantitative papers selected for this study were assessed by two independent reviewers for methodological validity prior to inclusion in the review using a revised standardized critical appraisal instrument from the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement (da Costa et al. 2011). This is a 40-item checklist used for observational studies (cross-sectional, cohort, case-control). Included studies were assessed according to the checklist and papers that achieved a score of at least 28 out of 40 were regarded as having high methodological quality (Yan et al. 2013).

2.3.5 Assessment of publication bias risk

Funnel plots were derived from pooled datasets using the Cochrane RevMan 5.3 software. Symmetrical funnel plots indicate no publication bias and asymmetrical plots are an indication of publication bias.

2.3.6 Statistical Analysis

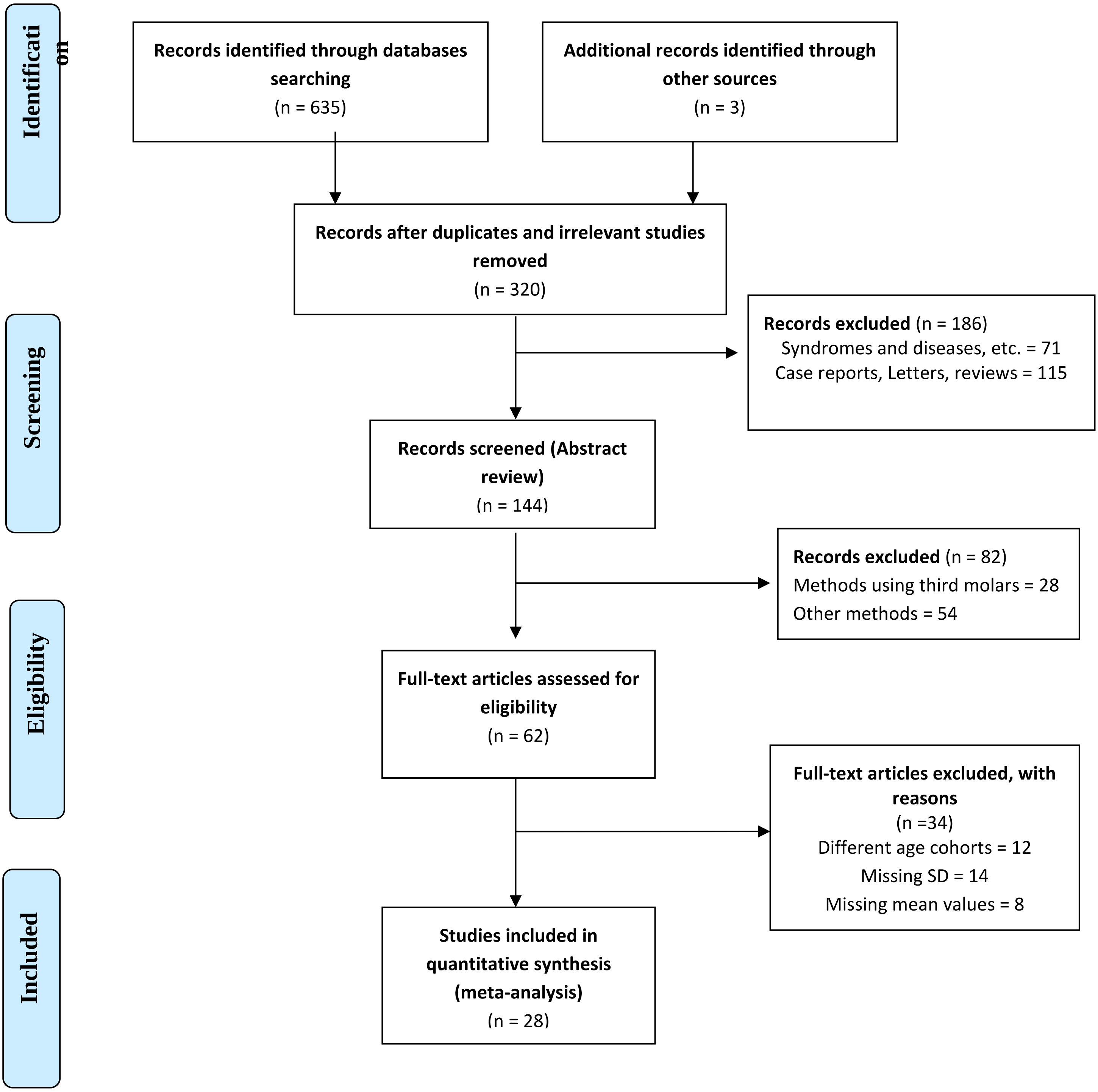
All statistical analyses were done using the Cochrane RevMan 5.3 software. Analysis was done separately for the two methods under review (Demirjian and Willems) with separate analyses of male and female data. The two methods were compared to determine their accuracy. The weighted mean difference (WMD) was used to assess accuracy of the methods in predicting the chronological age of the children. Heterogeneity and between study variability was assessed using the Tau and I^2 tests. A significant value of Tau ($p<0.05$) indicates significant heterogeneity. A value greater than 50% for the I^2 tests (with values ranging from 0 to 100%) is assumed to be significant. The effect sizes of the Demirjian method for different age groups were compared with those from the Willems method using a Student's t-test. Statistical significant was inferred at $p<0.05$.

2.4 Results

2.4.1 Literature Search

Figure 2.2 provides the flow diagram with details of how the identified studies were evaluated for final inclusion in this review. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) is an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses (Moher et al. 2009). PRISMA focuses on the reporting of reviews evaluating randomized trials, but it can also be used as a basis for reporting systematic reviews of other types of research, particularly evaluations of interventions (Moher et al. 2009). The common reasons for exclusion were that studies used a different age range (greater than 12 months cohort range, or different age cohort ranges, such as 3.5-4.5), absence of standard deviations, or lack of information regarding the methods for estimating the dental age.

Figure 2.2. PRISMA 2009 Flow Diagram for Systematic Review with Meta-analysis



All the cross-sectional studies met the inclusion criteria and were further analysed in this review. Information on these studies is provided in Table 2.2. Meta-analysis was performed on 28 published articles using the Demirjian and/or Willems methods to estimate chronological age in 14,109 children (6,581 males and 7,528 females) age 3-18 years in studies using the Demirjian method and 10,832 children (5176 males and 5656 females) age 4-18 years in studies using the Willems method. Most papers reported that the Demirjian method significantly overestimated the chronological age and was therefore not applicable for use in that specific population. This was observed in studies that used only the Demirjian method and in studies that compared the Demirjian method to other methods such as the Willems method. The Willems method was found to be a more accurate tool to estimate chronological age (Table 2.2).

Table 2.2. Table of Included Studies

Article	Type of study: Brief details	Details of participants and methods used	Main findings
Amberkova et al. 2014	Cross-sectional comparative: OPG of 7 left mandibular teeth. Study setting: Macedonia	966 children aged 6-13 analyzed using Willems and Demirjian methods	Willems method most accurate; Demirjian method overestimated chronological age
Asab et al. 2011	Cross-sectional: OPG of 7 left mandibular teeth. Study setting: Malaysia	905 children aged 6-16 analyzed using Demirjian method	Demirjian method less accurate by overestimating chronological age
Bagherpour et al. 2010	Cross-sectional. Study setting: Iran	311 boys and girls analyzed using Demirjian method	Demirjian method appropriate only for children 9-13 years
Caneiro et al. 2015	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Portugal	564 children analyzed using Demirjian method	Demirjian method not useful in predicting chronological age. Overestimation of dental age
Cavric et al. 2016	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Botswana	1760 children aged 6-23 analyzed using Demirjian method	Demirjian method not useful in predicting chronological age.
Djukic et al. 2013	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Serbia	686 children aged 4-15 analyzed using Demirjian and Willems methods	Demirjian method overestimated chronological age. Willems method provided better accuracy
El Bakary et al. 2010	Cross-sectional: OPG of 7 left mandibular teeth. Study setting: India	286 children aged 5-16 analyzed using Willems and Cameriere methods	Willems method predicts better than Cameriere method. Hence could be used in Egyptian population
Erdem et al. 2013	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: NW Turkey	425 children aged 7-13 analyzed using Demirjian method	Demirjian method overestimated chronological age and hence not suitable for estimating age
Feijoo et al. 2012	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Spain	1010 children 2-16 analyzed using Demirjian method	Demirjian method overestimated chronological age
Flood et al. 2013	Cross-sectional retrospective: OPG of 7 left mandibular teeth used. Study setting: Australia	504 children analyzed using the 4 Demirjian methods	All methods not accurate in predicting chronological age.
Galic et al. 2011	Cross-sectional comparative: Setting: Bosnia-Herzegovina	1089 children analyzed using Cameriere. Haavikko and Willems methods	Willems method overestimated chronological age hence not accurate
Hegde et al. 2016	Cross-sectional observational: OPG of 7 left mandibular teeth. Study setting: India	1200 children aged 5-15 analyzed using Willems I and Willems 2 methods	Willems 1method predicted age of boys more accurately
Ifesanya et al. 2012	Cross-sectional retrospective: OPG of 7 left mandibular teeth used. Study setting: Nigeria	124 children aged 4-16 analyzed using Demirjian method	Demirjian method overestimated chronological age
Javadinejad et al. 2013	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Iran	537 children aged 3.9-14 analyzed using Demirjian, Willems, Cameriere and Smith methods	Demirjian and Willems methods overestimated chronological age and hence less accurate
Khoja Fida and Shaikh 2015	Cross-sectional retrospective: OPG of 7 left mandibular teeth used. Study setting: Pakistan	403 children analyzed using Demirjian, Willems and Nolla methods	Willems method better predicts chronological age
Kirzioglu and Ceyhan 2012	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Turkey	425 children aged 7-13 analyzed using Demirjian, Nolla and Haavikko methods	All three methods not suitable for Turkish children

Koshy and Tandon 1998	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Southern India	184 children assessed using Demirjian method	Demirjian method overestimated chronological age hence not useful
Kumaresan et al. 2016	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Malaysia	426 children aged 5-15 analyzed using Demirjian, Willems and Nolla methods	Demirjian method least precise, overestimated chronological age
Leurs et al. 2005	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: Holland	451 children aged 3-17 analyzed using Demirjian method	Demirjian method overestimated chronological age hence not useful
Mani et al. 2008	Cross-sectional observational: Study setting: Malaysia	214 boys and 214 girls, selected by simple stratified random sampling. OPGs analyzed using Demirjian and Willems methods	Both overestimated chronological age but Willems had better accuracy
Mohammed et al. 2014	Cross-sectional comparative: OPG of 7 left mandibular teeth. Study setting: South India	660 children aged 6-13 analyzed using Willems, Demirjian, Nolla and Haavikko methods	All methods are reliable in estimating age
Mohammed et al. 2015	Cross-sectional comparative: OPG of 7 left mandibular teeth. Study setting: India	332 children aged 6-15.99 analyzed using Demirjian and Willems methods	Willems method is the best predictor of chronological age
Nik-Hussein and Kee Gan 2011	Cross-sectional study: OPG of 7 left mandibular teeth. Study setting: Malaysia	991 children aged 5-15; Willems and Demirjian methods compared for accuracy	Willems method more applicable for estimating dental age. Demirjian method overestimated chronological age
Patel et al. 2016	Cross-sectional comparative: OPG of 7 left mandibular teeth. Study setting: India	160 children aged 6-16 analyzed using Demirjian, Willem and Greulich and Pyle methods	Willems method can be accurately used in Southern India
Urzel and Bruzek 2015	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: France	743 children aged 4-15 analyzed using Demirjian, Willems I, II and Chaillet methods	Willems I method the most suitable when sex and ethnicity are known
Uys et al. 2014	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: South Africa	833 children aged 6-16 analyzed using Demirjian method	Demirjian method overestimated chronological age
Ye et al. 2014	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: China	941 children aged 7-14 analyzed using Demirjian and Willems methods	Willems method more applicable for estimating dental age. Demirjian method overestimated chronological age
Zhai et al. 2016	Cross-sectional retrospective: OPG of 7 left mandibular teeth. Study setting: China	1004 children aged 11-18 analyzed using Demirjian and Willems methods	Demirjian method overestimated chronological age but better accuracy with Demirjian method than with Willems method

OPG = Panoramic Radiographs

Table 2.3 provides the scores obtained when assessing the included studies using the STROBE checklist. The item scores are not intended to be a reflection of the quality of the included papers (von Elm et al. 2007), but are used to provide some insights on the methodological rigor of the individual papers. Most papers achieved scores of around 28, which has been used in previously published studies as an indication of high methodological quality (Yan et al. 2013).

Table 2.3 STROBE 40 Item Checklist Scores for Included Cross-sectional Studies

Amberkova et al. 2014	Cross-sectional comparative	27
Asab et al. 2011	Cross-sectional	27
Bagherpour et al. 2010	Cross-sectional	28
Caneiro et al. 2015	Retrospective	27
Cavric et al. 2016	Retrospective	28
Djukic et al, 2013	Retrospective	27
El Bakary et al. 2010	Cross-sectional	29
Erdem et al. 2013	Retrospective	26
Feijoo et al. 2012	Retrospective	26
Flood et al. 2013	Retrospective	28
Galic et al. 2011	Cross-sectional comparative	26
Hegde et al. 2016	Observational	26
Ifesanya et al. 2012	Retrospective	26
Javadinejad et al. 2013	Retrospective	26
Khoja and Shaikh 2015	Retrospective	27
Kirzioglu and Ceyhan 2012	Retrospective	26
Koshy and Tandon 1998	Cross-sectional	27
Kumaresan et al. 2016	Cross-sectional	25
Leurs et al. 2005	Retrospective	25
Mani et al. 2008	Cross-sectional	28
Mohammed et al. 2014	Cross-sectional comparative	26
Mohammed et al. 2015	Cross-sectional comparative	25
Nik-Hussein and Kee Gan 2011	Cross-sectional	26
Patel et al. 2016	Cross-sectional comparative	25
Urzel and Bruzek 2015	Retrospective	26
Uys et al. 2014	Retrospective	26
Ye et al. 2014	Retrospective	27
Zhai et al. 2016	Retrospective	27

2.4.2 Pooled meta-analysis of studies using the Demirjian method to determine difference in the dental age versus chronological age in males and females

The pooled effect estimates for ages 3-18 years in all the included studies were analyzed for males and females and a summary of the results obtained is presented in Figures 2.3 and 2.4. Considerable heterogeneity (I^2= 97% in males and 98% in females) was found in the pooled analyses for age groups 3-18 years. This can be explained by the pooling together of the ages and studies from different populations that have been found to grow at different rates (Cohen 2003). Overall, the meta-analysis showed a significant weighted mean difference (WMD) between the dental age and the chronological age in males (WMD=0.62 years, 95% CI (0.56, 0.66)) and in females (WMD=0.72 years, 95% CI (0.69, 0.75)). For males (Figure 2.3), the majority of the studies reported significant overestimation by the Demirjian method. The exception is that of Zhai et al. (2016), who reported a significant under-estimation of chronological age in males (WMD=-0.63 years, 95% CI (-0.85, -0.41). Three studies (Bagherpouret et al. 2010; Erdem et al. 2013; Mohammed et al. 2015) reported no significant difference between dental age estimation and chronological age for males. Similarly, for females most studies reported overestimation of the chronological age while only two studies (Erdem et al. 2015; Zhai et al. 2016) reported underestimation of the chronological age (Figure 2.4).

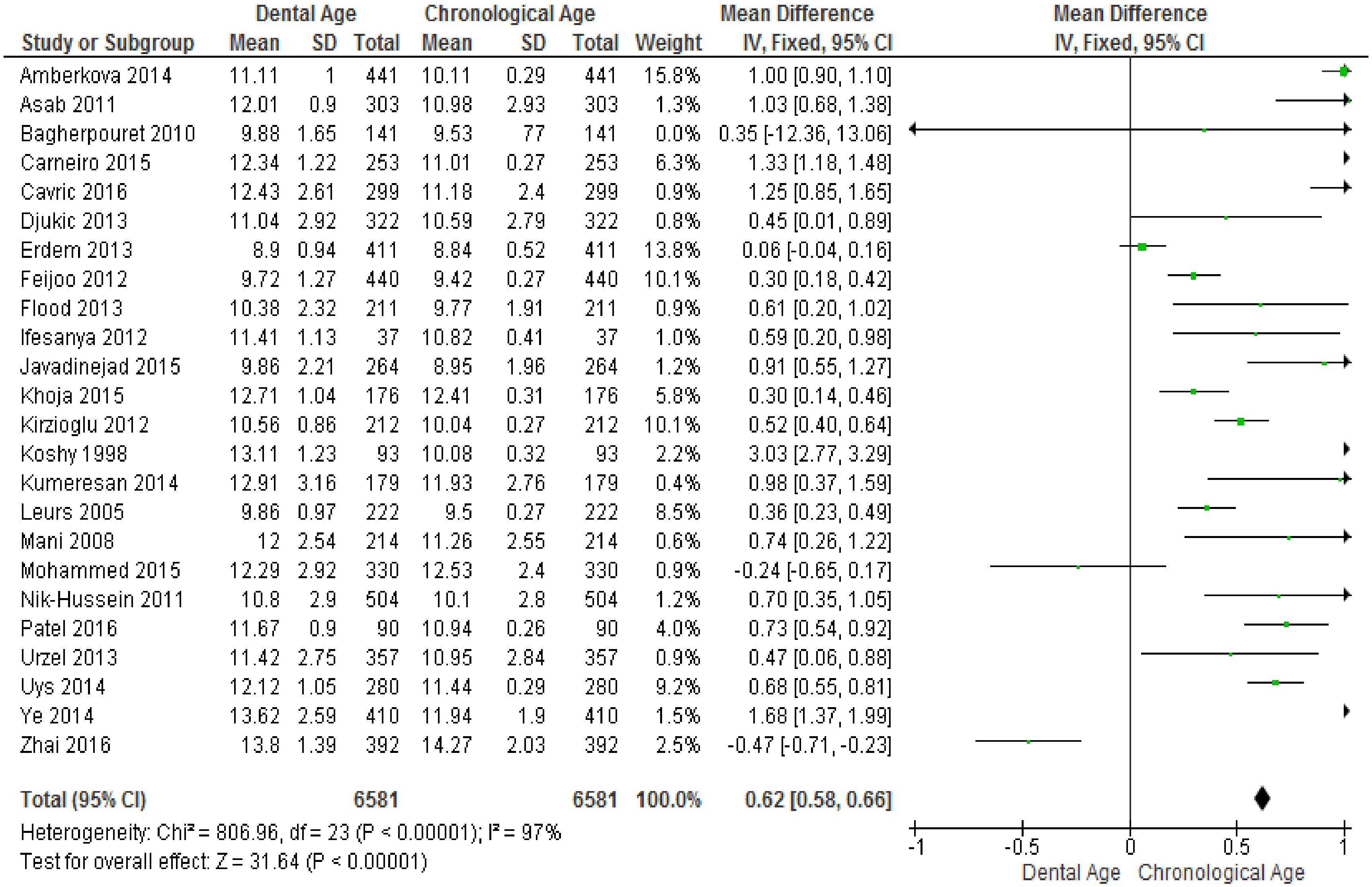
Meta-analysis of each age cohort in males and females demonstrated that the majority of the age cohorts had considerable heterogeneity (75-100%) with the exception of age cohorts 4 and 16 years in females. The heterogeneity may be due to the pooling of different studies into the meta-analyses. In males, significant overestimation of the chronological age by the Demirjian method was observed in the 3-15 year age cohorts. On the contrary, significant underestimation of the chronological ages was observed in the 16-18 year age cohorts (Table 2.4). Significant overestimation of the chronological ages of females was observed in all the age cohorts except 3 and 16-18 years where significant underestimation of chronological ages was observed (Table 2.4).

2.4.3 Pooled meta-analysis of studies using the Willems method to determine difference in the dental age versus chronological age in males and females

The pooled effect estimates of the Willems method for ages 4-18 in all the included studies were analyzed for males and females (Figures 2.5 and 2.6). Considerable heterogeneity (I^2 =85% in males and 93% in females) was detected in the pooled analyses for age groups 4-18 years. Again, this can be explained by the pooling together of the ages and studies from different populations, as mentioned above. The meta-analysis showed significant difference between the dental age and the chronological age in males (WMD=0.26 years, 95% CI (0.20, 0.32)) and in females (WMD=0.29, 95% CI (0.24, 0.35)). Six studies reported significant overestimation in males while only four studies reported significant overestimation in females. Furthermore, three studies reported significant underestimation in males, while only Zhai et al. (2016) reported significant underestimation in females. Seven studies of males and 11 of females did not report significant differences (Figures 2.5 and 2.6).

Variation in heterogeneity of the included studies was observed for both males and females when the studies were pooled by sex and age cohorts. The I^2 values ranged from “might not be important” (0-40%) to “considerable heterogeneity” (75-100%) in both males and females. Again, this can be attributed to the pooling together of different ages and populations. Meta-analysis of the age cohorts in males showed significant overestimation in age cohorts 5-14 years, while significant underestimation was found in age cohorts 16-18 years (Table 2.5). No significant differences were found between the dental ages and chronological ages of children in the age cohorts 4 and 15 years. In females, overestimation of the chronological age was observed in the age cohorts 5-8 and 11-13 years, while significant underestimation was found in the age cohorts 15-18 years (Table 2.5).

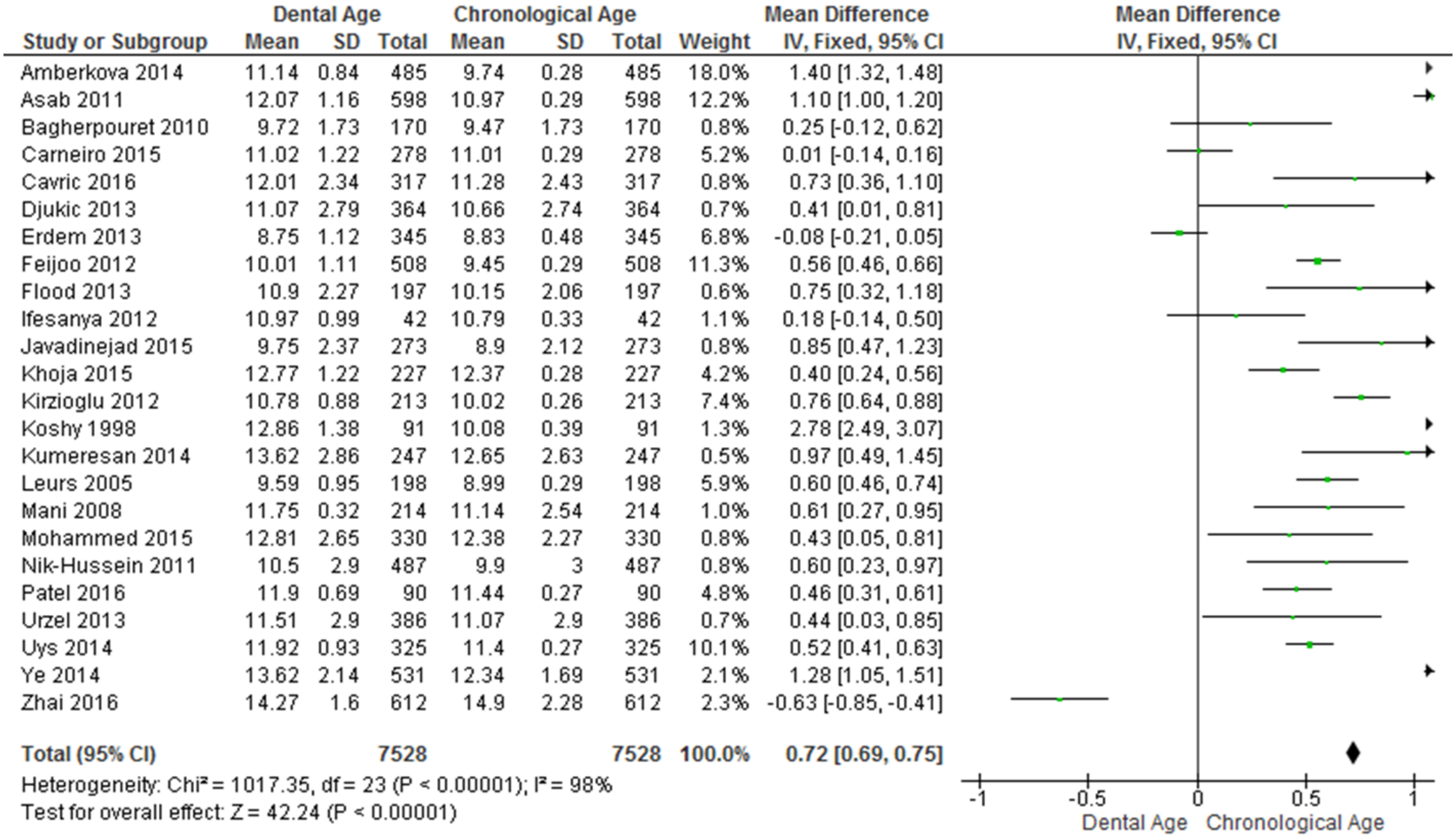
Figure 2.3: Comparison of dental age and chronological age pooled for sex (males) using the Demirjian method



Study or Subgroup	Dental Age Mean	Dental Age SD	Dental Age Total	Chronological Age Mean	Chronological Age SD	Chronological Age Total	Weight	Mean Difference IV, Fixed, 95% CI
Amberkova 2014	11.11	1	441	10.11	0.29	441	15.8%	1.00 [0.90, 1.10]
Asab 2011	12.01	0.9	303	10.98	2.93	303	1.3%	1.03 [0.68, 1.38]
Bagherpouret 2010	9.88	1.65	141	9.53	77	141	0.0%	0.35 [-12.36, 13.06]
Carneiro 2015	12.34	1.22	253	11.01	0.27	253	6.3%	1.33 [1.18, 1.48]
Cavric 2016	12.43	2.61	299	11.18	2.4	299	0.9%	1.25 [0.85, 1.65]
Djukic 2013	11.04	2.92	322	10.59	2.79	322	0.8%	0.45 [0.01, 0.89]
Erdem 2013	8.9	0.94	411	8.84	0.52	411	13.8%	0.06 [-0.04, 0.16]
Feijoo 2012	9.72	1.27	440	9.42	0.27	440	10.1%	0.30 [0.18, 0.42]
Flood 2013	10.38	2.32	211	9.77	1.91	211	0.9%	0.61 [0.20, 1.02]
Ifesanya 2012	11.41	1.13	37	10.82	0.41	37	1.0%	0.59 [0.20, 0.98]
Javadinejad 2015	9.86	2.21	264	8.95	1.96	264	1.2%	0.91 [0.55, 1.27]
Khoja 2015	12.71	1.04	176	12.41	0.31	176	5.8%	0.30 [0.14, 0.46]
Kirzioglu 2012	10.56	0.86	212	10.04	0.27	212	10.1%	0.52 [0.40, 0.64]
Koshy 1998	13.11	1.23	93	10.08	0.32	93	2.2%	3.03 [2.77, 3.29]
Kumeresan 2014	12.91	3.16	179	11.93	2.76	179	0.4%	0.98 [0.37, 1.59]
Leurs 2005	9.86	0.97	222	9.5	0.27	222	8.5%	0.36 [0.23, 0.49]
Mani 2008	12	2.54	214	11.26	2.55	214	0.6%	0.74 [0.26, 1.22]
Mohammed 2015	12.29	2.92	330	12.53	2.4	330	0.9%	-0.24 [-0.65, 0.17]
Nik-Hussein 2011	10.8	2.9	504	10.1	2.8	504	1.2%	0.70 [0.35, 1.05]
Patel 2016	11.67	0.9	90	10.94	0.26	90	4.0%	0.73 [0.54, 0.92]
Urzel 2013	11.42	2.75	357	10.95	2.84	357	0.9%	0.47 [0.06, 0.88]
Uys 2014	12.12	1.05	280	11.44	0.29	280	9.2%	0.68 [0.55, 0.81]
Ye 2014	13.62	2.59	410	11.94	1.9	410	1.5%	1.68 [1.37, 1.99]
Zhai 2016	13.8	1.39	392	14.27	2.03	392	2.5%	-0.47 [-0.71, -0.23]
Total (95% CI)			**6581**			**6581**	**100.0%**	**0.62 [0.58, 0.66]**

Heterogeneity: Chi² = 806.96, df = 23 (P < 0.00001); I² = 97%
Test for overall effect: Z = 31.64 (P < 0.00001)

Figure 2.4: Comparison of dental age and chronological age pooled for sex (females) using the Demirjian method



	Dental Age			Chronological Age				Mean Difference
Study or Subgroup	**Mean**	**SD**	**Total**	**Mean**	**SD**	**Total**	**Weight**	**IV, Fixed, 95% CI**
Amberkova 2014	11.14	0.84	485	9.74	0.28	485	18.0%	1.40 [1.32, 1.48]
Asab 2011	12.07	1.16	598	10.97	0.29	598	12.2%	1.10 [1.00, 1.20]
Bagherpouret 2010	9.72	1.73	170	9.47	1.73	170	0.8%	0.25 [-0.12, 0.62]
Carneiro 2015	11.02	1.22	278	11.01	0.29	278	5.2%	0.01 [-0.14, 0.16]
Cavric 2016	12.01	2.34	317	11.28	2.43	317	0.8%	0.73 [0.36, 1.10]
Djukic 2013	11.07	2.79	364	10.66	2.74	364	0.7%	0.41 [0.01, 0.81]
Erdem 2013	8.75	1.12	345	8.83	0.48	345	6.8%	-0.08 [-0.21, 0.05]
Feijoo 2012	10.01	1.11	508	9.45	0.29	508	11.3%	0.56 [0.46, 0.66]
Flood 2013	10.9	2.27	197	10.15	2.06	197	0.6%	0.75 [0.32, 1.18]
Ifesanya 2012	10.97	0.99	42	10.79	0.33	42	1.1%	0.18 [-0.14, 0.50]
Javadinejad 2015	9.75	2.37	273	8.9	2.12	273	0.8%	0.85 [0.47, 1.23]
Khoja 2015	12.77	1.22	227	12.37	0.28	227	4.2%	0.40 [0.24, 0.56]
Kirzioglu 2012	10.78	0.88	213	10.02	0.26	213	7.4%	0.76 [0.64, 0.88]
Koshy 1998	12.86	1.38	91	10.08	0.39	91	1.3%	2.78 [2.49, 3.07]
Kumeresan 2014	13.62	2.86	247	12.65	2.63	247	0.5%	0.97 [0.49, 1.45]
Leurs 2005	9.59	0.95	198	8.99	0.29	198	5.9%	0.60 [0.46, 0.74]
Mani 2008	11.75	0.32	214	11.14	2.54	214	1.0%	0.61 [0.27, 0.95]
Mohammed 2015	12.81	2.65	330	12.38	2.27	330	0.8%	0.43 [0.05, 0.81]
Nik-Hussein 2011	10.5	2.9	487	9.9	3	487	0.8%	0.60 [0.23, 0.97]
Patel 2016	11.9	0.69	90	11.44	0.27	90	4.8%	0.46 [0.31, 0.61]
Urzel 2013	11.51	2.9	386	11.07	2.9	386	0.7%	0.44 [0.03, 0.85]
Uys 2014	11.92	0.93	325	11.4	0.27	325	10.1%	0.52 [0.41, 0.63]
Ye 2014	13.62	2.14	531	12.34	1.69	531	2.1%	1.28 [1.05, 1.51]
Zhai 2016	14.27	1.6	612	14.9	2.28	612	2.3%	-0.63 [-0.85, -0.41]
Total (95% CI)			**7528**			**7528**	**100.0%**	**0.72 [0.69, 0.75]**

Heterogeneity: Chi² = 1017.35, df = 23 (P < 0.00001); I² = 98%

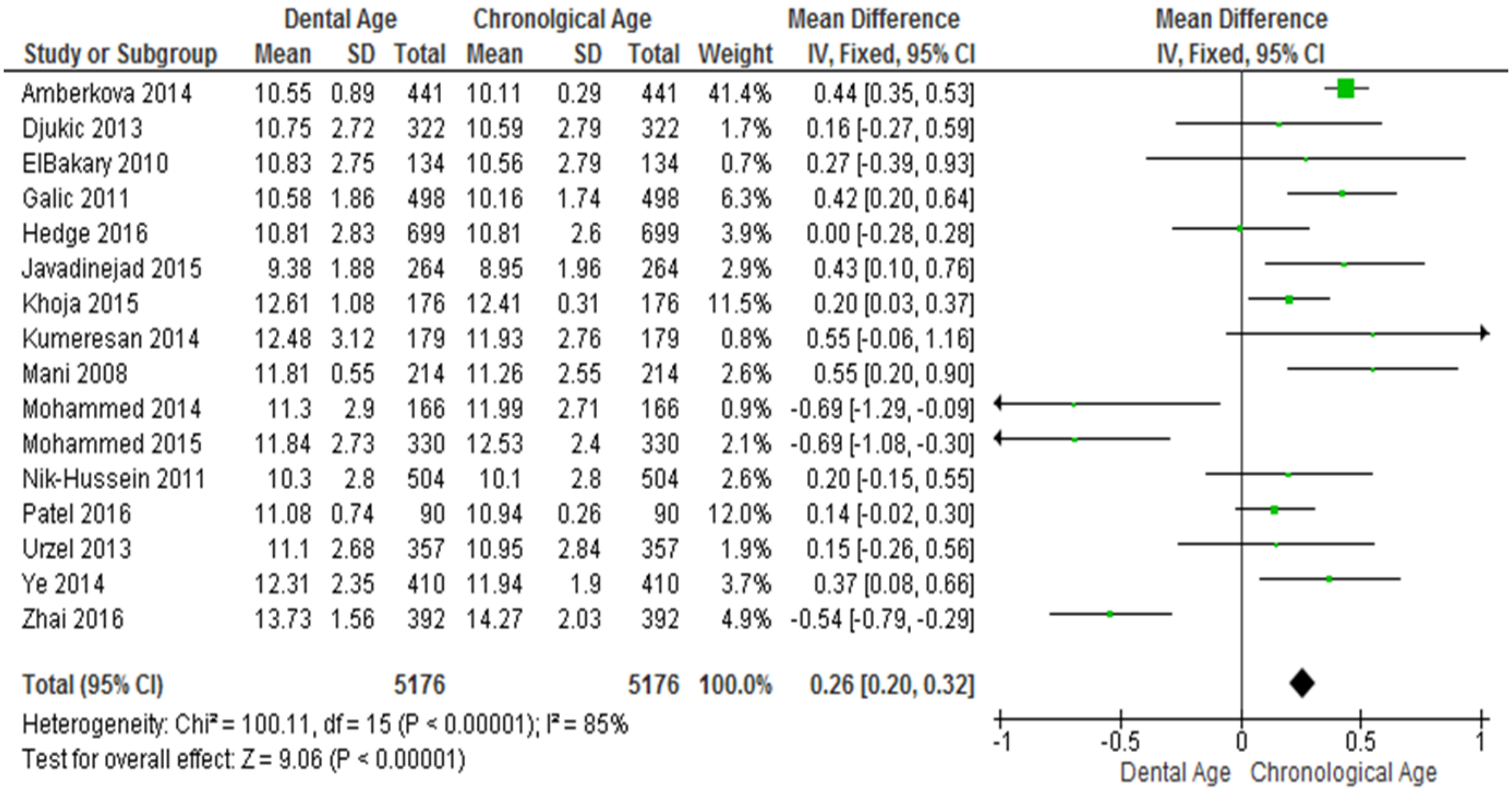
Test for overall effect: Z = 42.24 (P < 0.00001)

Table 2.4: Pooled effect estimates (dental age versus chronological age) for ages 3-18 and sex (males and females) using the Demirjian method

Age cohort	**Male**					**Female**				
	Number of studies	**n**	**I^2 (%)**	**Effect estimate (95% CI)**	**SD**	**Number of studies**	**n**	**I^2 (%)**	**Effect estimate (95% CI)**	**SD**
3	1	26	NA	**0.57 [0.03, 1.11]**	1.32	1	14	NA	-0.19 [-0.60, 0.22]	0.74
4	4	106	71	**0.61 [0.42, 0.81]**	1.25	4	100	44	**0.28 [0.08, 0.48]**	1.24
5	8	270	93	**1.39 [1.26, 1.51]**	1.28	8	244	82	**1.16 [1.02, 1.30]**	1.36
6	15	614	82	**1.11 [1.04, 1.17]**	1.00	15	608	83	**0.88 [0.81, 0.95]**	1.07
7	19	968	96	**0.76 [0.71, 0.82]**	1.06	19	1084	76	**0.52 [0.46, 0.57]**	1.12
8	20	1360	87	**0.53 [0.46, 0.60]**	1.60	20	1400	76	**0.49 [0.42, 0.55]**	1.51
9	20	1366	82	**0.49 [0.41, 0.58]**	1.95	20	1412	83	**0.57 [0.48, 0.66]**	2.10
10	20	1348	89	**0.75 [0.65, 0.84]**	2.17	20	1367	86	**0.64 [0.55, 0.72]**	1.95
11	21	1556	97	**0.84 [0.77, 0.92]**	1.84	20	1564	91	**0.90 [0.82, 0.97]**	1.84
12	21	1354	95	**0.88 [0.79, 0.96]**	1.94	20	1679	95	**0.87 [0.82, 0.93]**	1.40
13	20	1146	96	**1.08 [1.00, 1.17]**	1.79	19	1420	98	**1.14 [1.08, 1.21]**	1.52
14	17	784	95	**1.06 [0.99,1.14]**	1.30	16	1108	97	**0.60 [0.55, 0.65]**	1.03
15	13	544	95	**0.11 [0.04, 0.18]**	1.01	12	658	96	**-0.20 [-0.27, -0.13]**	1.12
16	4	112	NA	**-1.48 [-1.79, -1.17]**	2.04	5	224	56	**-0.81 [-0.96, -0.66]**	1.39
17	1	76	NA	**-1.95 [-2.17, -1.73]**	1.19	1	148	NA	**-1.52 [-1.67, -1.37]**	1.13
18	1	36	NA	**-2.67 [-2.92, -2.42]**	0.72	1	176	NA	**-2.52 [-2.65, -2.39]**	1.07

Significant values in bold

Figure 2.5: Comparison of dental age and chronological age pooled for sex (males), using Willems method

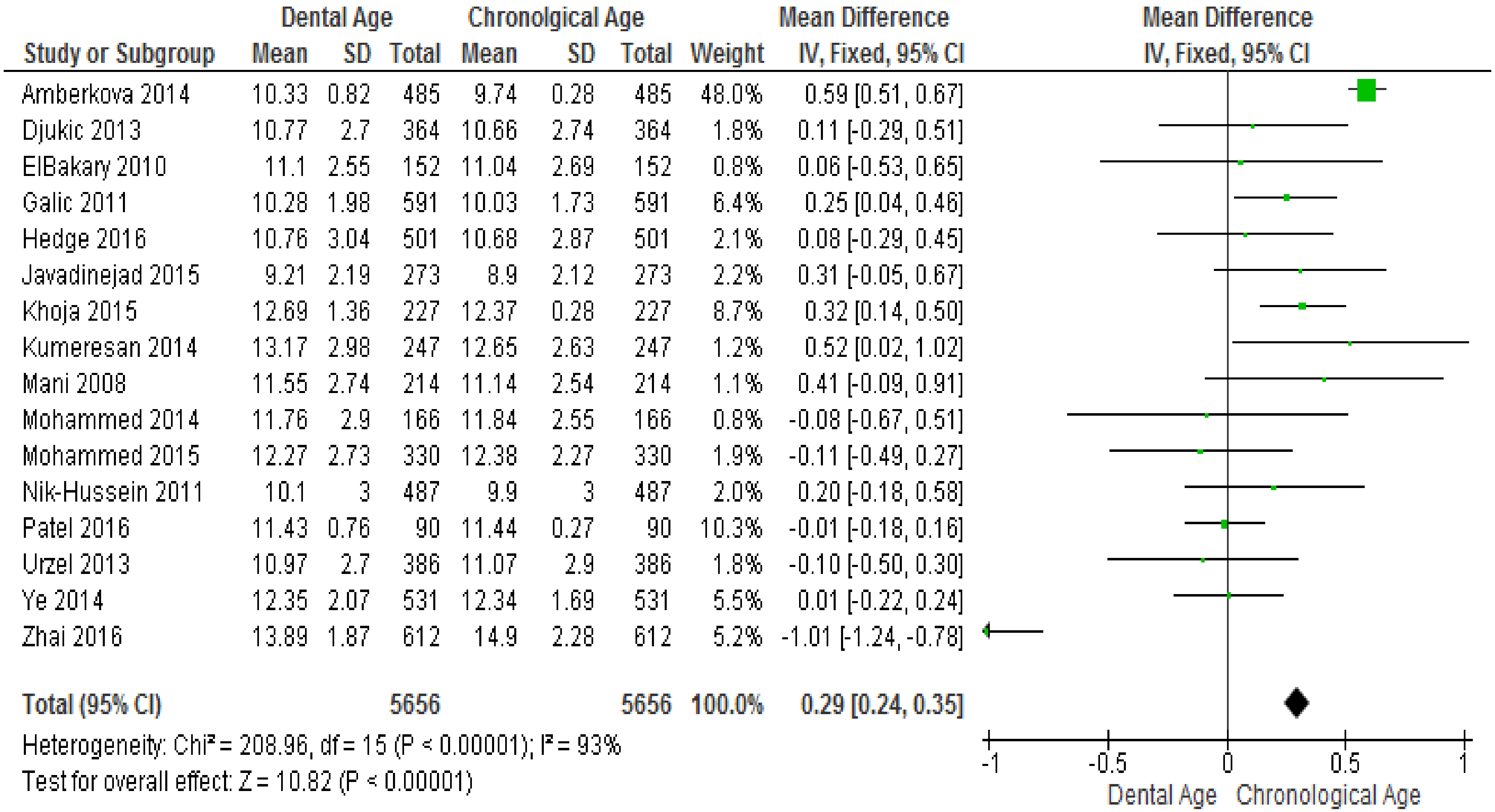


Study or Subgroup	Dental Age Mean	Dental Age SD	Dental Age Total	Chronolgical Age Mean	Chronolgical Age SD	Chronolgical Age Total	Weight	Mean Difference IV, Fixed, 95% CI
Amberkova 2014	10.55	0.89	441	10.11	0.29	441	41.4%	0.44 [0.35, 0.53]
Djukic 2013	10.75	2.72	322	10.59	2.79	322	1.7%	0.16 [-0.27, 0.59]
ElBakary 2010	10.83	2.75	134	10.56	2.79	134	0.7%	0.27 [-0.39, 0.93]
Galic 2011	10.58	1.86	498	10.16	1.74	498	6.3%	0.42 [0.20, 0.64]
Hedge 2016	10.81	2.83	699	10.81	2.6	699	3.9%	0.00 [-0.28, 0.28]
Javadinejad 2015	9.38	1.88	264	8.95	1.96	264	2.9%	0.43 [0.10, 0.76]
Khoja 2015	12.61	1.08	176	12.41	0.31	176	11.5%	0.20 [0.03, 0.37]
Kumeresan 2014	12.48	3.12	179	11.93	2.76	179	0.8%	0.55 [-0.06, 1.16]
Mani 2008	11.81	0.55	214	11.26	2.55	214	2.6%	0.55 [0.20, 0.90]
Mohammed 2014	11.3	2.9	166	11.99	2.71	166	0.9%	-0.69 [-1.29, -0.09]
Mohammed 2015	11.84	2.73	330	12.53	2.4	330	2.1%	-0.69 [-1.08, -0.30]
Nik-Hussein 2011	10.3	2.8	504	10.1	2.8	504	2.6%	0.20 [-0.15, 0.55]
Patel 2016	11.08	0.74	90	10.94	0.26	90	12.0%	0.14 [-0.02, 0.30]
Urzel 2013	11.1	2.68	357	10.95	2.84	357	1.9%	0.15 [-0.26, 0.56]
Ye 2014	12.31	2.35	410	11.94	1.9	410	3.7%	0.37 [0.08, 0.66]
Zhai 2016	13.73	1.56	392	14.27	2.03	392	4.9%	-0.54 [-0.79, -0.29]
Total (95% CI)			**5176**			**5176**	**100.0%**	**0.26 [0.20, 0.32]**

Heterogeneity: Chi² = 100.11, df = 15 (P < 0.00001); I² = 85%

Test for overall effect: Z = 9.06 (P < 0.00001)

Figure 2.6: Comparison of dental age and chronological age pooled for sex (females) using Willems method



	Dental Age			Chronolgical Age				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI
Amberkova 2014	10.33	0.82	485	9.74	0.28	485	48.0%	0.59 [0.51, 0.67]
Djukic 2013	10.77	2.7	364	10.66	2.74	364	1.8%	0.11 [-0.29, 0.51]
ElBakary 2010	11.1	2.55	152	11.04	2.69	152	0.8%	0.06 [-0.53, 0.65]
Galic 2011	10.28	1.98	591	10.03	1.73	591	6.4%	0.25 [0.04, 0.46]
Hedge 2016	10.76	3.04	501	10.68	2.87	501	2.1%	0.08 [-0.29, 0.45]
Javadinejad 2015	9.21	2.19	273	8.9	2.12	273	2.2%	0.31 [-0.05, 0.67]
Khoja 2015	12.69	1.36	227	12.37	0.28	227	8.7%	0.32 [0.14, 0.50]
Kumeresan 2014	13.17	2.98	247	12.65	2.63	247	1.2%	0.52 [0.02, 1.02]
Mani 2008	11.55	2.74	214	11.14	2.54	214	1.1%	0.41 [-0.09, 0.91]
Mohammed 2014	11.76	2.9	166	11.84	2.55	166	0.8%	-0.08 [-0.67, 0.51]
Mohammed 2015	12.27	2.73	330	12.38	2.27	330	1.9%	-0.11 [-0.49, 0.27]
Nik-Hussein 2011	10.1	3	487	9.9	3	487	2.0%	0.20 [-0.18, 0.58]
Patel 2016	11.43	0.76	90	11.44	0.27	90	10.3%	-0.01 [-0.18, 0.16]
Urzel 2013	10.97	2.7	386	11.07	2.9	386	1.8%	-0.10 [-0.50, 0.30]
Ye 2014	12.35	2.07	531	12.34	1.69	531	5.5%	0.01 [-0.22, 0.24]
Zhai 2016	13.89	1.87	612	14.9	2.28	612	5.2%	-1.01 [-1.24, -0.78]
Total (95% CI)			**5656**			**5656**	**100.0%**	**0.29 [0.24, 0.35]**

Heterogeneity: Chi² = 208.96, df = 15 (P < 0.00001); I² = 93%

Test for overall effect: Z = 10.82 (P < 0.00001)

Table 2.5: Pooled effect estimates (dental age versus chronological age) for ages 4-18 and sex (males and females) using Willems method

Age cohort	**Male**					**Female**				
	Number of studies	N	**I^2 (%)**	**Effect estimate (95% CI)**	**SD**	**Number of Studies**	**N**	**I^2 (%)**	**Effect estimate (95% CI)**	**SD**
4	2	18	0	-0.05 [-0.39, 0.30]	0.91	2	20	0	0.02 [-0.35, 0.40]	0.80
5	4	140	0	**0.31 [0.12, 0.50]**	1.40	4	134	65	**0.45 [0.28, 0.62]**	1.22
6	6	348	73	**0.54 [0.42, 0.65]**	1.33	6	326	83	**0.17 [0.07, 0.26]**	1.07
7	8	510	91	**0.55[0.47, 0.63]**	1.12	8	558	70	**0.18 [0.09, 0.27]**	1.32
8	9	654	89	**0.24 [0.15, 0.33]**	1.43	9	738	55	**0.16 [0.08, 0.25]**	1.43
9	9	764	0	**0.23 [0.15, 0.30]**	1.29	9	694	28	0.07 [-0.03, 0.17]	1.64
10	9	788	53	**0.36 [0.26, 0.46]**	1.74	9	696	55	0.09 [-0.02, 0.19]	1.72
11	10	976	78	**0.30 [0.21, 0.38]**	1.65	10	924	70	**0.19 [0.09, 0.29]**	1.89
12	10	916	97	**0.76 [0.67, 0.85]**	1.69	10	1048	36	**0.13 [0.03, 0.22]**	1.91
13	10	874	97	**0.58 [0.50, 0.65]**	1.38	10	874	99	**0.36 [0.27, 0.45]**	1.65
14	9	574	85	**0.20 [0.08, 0.33]**	1.86	9	764	91	-0.06 [-0.19, 0.06]	2.15
15	8	438	91	0.00 [-0.10, 0.11]	1.36	8	494	79	**-0.21 [-0.33, -0.09]**	1.66
16	2	98	NA	**-1.63 [-2.01, -1.25]**	2.34	3	196	0	**-0.94 [-1.13, -0.74]**	1.70
17	1	76	NA	**-2.15 [-2.46, -1.84]**	1.68	1	148	NA	**-1.64 [-1.77, -1.51]**	0.98
18	1	36	NA	**-2.72 [-3.10, -2.34]**	1.42	1	176	NA	**-2.66 [-2.78, -2.54]**	0.99

Significant values in bold.

2.4.4 Pooled meta-analysis of studies comparing the Willems and Demirjian methods in males

At age 4 years there was no significant difference ($p>0.05$) in the effect size between the Willems and the Demirjian methods in age estimation. From age cohorts 5-14 years there were significant differences in the effect estimate between the two methods ($p<0.001$), with the magnitude of deviation of the dental age from the chronological age significantly greater with the Demirjian method compared to the Willems method (Table 2.6). It should be noted that the two methods overestimated the chronological ages for these age groups. The Willems method estimated age group 13 accurately, judging from the WMD of 0.00 found in this review. From ages 14-18 years, no significant difference ($p>0.05$) exists between the effect sizes of Demirjian's method and the Willems method (Table 2.6). Overall, the Demirjian method significantly overestimated chronological age compared to the Willems method in males ($p=0.000$).

2.4.5 Pooled meta-analysis of studies comparing the Willems and Demirjian methods in females

There was no significant difference ($p>0.05$) in the effect estimate of the Demirjian and the Willems methods at age 4 years. However, significant differences were noted in the effect sizes of the two methods from ages 5-14 years ($p<0.001$), while no significant differences were noted for ages 15-18 years ($p>0.05$). Demirjian's method overestimated chronological age from 4-14 years and thereafter underestimated ages for 15-18 years. The Willems method overestimated dental age from 4-13 years and thereafter underestimated the chronological age from 15-18 years (Table 2.7). Overall, the Demirjian method significantly overestimated the chronological age of the females compared to the Willems method ($p=0.000$).

Table 2.6: Comparison of the effect estimate (pooled for age cohorts) of the Demirjian and Willems methods in males

Demirjian method				**Willems method**				**t**	**p**
Age cohort	N	**Effect estimate**	**SD**	**Age cohort**	**n**	**Effect estimate**	**SD**		
3	26	0.57	1.32						
4	106	0.61	1.25	4	18	-0.05	0.91	2.14	**0.03**
5	270	1.39	1.28	5	140	0.31	1.40	7.92	**0.00**
6	614	1.11	1.00	6	348	0.54	1.33	7.51	**0.00**
7	968	0.76	1.06	7	510	0.55	1.12	3.55	**0.00**
8	1360	0.53	1.60	8	654	0.24	1.43	3.80	**0.00**
9	1366	0.49	1.95	9	764	0.23	1.29	3.30	**0.00**
10	1348	0.75	2.17	10	788	0.36	1.74	4.30	**0.00**
11	1556	0.84	1.84	11	976	0.30	1.65	7.48	**0.00**
12	1354	0.88	1.94	12	916	0.76	1.69	1.70	0.09
13	1146	1.08	1.79	13	874	0.58	1.38	6.85	**0.00**
14	784	1.06	1.30	14	574	0.20	1.86	9.65	**0.00**
15	544	0.11	1.01	15	438	0.00	1.36	1.45	0.15
16	112	-1.48	2.04	16	98	-1.63	2.34	0.50	0.62
17	76	-1.95	1.19	17	76	-2.15	1.68	0.85	0.40
18	36	-2.67	0.72	18	36	-2.72	1.42	0.19	0.85
OVERALL	**6581**	**0.62**	**1.47**	**OVERALL**	**5176**	**0.26**	**1.51**	**13.02**	**0.00**

Significant values in bold.

Table 2.7: Comparison of the effect estimate (pooled for age cohorts) of the Demirjian and Willems methods in females

Demirjian method				**Willems method**				**t**	**p**
Age cohort	**N**	**Effect estimate**	**SD**	**Age cohort**	**n**	**Effect estimate**	**SD**		
3	14	-0.19	0.74						
4	100	0.28	1.24	4	20	0.02	0.80	0.90	0.37
5	244	1.16	1.36	5	134	0.45	1.22	5.03	**0.00**
6	608	0.88	1.07	6	326	0.17	1.07	9.67	**0.00**
7	1084	0.52	1.12	7	558	0.18	1.32	5.48	**0.00**
8	1400	0.49	1.51	8	738	0.16	1.43	4.89	**0.00**
9	1412	0.57	2.10	9	694	0.07	1.64	5.50	**0.00**
10	1367	0.64	1.95	10	696	0.09	1.72	6.30	**0.00**
11	1564	0.90	1.84	11	924	0.19	1.89	9.21	**0.00**
12	1679	0.87	1.40	12	1048	0.13	1.91	11.64	**0.00**
13	1420	1.14	1.52	13	874	0.36	1.65	11.55	**0.00**
14	1108	0.60	1.03	14	764	-0.06	2.15	8.86	**0.00**
15	658	-0.20	1.12	15	494	-0.21	1.66	0.12	0.90
16	224	-0.81	1.39	16	196	-0.94	1.70	0.86	0.39
17	148	-1.52	1.13	17	148	-1.64	0.98	0.98	0.33
18	176	-2.52	1.07	18	176	-2.66	0.99	1.27	0.20
OVERALL	**7528**	**0.72**	**1.35**	**OVERALL**	**5656**	**0.29**	**1.48**	**17.36**	**0.00**

Significant values

2.4.6 Evaluation of heterogeneity and publication bias

No significant difference was noted in the sensitivity test done to determine the influence of individual studies on the overall effect size by omitting each study in turn. Funnel plots were generated to determine the publication bias of the included studies. Visual analysis of the funnel plots does not indicate any evidence of asymmetry as points are distributed across the baseline (Figures 2.6 and 2.7).

Figure 2.7a Funnel plot for males, Demirjian method

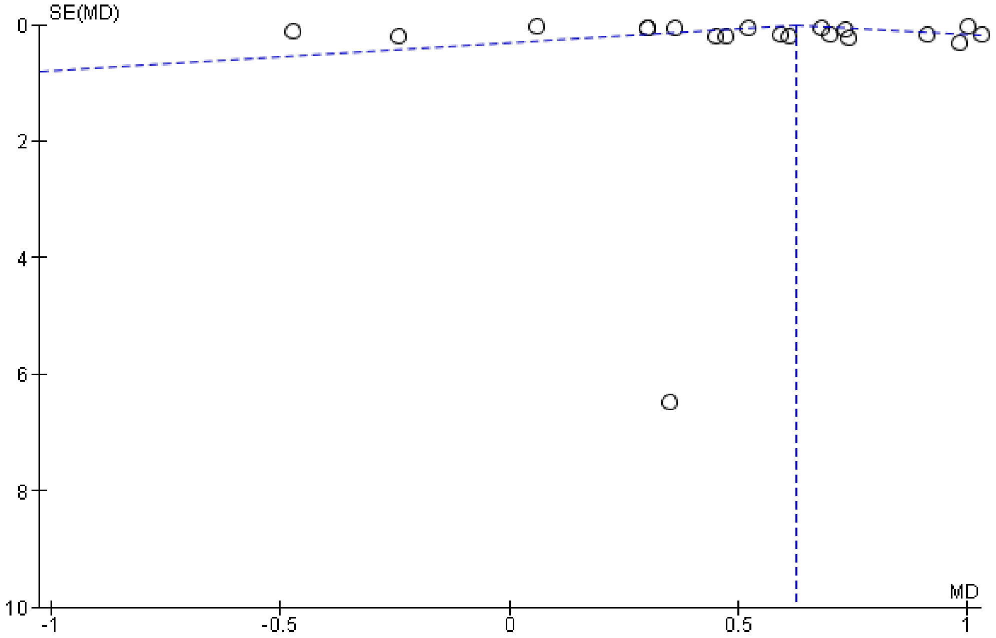


Figure 2.7b Funnel plot for females, Demirjian method

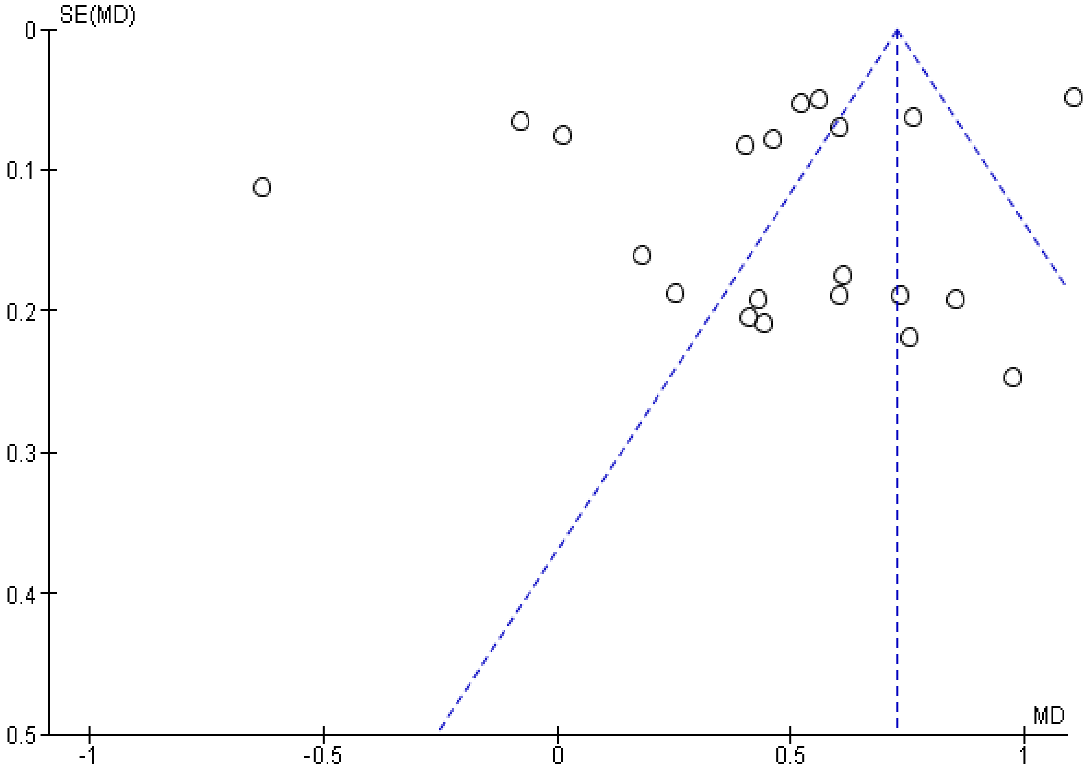


Figure 2.8a: Funnel plot for males, Willems method

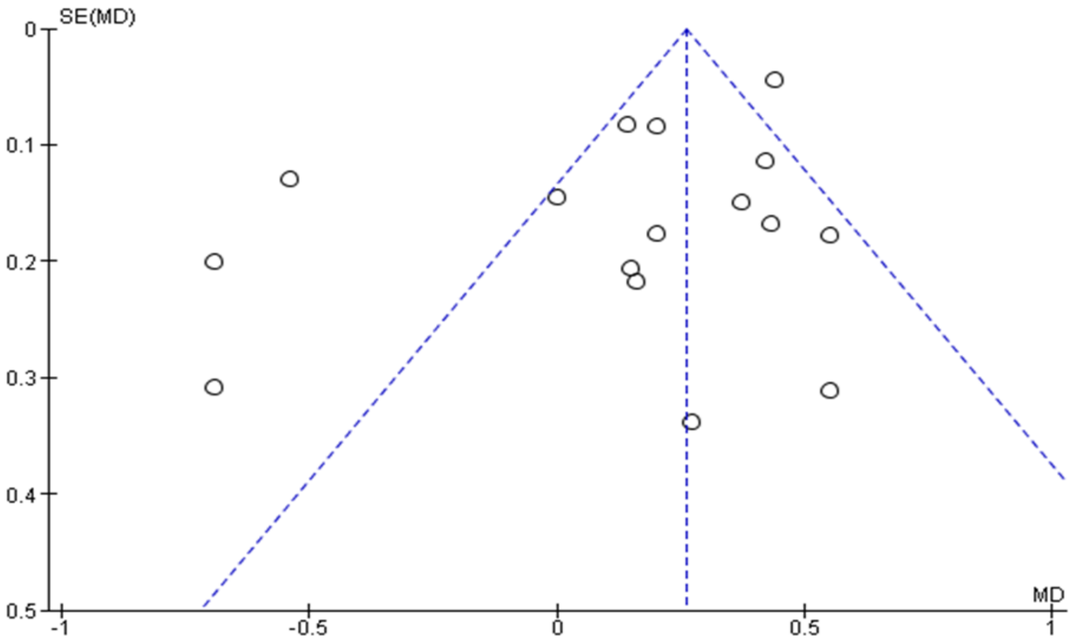
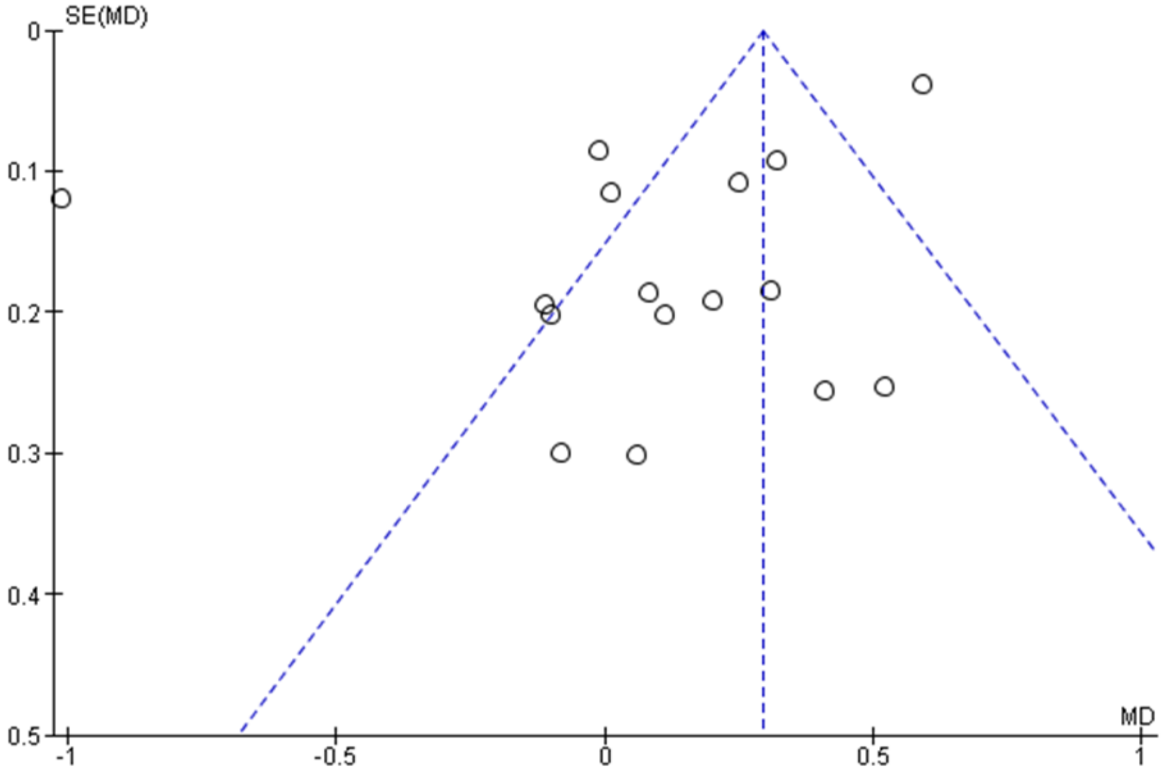


Figure 2.8b: Funnel plot for females, Willems method



2.5 Discussion

Standards for growth and development are desirable for forensic, anthropological and clinical purposes (Scheuer and Black 2000). Most methods for assessing growth and development, especially those based on the skeleton, are not highly reliable for estimating age due to the influence of genetic and environmental factors. Dental development is viewed as a more reliable gauge for assessing the age of children and juveniles in forensic and anthropological contexts (Demirjian et al. 1973; Liversidge 2012), although population variability in dental development has been reported (Pahkala et al. 1991; Willem et al. 2001; Liversidge 2003; Liversidge et al. 2006; Tunc and Koyuturk 2008). The accuracies of the methods derived from dental maturity, such as the Demirjian and Willems methods, for estimating chronological age across populations is still a subject of debate. Hence this systematic review focused on studies investigating the Demirjian and Willems methods in different populations with the aim of determining the method with a better accuracy.

A limitation of this review is the considerable heterogeneity observed in our results when the results were pooled and also stratified by age and sex. The reason could be due to differences in population characteristic in terms of differences in growth patterns. Furthermore, Demirjian and colleagues stated that their method is based entirely on a French-Canadian population and that variation may occur when it is used in other populations. They therefore cautioned that although the stages of the dental maturity scoring system may be universal in application, population differences may affect the accuracy levels when maturity scores are converted to dental ages (Demirjian et al. 1973). This observation highlights the need for population-specific standards for age estimation, especially for forensic and anthropological applications where there are demands for high levels of accuracy.

2.5.1 Comparison between chronological age and dental age using Demirjian’s method

This review found the Demirjian method significantly overestimates the ages of males and females aged up to 16 years by 0.62 and 0.74 years respectively. The level of overestimation from the Demirjian method makes it unsuitable for forensic purposes in other populations. Other systematic reviews found similar results of age overestimation with Demirjian’s method (Yan et al, 2013; Jayaraman et al, 2013). The overestimation was greater in females than in males. The reason for this difference is not clear, but it may be due to varying levels of sexual dimorphism or sex based differences in environmental stresses.

The underestimation of the chronological age by the Demirjian method in age cohorts 16-18 years in both males and females is due to the non-availability of values for ages 16 years and above in the Demirjian conversion tables of maturity scores to dental age. By that age, all individuals have attained full maturity of the seven tooth (I1-M2) dental sequence. Hence, all ages above 16 years are underestimated.

2.5.2 Comparison between chronological age and dental age using Willems method

This review found no significant mean difference between dental age estimated by the Willems method and chronological age. Overall the Willems method overestimated the chronological age of males by 0.26 years, while it overestimated females by only 0.29 years. This pattern is similar to the result for Demirjian’s method where the ages of females were overestimated more than the males. Similar to the Demirjian method, the Willems method cannot be used to estimate chronological age above 16 years because the upper limit of the total maturity score, which is the dental age of 15.77 years. Therefore, anyone above 16 years of age is underestimated.

2.5.3 Comparison between the Willems and Demirjian methods

This is the first systematic review and meta-analysis comparing the Willems and Demirjian methods. Significant differences between dental ages estimated by the two methods were found. The wide gap between the estimates of the two methods is due to Demirjian's method significantly overestimating the dental age in all age groups (except for older children aged 15-18 years, primarily due to the contraints of the method, as described above).

Based on our results, the Willems method may be used for age estimation for anthropological or forensic purposes in populations where specific reference values are unknown and the levels of accuracy reported here are deemed acceptable. Nevertheless, it is important to emphasize that both methods significantly overestimated chronological age. Hence, our results illustrate that there is a need for population-specific standards for age estimation when the highest levels of accuracy is required.

2.5.4 Variation in dental development in human populations: Implications for age estimation

The debate is still ongoing whether tooth development is influenced by factors such as nutrition, climate and chronic or infectious diseases. Studies of fluctuating dental asymmetry, thought to be caused by response to stresses, are inconclusive (Perzigian 1977: Smith et al. 1982). Although tooth size and basic morphology are generally perceived to be relatively immune to major disruptions compared to other growth indicators, the widespread presence of enamel hypoplasias in human populations attests to some level of disruption affecting dental morphology one counter example among many. The investigation of differences in the timing of dental maturation is challenging. The relationship between malnutrition and tooth formation is difficult to evaluate, with some researchers reporting no effect of malnutrition on tooth formation (Eid et al. 2002 Cameriere et al. 2007, Elamin and Liversidge 2014), while others observed a delay in formation

(Hilgers et al. 2006, Mani et al 2008). Such studies are based on selected proxies of nutritional status such as height, weight and body mass index (BMI). Well-designed studies on severely malnourished children are lacking and constrained by ethical considerations. Recent research on Southern African Black children documents significant differences in the timing of tooth formation in children of different BMI statuses (Esan and Schepartz, n.d.).

Fewer researchers have considered whether the timing of tooth formation varies significantly among human populations. The consistent pattern of variability in overestimation of ages documented by the published studies considered here suggests that variation in the timing of tooth formation may be influenced by genetic as well as environmental factors. Tables of tooth formation and age of attainment of specific developmental stages from one region of the world may not apply in a different setting, as is clearly demonstrated by our analysis. The documentation of significant variation in dental maturation among human populations, which is growing with expanded research that includes a broader range of populations, needs to be recognized and accounted for in the same way that skeletal and other aspects of growth variation are considered. When the highest levels of accuracy in age estimation are required, population-specific standards need be developed, rather than working toward a global standard.

In conclusion, the Willems method of dental age estimation provides a better and more accurate estimation of chronological age in different populations than the Demirjian method. The Demirjian scoring system has broad application in terms of determining maturity scores, but the accuracies of Demirjian age estimates are confounded by population variation when converting maturity scores to dental ages. Both of the methods reviewed here, when applied to other populations, do not yield a level of accuracy comparable to estimates from population-specific reference data, which should be employed when the highest accuracy is needed.

Conflicts of interest

There are no conflicts of interest for any of the authors.

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Appendix I

Modified STROBE quality score systems.	
Criteria items	Score
	(0 to 40)
Title and Abstract	☐ 0 ☐ 1
Title and Abstract: Indicate the study design (case-control or cohort study) in the title or the abstract	☐ 0 ☐ 1
Abstract: Provide an informative and balanced summary of the study	☐ 0 ☐ 1
Introduction: Explain the scientific background and rationale for the investigation	☐ 0 ☐ 1
Introduction: State specific objectives, including any prespecified hypotheses	☐ 0 ☐ 1
Methods	☐ 0 ☐ 1
Study Design: Present key elements of study design	☐ 0 ☐ 1
Setting: Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	☐ 0 ☐ 1
Participants: Give the eligibility criteria of case	☐ 0 ☐ 1
Participants: Give the sources and methods of case ascertainment and control selection	☐ 0 ☐ 1
Participants: Give matching criteria and the number of controls	☐ 0 ☐ 1
Variables: Clearly define all outcomes, exposures, predictors, potential confounders, effect modifiers	☐ 0 ☐ 1
Data sources/Measurement: Give sources of data and details of methods of assessment	☐ 0 ☐ 1
Data sources/Measurement: Describe comparability of assessment methods	☐ 0 ☐ 1
Bias: Describe any efforts to address potential sources of bias	☐ 0 ☐ 1
Study size: Explain and describe the estimation of the study size	☐ 0 ☐ 1
Quantitative variables: Explain how quantitative variables were handled in the analyses	☐ 0 ☐ 1
Quantitative variables: Give group included criteria in the analyses	☐ 0 ☐ 1
Statistical methods: Describe all statistical methods, including those used to control for confounding	☐ 0 ☐ 1
Statistical methods: Describe any methods used to examine subgroups and interactions	☐ 0 ☐ 1
Statistical methods: Explain how missing data were addressed	☐ 0 ☐ 1
Statistical methods: Explain how matching of cases and controls was addressed	☐ 0 ☐ 1
Statistical methods: Describe any sensitivity analyses	☐ 0 ☐ 1
Hardy-Weinberg equilibrium: HWE was assessed	☐ 0 ☐ 1
Hardy-Weinberg equilibrium: HWE of control group was assessed	☐ 0 ☐ 1
Results	
Participants: Report the numbers of individuals at each stage of the study, such as numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up and analyzed	☐ 0 ☐ 1
Participants: Give reasons for non-participation at each stage	☐ 0 ☐ 1
Participants: Give a flow diagram	☐ 0 ☐ 1

Descriptive data: Give characteristics of study participants (e.g. demographic, clinical diagnosis, ethnicity, sex ratio, etc.)	☐ 0 ☐ 1
Descriptive data: Indicate the number of participants with missing data	☐ 0 ☐ 1
Outcome data: Report numbers in each exposure category, or summary measures of exposure	☐ 0 ☐ 1
Main results: Give unadjusted estimates and confounder-adjusted estimates and their 95% confidence intervals	☐ 0 ☐ 1
Main results: Make clear which confounders were adjusted for and why they were included	☐ 0 ☐ 1
Main results: If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	☐ 0 ☐ 1
Other analyses: Report other analyses such subgroups, interactions, and sensitivity analyses	☐ 0 ☐ 1
Discussion	☐ 0 ☐ 1
Key results: Summarize key results with reference to study objectives	☐ 0 ☐ 1
Main results: Report category boundaries when continuous variables were categorized	☐ 0 ☐ 1
Limitations: Discuss both direction and magnitude of any potential bias	☐ 0 ☐ 1
Interpretation: Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	☐ 0 ☐ 1
Generalizability: Discuss the generalizability (external validity) of the study results	☐ 0 ☐ 1
Other	☐ 0 ☐ 1
Funding: Give the source of funding and the role of the funders for the present study	☐ 0 ☐ 1

Contents

Chapter 3

Tooth formation: Assessment of maturity scores and dental age estimation of Black Southern African children using Demirjian's method

Abstract

Background: Tooth formation is an important aspect of growth and development due to its relative immunity from environmental influences. Other aspects of growth have been extensively documented for Black Southern African children, yet their timing oftooth formation has not been comprehensively investigated.

Aim: The present study was designed to provide information on the process of tooth formation in a similar sample of Black Southern African children.

Method: This was a quantitative cross-sectional study of 642 Southern African Black children comprising of 270 males and 372 females. Panoramic radiographs of the children were obtained and the stages of tooth formation of the left seven mandibular teeth were analysed according to the Demirjian et al. (1973) method. Dental ages obtained by the method were compared to the chronological ages. Probit regression analysis was employed to calculate the mean age of attainment of stages of tooth formation. Maturity scores and age of attainment were compared by sex and with published data on other populations.

Results: Females show significantly advanced dental maturity and dental ages, as well as earlier attainment of all the stages of formation ($p<0.05$). The Demirjian method generally overestimated dental age in both males and females. For males, there was an overestimation of the mean age by 0.8 years, while it is about 1.0 years in females. Cross-population comparisons illustrate that the Southern African children are generally advanced in dental maturity compared to children from Europe and Asia.

Conclusion: The Demirjian method overestimated the chronological ages of Black Southern African males and females. Similarly, the age of attainment of specific developmental stages shows that the Southern Africans attain maturity earlier than South Korean, Canadian and Belgian children. Females were more advanced in dental maturity than males. These differences in dental maturity clearly illustrate the need for population-specific, rather than global, dental maturity standard.

3.1 Introduction

The timing of tooth formation for any given population is important, as this aspect of dental development serves as an index for evaluating dental maturation and estimating the age of children and young adults (Haavikko 1974; Demirjian and Goldstein 1976; Lewis and Rutty 2003; Teivens and Mörnstad 2001; Cattaneo et al. 2009). The data are relevant for archaeological and forensic applications (Scheuer and Black 2007), particularly when the assessment of growth changes are part of the biological profile (Scheuer and Black 2006). The timing of tooth formation is also used for comparison of growth between populations or species when evolutionary trends are under investigation (Liversidge 2003).

Major advances in developing growth and anthropometric reference values for South African children resulted from the “Birth to Twenty” (BTT) projects that were initiated around the time that the apartheid system was ending (Richter et al. 1995; Cameron 2003; Vidulich et al. 2006; Richter et al. 2007). A massive longitudinal study, the BTT research focuses on a wide array of biological parameters, but dental development was not investigated. The present study was designed to provide information on the process of tooth formation in a similar sample of Black Southern African children.

Age estimation using dental maturity is the most reliable aging method because teeth are relatively indestructible and exhibit the least amount of turnover (remodelling) of their natural structure (Carvalho et al. 2009; Masthan 2009). Another advantage is that the timing of tooth formation displays less variability than other major developmental indicators, including tooth emergence (Demirjian et al. 1985; Demirjian 1986). Furthermore, stronger associations are found between chronological age and dental age than between skeletal age and dental age (Lewis and Garn 1960; Demirjian et al. 1985; Demirjian 1986).

3.1.1 Method of estimating dental age

Dental age is assessed by matching the dental maturity status of an individual of unknown chronological age with population references. Most of the methods developed for dental age estimation are based on a comparison of the development of teeth with standard charts based on large samples drawn from a well-defined geographic region.

The most widely used of these age estimation procedures is commonly known as the Demirjian method (Demirjian et al. 1973; Demirjian and Goldstein 1976). The work was based on a large, random sample of French Canadian children. The changes from initial calcium deposition to complete apex formation are divided into eight observable stages (A through H). The seven left mandibular teeth, I1-M2, are evaluated and the individual "score" from each tooth is totaled. The resulting sum is then referenced on a corresponding conversion chart with ages ranging from 3 to 17 years in increments of one-tenth year. The underlying basis of the conversion chart incorporates information on skeletal aging derived from the work of Tanner et al. (1962).

Although the Demirjian method worked well for the original French-Canadian sample, subsequent studies have questioned its effectiveness when applied to other populations (Davis and Hagg 1993; Willems et al. 2001; Chen et al. 2010; Cruz-Landeira et al. 2010; Ogodescu et al. 2011; Baghdadi and Pani 2012; Erdem et al. 2013). A study of Belgian children (Willems et al. 2001) confirmed that the Demirjian method has a tendency to overestimate age. Similarly, Tunc and Koyuturk (2008) found that the method was less accurate with Turkish children, who displayed advanced dental maturity compared to the French-Canadian population. However, after an extensive review, Liversidge et al. (2012) concluded that the Demirjian technique remains a valuable forensic tool for estimating age in developing children.

3.1.2 Variation in tooth maturation

Dental development is widely regarded as relatively immune from environmental factors (Elamin and Liversidge 2013), but there is evidence that tooth formation varies by sex and population (Willems et al. 2001; McKenna et al. 2002; Tunc and Koyuturk 2008; Qudeimat and Behbehani 2009). Sexual dimorphism of dental development is present in all populations. Universally, it appears that females in any given population are more advanced in tooth formation than their male counterparts (Fanning 1961; Demirjian et al. 1973; Demirjian and Levesque 1980; Hägg and Matsson 1985; Liversidge et al. 1999; Nykänen et al. 1998; Uys et al. 2014). This pattern is similar to other growth indicators, where females are always ahead of males in growth and development until they reach adulthood. This has been attributed to the earlier fetal development seen in females (Almonaitiene et al. 2010) as well as the greater vulnerability of males to environmental stresses (Stinson 1985).

The patterns of advanced or delayed dental maturity obtained from using the Demirjian method on other populations can be interpreted as documentation of cross-populational variation (Haavikko 1974; Hägg and Matsson 1985; Davis and Hägg 1993; Mörnstad et al. 1994; Liversidge et al. 1999; Chaillet et al. 2005; Baghdadi and Pani 2012). As a result, several authors questioned the validity of a “one fits all” method for estimating dental maturity and argued for population-specific databases that can more accurately describe variation in tooth formation (Baghdadi and Pani 2012; Chaillet and Willems 2004; Chaillet et al. 2005; Lee et al. 2011).

The reason for the observed variation in dental maturation among human populations is not fully understood, although several explanations involving genetic or environmental factors have been adduced (Chaillet et al. 2005). Eveleth (1966) found that groups living in tropical regions are dentally advanced, suggesting that the climate had an accelerating effect on maturation. Similarly, Cantekin et al. (2014), comparing dental maturity of Turkish children living in cold high altitudes with children from the lower altitude and warmer regions, found that the latter group was more dentally advanced.

There is no general agreement that the degree of variation in tooth formation among populations is significant, or that it is of a magnitude that necessitates individual population references. This is also the case for sexual dimorphism in developmental timing. Liversidge et al. (2006) did an extensive systematic review of European, South Korean and Australian studies and concluded that there were no significant differences in the age of attainment of tooth developmental stages. Therefore, they formulated a combined table of mean age of attainment for males and females from several populations (Liversidge et al. 2006). No data from Africa were included. In general, comparative data on maturity scores for African children are lacking; where they do exist, they are only expressed as dental age (Uys et al. 2014) or they are not sex specific (Phillips and van Wyk Kotze 2009).

In sum, there are few maturity references for populations in Africa. Therefore, the aim of this study is to provide the baseline data on the timing of maturation stages of tooth formation and dental age estimation using the Demirjian tables and to compare this information with published data on other populations.

3.2 Materials and Methods

3.2.1 Study design

This was a quantitative cross-sectional study of Southern African Black children. The sample population was drawn from children whose parents and grandparents are indigenous Southern Africans.

3.2.2 Study population

The sample population was randomly selected from primary schools and secondary schools in the Johannesburg Municipality, South Africa. Children who were screened for dental diseases by the Community Oral Health Outreach Program (COHOP) of the Department of Community Dentistry of the School of Oral Health Sciences, University of the Witwatersrand and who met the inclusion criteria were selected. Ethical approval (NO. M141001) was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. Permission to carry out the study on the school children was obtained from the local education authority and respective school heads. Consent was obtained from the parents while assent was obtained from the children.

3.2.3 Inclusion and exclusion criteria

The selected age range was 5-20 years. Studies from other populations showed that at age 16 years, all the children would have reached the highest maturity score of 100. However, age children aged 17-20 years were included to determine if there are variation in dental maturity of Black Southern African children beyond age 16 years. Children with systemic diseases, mandibular hypodontia (except third molars), and those who had lost their teeth on both sides of the mandible were excluded.

3.2.4 Data collection

Panoramic radiographs of children screened for treatment during visits of the Community Oral Health Outreach Program were collected and analyzed.

3.2.5 Sample

A total of 642 children comprising of 270 males and 372 females were sampled (Table 1). The sample size formula used to determine the minimum sample for statistical significance testing is $N = 4z_{\alpha}^{2}S^{2} \div W^{2}$, where S = standard deviation, W = desired total width and Z_{α} is the standard normal deviate for the 95% confidence level. In a similar study, Cameriere et al. (2008) found a mean of 1.076 and a standard deviation of 0.824 derived from a standard error of 0.030. Using a width of 0.2, the minimum sample size required for the 14 cohorts (children aged 18-20 were placed in one cohort) was 280. However, to improve the power of the study, 642 children were recruited.

3.2.6 Pilot test

Prior to data collection, a reliability study to assess the magnitude of the intra-observer error of interpretation and detection was conducted. Firstly, two trained (calibrated) examiners assessed the maturation stage of the 7-left mandibular permanent teeth without the knowledge of chronological age or sex. To evaluate reproducibility, twenty-five radiographs (with 175 tooth ratings) were randomly selected and assessed by both examiners at day one and day three. The investigator was the only rater for the developmental stages of the teeth. Intra-examiner reliability of dental age assessment for the Demirjian method was calculated using Cohen's Kappa (Landis and Koch 1977) and found to be acceptable at 0.97.

3.2.7 Dental maturity score and age assessment

Dental age assessment was performed according to the original version of Demirjian's method (Demirjian et al. 1973). The investigator did not have access to the chronological age of the participants. The digital radiographs of each child were enhanced using Microsoft Office Picture Manager, properly labeled with a unique identity number, and digitally archived. Each radiograph was assessed for the development of the seven-left permanent mandibular teeth and was rated on the 8-stage scale from A to H, with stage 0 for non-appearance. Each stage of the teeth was allocated a sex-specific biologically weighted score and the sum of the scores for each participant was used to determine the dental maturity measured on a scale of 0 to 100. The dental maturity score of each child was converted to dental age using the standard tables and percentile curves for males and females (Demirjian et al. 1973; Demirjian and Goldstein 1976).

3.2.8 Data analysis

The data were analyzed using IBM SPSS (version 22) software for Windows and STATA 12. Analyses were done for the entire group as well as for each sex and age cohort. The maturity scores were computed and sex differences were calculated using independent sample t-tests. Dental age (DA), as calculated from the reference tables (Demirjian et al. 1973), was compared to chronological age (CA) for males and females separately. The difference between the DA and CA was tested using paired t-tests at a significance level of $p<0.05$. The absolute mean difference between the DA and CA was calculated to express accuracy independent of bias. To calculate the mean age of attainment of the developmental stages, the data for the developmental stages were recoded as present or absent. Probit regression analysis was used to analyze and calculate the probable age of attainment of the specific developmental stage for each tooth. Statistically significant values were inferred at $p\leq0.05$.

3.3 Results

The age group and sex distributions of the study participants are presented in Table 3.1. There are significantly more females in the sample ($p<0.05$). The mean age is 10.69±3.08 for males and 11.15±2.89 for females (Table 3.2). There is no significant difference in the mean age of the males and females ($p=0.053$).

3.3.1 Dental age estimation using Demirjian's method

Females show advanced dental maturity and dental ages compared to males (Table 3.2). There is general overestimation by the Demirjian method of the dental age in both sexes. For males, there is an overestimation of the mean age by 0.8 years, while it is about 1.0 years in females (Table 3.2).

Table 3.1. Distribution of participants by chronological age cohorts and sex

Age cohort (Years)	**Number of participants**		**Total (%)**
	Males (%)	**Females (%)**	
5 – 5.99	10 (3.7)	13 (3.5)	23 (3.6)
6 – 6.99	27 (10.0)	28 (7.5)	55 (8.6)
7 – 7.99	6 (2.2)	9 (2.4)	15 (2.3)
8 – 8.99	33 (12.2)	27 (7.3)	60 (9.3)
9 – 9.99	36 (13.3)	30 (8.1)	67 (10.4)
10 – 10.99	16 (5.9)	22 (5.9)	38 (5.9)
11 – 11.99	17 (6.3)	36 (9.7)	53 (8.3)
12 – 12.99	21 (7.8)	44 (11.8)	64 (10.0)
13 – 13.99	15 (5.6)	39 (10.5)	54 (8.4)
14 – 14.99	28 (10.4)	32 (8.6)	60 (9.3)
15 – 15.99	25 (9.3)	27 (7.3)	52 (8.1)
16 – 16.99	11 (4.1)	28 (7.5)	39 (6.1)
17 – 17.99	20 (7.4)	25 (6.7)	45 (7.0)
18 – 20.00	5 (1.9)	12 (3.2)	17 (2.6)
Total	**270**	**372**	**642 (100.0)**

Table 3.2. Mean values for dental age measures and chronological age[1]

Variable	Males (N=233)		Females (N=307)		t	p
	Mean	**SD**	**Mean**	**SD**		
Maturity score	85.98	16.50	90.81	14.63	-3.54	**0.00**
Dental age	11.50	2.99	12.18	2.90	-2.64	**0.01**
Chronological age	10.69	3.08	11.15	2.89	-1.77	0.08
Mean overestimation	0.82	1.02	1.03	0.98	-2.41	**0.02**

[1] Age cohorts above 16 years were removed from this analysis because Demirjian's table ends at 16 years.

Significant differences are in bold.

Among the males, the estimated dental age is significantly higher than the chronological age ($p<0.05$) in all the age groups except for age 12 (Tables 3.3). For females, the estimated dental age is significantly higher than the chronological age in all the age groups ($p<0.05$) (Tables 3.3 and 3.4). Overestimation of age is particularly pronounced (over one year) in the younger age groups (5 to 8.99 years), but the gap between the chronological age and the estimate begins to decrease at age 9 in males and age 10 in females. The smallest gap between chronological age and dental age is seen in the age cohort 16 years in both males and females (Tables 3.3 and 3.4).

Table 3.3. Comparison of mean dental and chronological ages in males

Age cohort (years)	Chronological age (CA)		Dental age (DA)		Mean diff (CA-DA)		t	p
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
5 – 5.99	5.71	0.15	7.03	0.23	-1.32	0.31	13.58	**0.000**
6 – 6.99	6.57	1.98	7.64	1.74	-1.07	0.52	10.76	**0.000**
7 – 7.99	7.63	0.34	8.85	1.04	-1.23	0.92	3.26	**0.022**
8 – 8.99	8.54	0.28	9.75	1.11	-1.21	1.05	6.64	**0.000**
9 – 9.99	9.38	0.31	10.31	1.44	-0.93	1.34	4.21	**0.000**
10 – 10.99	10.29	0.29	10.82	0.72	-0.53	0.76	2.76	**0.014**
11 – 11.99	11.47	0.29	12.04	0.91	-0.57	0.88	2.67	**0.017**
12 – 12.99	12.27	0.24	12.42	0.91	-0.13	0.86	0.66	0.519
13 – 13.99	13.31	0.25	14.73	1.20	-1.42	1.14	4.81	**0.000**
14 – 14.99	14.45	0.32	15.01	1.14	-0.56	1.12	2.67	**0.013**
15 – 15.99	15.47	0.26	15.86	0.48	-0.39	0.56	3.47	**0.000**
16 – 16.99	16.52	0.29	16.00	0.00	0.52	0.29	-	-
17 – 17.99	17.43	0.24	16.00	0.00	1.43	0.24	-	-
18 – 20.00	19.65	0.77	16.00	0.00	3.65	0.77	-	-

Cannot compute t & p values for the last three rows because SD = 0.00

Significant differences in bold.

Table 3.4. Comparison of mean dental and chronological ages by age cohort in females

Age cohort (years)	Chronological age (CA)		Dental age (DA)		Mean diff (DA-CA)		t	p
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
5 – 5.99	5.77	0.17	7.03	0.28	-1.26	0.32	13.95	**0.000**
6 – 6.99	6.31	0.24	7.37	0.61	-1.05	0.67	8.38	**0.000**
7 – 7.99	7.64	0.35	8.69	1.25	-1.05	1.11	2.85	**0.021**
8 – 8.99	8.54	0.28	9.85	0.99	-1.31	0.98	6.95	**0.000**
9 – 9.99	9.46	0.26	10.83	1.02	-1.37	0.95	7.88	**0.000**
10 – 10.99	10.42	0.33	11.35	1.16	-0.93	1.09	4.01	**0.001**
11 – 11.99	11.41	0.28	12.33	0.93	-0.92	0.94	5.80	**0.000**
12 – 12.99	12.46	0.26	13.49	1.08	-1.03	1.13	6.02	**0.000**
13 – 13.99	13.41	0.28	14.76	1.30	-1.35	1.30	6.49	**0.000**
14 – 14.99	14.40	0.20	14.99	0.87	-0.59	0.79	4.26	**0.000**
15 – 15.99	15.41	0.30	15.95	0.27	-0.54	0.35	7.97	**0.000**
16 – 16.99	16.48	0.33	16.00	0.00	0.48	0.33	-	-
17 – 17.99	17.42	0.28	16.00	0.00	1.42	0.28	-	-
18 – 20.00	18.77	0.50	16.00	0.00	2.77	0.50	-	-

Cannot compute t & p values for the last three rows because SD = 0.00
Significant differences in bold.

3.3.2 Sex variation in dental maturity

Females are significantly more advanced in dental maturity than their male counterparts in all age groups except age 6 years. This demonstrates that the females attain specific maturity stages in tooth formation earlier than their male agemates (Table 3.5). In addition, both males and females complete the maturation stages of all seven teeth by age 16 years (Table 3.5). Further analysis to ascertain the proportion of males and females who complete the dental maturation stages illustrates that only 18% of the males and 38% of the females attain a maturity score of 100 at age 14. However, at age 15 years, the majority of males (76%) and females (96%) attain maturity scores of 100 (Table 3.6).

Table 3.5. Mean maturity scores in males and females by age cohort

Age cohort (years)	Males			Females			t	p	95% CI
	Maturity score			Maturity score					
	N	Mean	SD	n	Mean	SD			
5 – 5.99	10	46.85	4.00	13	51.81	5.73	-2.32	**0.03**	-9.39, -0.53
6 – 6.99	27	54.85	12.88	28	59.78	11.07	-1.52	0.13	-11.52, 1.56
7 – 7.99	6	77.63	11.13	9	79.27	14.39	-0.23	0.82	-16.70, 13.44
8 – 8.99	33	85.98	5.88	27	90.03	4.69	-2.89	**0.01**	-6.84, -1.25
9 – 9.99	36	87.79	6.58	30	93.36	3.43	-4.46	**0.00**	-8.08, -3.07
10 – 10.99	16	91.16	2.16	22	94.52	2.83	-3.99	**0.00**	-5.08, -1.65
11 – 11.99	17	93.87	1.49	36	96.51	1.06	-7.38	**0.00**	-3.36, -1.92
12 – 12.99	21	94.52	1.28	44	97.78	1.05	-10.73	**0.00**	-3.87, -2.65
13 – 13.99	15	97.28	1.20	39	98.93	1.17	-4.62	**0.00**	-2.37, -0.94
14 – 14.99	28	97.83	1.56	32	99.19	0.72	-4.14	**0.00**	-2.01, -0.69
15 – 15.99	25	99.52	0.97	27	99.96	0.21	-2.19	**0.04**	-0.84, -0.03
16 – 16.99	11	100.00	0.00	28	100.00	0.00	-	-	-
17 – 17.99	20	100.00	0.00	25	100.00	0.00	-	-	-
18 – 20.00	5	100.00	0.00	12	100.00	0.00	-	-	-

Cannot compute t & p values for the last three rows because SD=0.00

Significant differences in bold.

Table 3.6. Proportion of participants to have attained maturity score of 100 at cohorts 14 and 15 years

Age cohort 14 years					
Males			**Females**		
Maturity score	**n**	**%**	**Maturity score**	**n**	**%**
93.10	1	3.6	-	-	-
95.40	1	3.6	-	-	-
96.20	2	7.1	-	-	-
97.00	8	28.6	97.40	1	3.1
98.20	8	28.6	98.10	3	9.4
98.80	1	3.6	98.60	1	3.1
99.10	1	3.6	98.90	14	43.8
99.10	1	3.6	99.10	1	3.1
100.00	5	17.9	100.00	12	37.5
Total	**28**	**100.0**	**Total**	**32**	**100.0**
Age cohort 15 years					
Males			**Females**		
Maturity score	**n**	**%**	**Maturity score**	**n**	**%**
96.20	1	4.0	-	-	-
98.20	4	16.0	-	-	-
99.10	1	4.0	98.90	1	3.7
100.00	19	76.0	100.00	26	96.3
Total	**25**	**100.0**	**Total**	**27**	**100.0**

3.3.3 Sex comparison of the age of attainment of tooth developmental stages

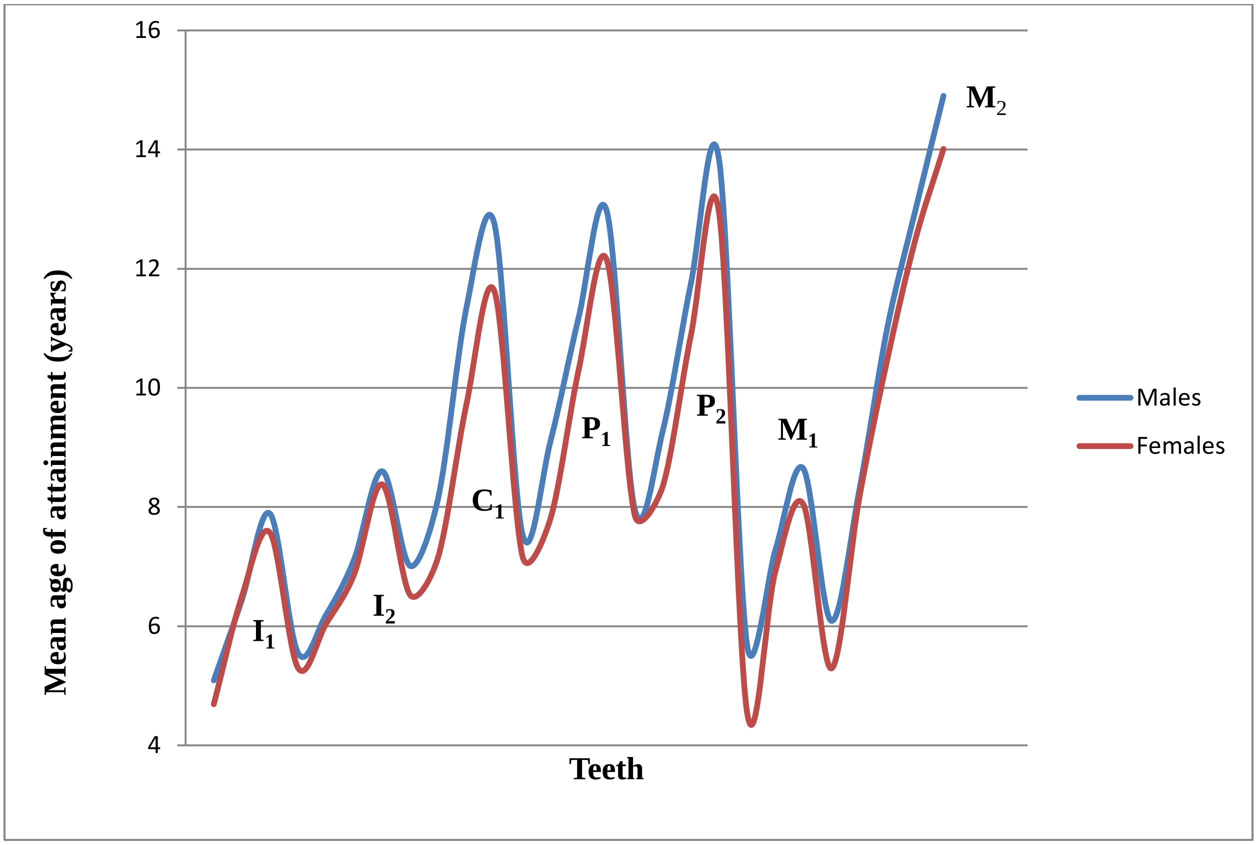
Table 3.7 provides the age of attainment of the maturation stages. Females show significantly earlier age of attainment in all of the maturation stages than their male counterparts ($p<0.05$) except for the G stage of the central incisor and the E stage of the lateral incisor. It is noteworthy that the biggest difference between males and females occurs at the G stage of canine development, followed by the F stage of the first premolar. Figure 3.1 show the mean age of attainment of the H stage of the seven mandibular teeth in males and females.

Table 3.7. Age of attainment of permanent tooth maturational stages in males and females

Tooth	Stage	Male			Female			Mean diff	t	p
		Mean age	SD	n	Mean age	SD	n			
I_1	E	3.55	0.71	4	-	-				
	F	5.09	0.17	24	4.69	0.28	22	0.4	5.91	**0.00**
	G	6.44	0.05	24	6.50	0.11	19	-0.06	-2.38	0.99
	H	7.89	0.19	217	7.57	0.11	325	0.32	24.78	**0.00**
I_2	E	5.55	0.71	20	5.30	0.08	17	0.25	1.44	0.08
	F	6.19	0.10	14	6.04	0.13	18	0.15	3.57	**0.00**
	G	7.12	0.10	34	6.87	0.08	28	0.25	10.70	**0.00**
	H	8.60	0.15	201	8.38	0.14	307	0.22	16.83	**0.00**
C_1	D	3.17	0.47	33	-	-	-	-	-	-
	E	7.01	0.10	24	6.52	0.11	11	0.49	13.05	**0.00**
	F	8.16	0.15	77	7.17	0.12	59	0.99	41.52	**0.00**
	G	11.34	0.12	27	9.72	0.09	59	1.62	69.55	**0.00**
	H	12.75	0.07	108	11.62	0.10	372	1.13	109.84	**0.00**
P_1	D	3.17	0.47	39	-	-	-	-	-	-
	E	7.54	0.09	42	7.17	0.10	41	0.37	17.73	**0.00**
	F	9.11	0.09	50	7.81	0.12	64	1.30	63.83	**0.00**
	G	11.18	0.11	34	10.30	0.10	62	0.88	38.35	**0.00**
	H	12.97	0.10	104	12.15	0.10	194	0.82	64.47	**0.00**
P_2	D	4.74	0.27	44	-	-	-	-	-	-
	E	7.96	0.12	37	7.86	0.14	12	0.1	2.41	**0.01**
	F	9.28	0.11	56	8.35	0.15	69	0.93	38.70	**0.00**
	G	11.75	0.12	41	10.88	0.10	75	0.87	41.69	**0.00**
	H	13.84	0.09	86	12.95	0.08	164	0.89	74.99	**0.00**
M_1	F	5.69	0.09	27	4.56	0.31	31	1.13	18.26	**0.00**
	G	7.26	0.12	32	6.90	0.10	20	0.36	11.19	**0.00**
	H	8.65	0.12	201	8.05	0.15	315	0.6	47.78	**0.00**
M_2	C	5.06	0.09	18	-	-		-	-	-
	D	6.09	0.16	42	5.29	0.19	51	0.8	21.68	**0.00**
	E	8.28	0.17	68	8.15	0.15	60	0.13	4.56	**0.00**
	F	11.01	0.13	38	10.48	0.06	69	0.53	28.82	**0.00**
	G	12.98	0.07	39	12.49	0.07	59	0.49	33.92	**0.00**
	H	14.90	0.05	64	14.01	0.08	124	0.89	81.14	**0.00**

Significant differences in bold.

Figure 3.1. Age of attainment of H stages in the seven left mandibular teeth for males and females



Age of attainment of H stage in males and females respectively: I_1, 7.89 and 7.57 years; I_2, 8.60 and 8.38 years; $C_{1,}$ 12.75 and 11.62 years; P_1, 12.92 and 12.15 years, P_2, 13.89 and 12.95 years; M_1, 8.68 and 8.05 years; M_2, 14.90 and 14.01 years.

3.3.4 Comparisons of population dental maturity scores

One advantage of the maturity score is that it allows easy comparison among populations using the same instrument. When the mean maturity scores obtained from this study are matched with the maturity scores in the Demirjian table, the Southern African males have significantly higher maturity scores ($p<0.05$) than the French-Canadian sample from which the reference values were derived except at ages 10, 11 and 12 (Table 3.8). The Southern African females have significantly higher ($p<0.05$) maturity scores in all the age cohorts compared to the French Canadians (Table 3.9).

Table 3.8. Comparison of the mean maturity score obtained in the present study with the mean score for the Demirjian reference population in the same age cohort in males

Age cohort	Group	Mean	n	SD	Mean diff	t	p
5 - 5.99	SA study	46.85	10	4.00	15.42	10.89	**0.00**
	Demirjian	31.43	10	1.14			
6 - 6.99	SA study	53.11	26	9.37	17.31	10.29	**0.00**
	Demirjian	35.80	26	2.17			
7 - 7.99	SA study	77.63	6	11.13	16.45	3.80	**0.01**
	Demirjian	61.18	6	8.68			
8 - 8.99	SA study	85.98	33	5.88	6.7	6.11	**0.00**
	Demirjian	79.24	33	3.20			
9 - 9.99	SA study	87.79	37	6.58	2.07	2.06	**0.04**
	Demirjian	85.72	37	2.49			
10 - 10.99	SA study	91.16	16	2.16	1,14	1.95	0.07
	Demirjian	90.01	16	1.00			
11- 11.99	SA study	93.87	17	1.50	7.18	2.02	0.06
	Demirjian	93.15	17	0.79			
12 - 12.99	SA study	94.52	20	1.28	0.03	0.09	0.93
	Demirjian	94.50	20	0.36			
13 - 13.99	SA study	97.28	15	1.20	1.35	4.64	**0.00**
	Demirjian	95.93	15	0.27			
14 - 14.99	SA study	97.84	28	1.59	0.72	2.72	**0.01**
	Demirjian	97.06	28	0.31			
15 - 15.99	SA study	99.52	25	0.97	1.54	7.72	**0.00**
	Demirjian	97.99	25	0.21			
16 - 16.99	SA study	100.00	12	-	-	-	-
	Demirjian	100.00	12	-			
17 - 17.99	SA study	100.00	20	-	-	-	-
	Demirjian	100.00	20	-			
18 - 20.00	SA study	100.00	5	-	-	-	-
	Demirjian	100.00	5	-			

Significant differences in bold.

Table 3.9. Comparison of the mean maturity score obtained in the present study with the mean score for the Demirjian reference population in the same age cohort in females

Age group	Study	Mean	n	SD	Mean diff	t	P
5 - 5.99	SA study	51.81	13	5.73	15.33	7.42	**0.000**
	Demirjian	36.48	13	3.47			
6 - 6.99	SA study	59.78	28	11.07	16.68	7.02	**0.000**
	Demirjian	43.11	28	7.90			
7 - 7.99	SA study	79.27	9	14.40	9.52	3.86	**0.005**
	Demirjian	69.74	9	10.19			
8 - 8.99	SA study	90.03	27	4.70	5.96	6.42	**0.000**
	Demirjian	84.07	27	2.22			
9 - 9.99	SA study	93.36	30	3.43	3.86	6.79	**0.000**
	Demirjian	89.51	30	1.20			
10 - 10.99	SA study	94.53	22	2.83	1.55	2.73	**0.013**
	Demirjian	92.97	22	0.96			
11- 11.99	SA study	96.52	36	1.06	1,42	4.10	**0.000**
	Demirjian	95.09	36	1.89			
12 - 12.99	SA study	97.78	44	1.05	0.97	5.63	**0.000**
	Demirjian	96.82	44	0.40			
13 - 13.99	SA study	98.93	39	1.17	1.21	6.33	**0.000**
	Demirjian	97.72	39	0.33			
14 - 14.99	SA study	99.19	32	0.72	0.50	4.34	**0.000**
	Demirjian	98.69	32	0.21			
15 - 15.99	SA study	99.96	27	0.21	0.44	8.67	**0.000**
	Demirjian	99.51	27	0.21			
16 - 16.99	SA study	100.00	28	-	-	-	-
	Demirjian	100.00	28	.-			
17 - 17.99	SA study	100.00	25	-	-	-	-
	Demirjian	100.00	25	-			
18 - 20.00	SA study	100.00	12	-	-	-	-
	Demirjian	100.00	12	-			

Significant differences in bold.

3.3.5 Population comparisons of age of attainment of developmental stages

A broader cross-population comparison is presented in Tables 3.10 and 3.11. Here it is clear that among the females, the Southern African children show advanced dental maturity compared to Belgian, South Korean and Canadian children while the Australian females have advanced dental maturity in the canines and both premolars compared to Southern African females. Additionally, the age of attainment of any stage in the central incisor and first molar is earlier for Southern Africans than for children from South Korea, Canada, Australia and Belgium (Table 3.11).

The Black Southern African males generally have an earlier age of attainment of the tooth developmental stages than their male counterparts from South Korea, Canada, Australia, and Belgium. However, they show delayed age of attainment in the second molar compared to males from Australia and Belgium.

Table 3.10. Mean age of attainment of dental maturity of Black Southern African males in comparison with males from other continents

Tooth	Stage	Belgium[1]		Canada[2]		South Korea[3]		Australia[4]		**Present study**	
		Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**
I_1	E	4.61	0.08	-	-	4.56	0.24	-	-	**3.55**	**0.20**
	F	6.17	0.08	4.75	0.63	6.25	0.14	-	-	**5.09**	**0.02**
	G	7.22	0.08	7.05	0.10	7.01	0.15	6.72	0.11	**6.44**	**0.01**
	H	8.52	0.08	8.79	0.08	8.54	0.26	7.75	0.13	**7.89**	**0.01**
I_2	E	5.27	0.08	-	-	5.33	0.17	-	-	**5.55**	**0.09**
	F	6.83	0.08	6.37	0.15	6.49	0.16	6.07	0.29	**6.19**	**0.02**
	G	8.11	0.09	7.97	0.08	7.70	0.22	7.92	0.13	**7.12**	**0.01**
	H	9.76	0.09	9.70	0.08	9.94	0.20	8.75	0.11	**8.60**	**0.01**
C_1	E	6.94	0.09	-	-	6.25	0.13	6.66	0.10	**7.01**	**0.01**
	F	8.38	0.08	8.30	0.08	9.39	0.24	8.74	0.10	**8.16**	**0.01**
	G	11.68	0.09	10.49	0.08	11.60	0.20	11.50	0.16	**11.34**	**0.01**
	H	13.39	0.09	13.10	0.09	13.54	0.20	13.18	0.12	**12.75**	**0.01**
P_1	C	3.60	0.11	-	-	-	-	-	-	**-**	**-**
	D	5.81	0.08	-	-	4.80	0.16	-	-	**3.17**	**0.01**
	E	7.82	0.08	6.87	0.13	6.94	0.16	7.36	0.13	**7.54**	**0.04**
	F	9.14	0.08	9.34	0.08	9.38	0.22	9.42	0.09	**9.11**	**0.01**
	G	11.91	0.09	11.40	0.08	11.75	0.22	11.55	0.14	**11.18**	**0.01**
	H	13,02	0.09	13.17	0.09	13.26	0.23	12.85	0.19	**12.97**	**0.01**
P_2	B	3.81	0.12	-	-	4.16	0.19	-	-	**-**	**-**
	C	5.00	0.08	-	-	4.29	0.15	-	-	**-**	**-**
	D	6.61	0.09	-	-	5.86	0.16	5.88	0.42	**4.74**	**0.02**
	E	8.43	0.09	7.69	0.12	7.48	0.18	8.52	0.12	**7.96**	**0.01**
	F	9.75	0.09	10.10	0.09	10.02	0.24	10.18	0.13	**9.28**	**0.01**
	G	12.63	0.10	12.32	0.09	12.77	0.16	12.75	0.19	**11.75**	**0.01**
	H	13.99	0.09	14.10	0.10	14.33	0.23	13.88	0.21	**13.84**	**0.01**
M_1	E	3.93	0.06	-	-	3.89	0.26	-	-	**-**	**-**
	F	5.14	0.07	-	-	5.79	0.10	6.09	0.24	**5.69**	**0.01**
	G	7.17	0.09	5.83	0.34	7.34	0.18	7.02	0.12	**7.26**	**0.01**
	H	9.68	0.10	10.72	0.07	9.45	0.19	9.43	0.13	**8.65**	**0.00**
M_2	B	3.77	0.12	-	-	4.18	0.17	-	-	**-**	**-**
	C	4.95	0.09	-	-	5.14	0.15	-	-	**5.06**	**0.01**
	D	6.80	0.09	5.53	0.35	6.58	0.15	6.90	0.17	**6.09**	**0.01**
	E	8.73	0.08	8.57	0.10	9.05	0.20	8.77	0.19	**8.28**	**0.01**
	F	10.40	0.08	10.69	0.14	11.21	0.20	11.04	0.14	**11.01**	**0.01**
	G	12.42	0.09	11.80	0.08	13.10	0.17	12.74	0.19	**12.98**	**0.01**
	H	14.86	0.10	15.51	0.14	15.41	0.17	14.62	0.20	**14.9**	**0.01**

[1] Willems et al. 2001

[2] Chaillet and Demirjian 2004

[3] Teivens and Mörnstad 2001

[4] McKenna et al. 2002

Table 3.11. Mean age of attainment of dental maturity of Black Southern African females in comparison with females from other continents

Tooth	Stage	Belgium[1]		Canada[2]		South Korea[3]		Australia[4]		Present study	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
I_1	E	4.49	0.11	-	-	4.45	0.03	-	-	-	-
	F	5.38	0.08	5.38	0.42	6.40	0.18	5.63	0.28	4.69	0.03
	G	6.75	0.08	6.66	0.14	6.86	0.15	6.22	0.13	6.50	0.01
	H	7.92	0.08	8.40	0.09	8.38	0.26	7.31	0.14	7.57	0.00
I_2	E	5.00	0.1	-	-	5.03	0.24	-	-	5.30	0.01
	F	6.39	0.08	5.92	0.20	6.56	0.02	6.21	0.14	6.04	0.02
	G	7.53	0.07	7.48	0.09	7.86	0.25	7.31	0.13	6.87	0.01
	H	8.94	0.08	9.17	0.08	9.12	0.20	8.30	0.11	8.38	0.01
C_1	E	6.39	0.08	-	-	5.91	0.19	6.03	0.22	6.52	0.02
	F	7.64	0.07	7.51	0.1	8.47	0.24	7.23	0.16	7.17	0.01
	G	10.22	0.07	9.66	0.08	10.30	0.27	10.17	0.11	9.72	0.01
	H	11.96	0.1	11.75	0.09	12.19	0.16	11.23	0.12	11.62	0.01
P_1	C	3.09	0.21	-	-	-	-	-	-	-	-
	D	5.35	0.09	-	-	4.63	0.25	-	-	-	-
	E	7.20	0.07	6.48	0.14	6.72	0.01	6.57	0.19	7.17	0.01
	F	8.67	0.06	8.99	0.08	9.09	0.01	9.17	0.1	7.81	0.01
	G	10.89	0.08	10.88	0.08	11.31	0.20	10.76	0.12	10.30	0.01
	H	12.32	0.08	12.52	0.09	12.56	0.22	12.01	0.15	12.15	0.01
P_2	B	3.63	0.16	-	-	4.31	0.29	-	-	-	-
	C	4.70	0.1	-	-	4.69	0.32	-	-	-	-
	D	6.32	0.1	-	-	5.65	0.22	5.64	0.44	-	-
	E	8.09	0.08	7.50	0.11	7.42	0.19	7.60	0.19	7.86	0.02
	F	9.18	0.08	9.71	0.08	9.36	0.20	9.70	0.1	8.35	0.01
	G	11.80	0.08	11.87	0.09	12.19	0.21	11.75	0.15	10.88	0.01
	H	13.47	0.09	13.31	0.09	13.93	0.25	12.93	0.17	12.95	0.01
M_1	E	3.71	0.1	-	-	4.01	0.31	-	-	-	-
	F	4.87	0.08	-	-	5.51	0.15	5.63	0.35	4.56	0.03
	G	6.62	0.08	6.19	0.18	6.62	0.22	8.83	0.21	6.90	0.01
	H	9.05	0.08	10.10	0.09	9.04	0.27	9.26	0.15	8.05	0.01
M_2	B	3.42	0.18	-	-	4.01	0.31	-	-	-	-
	C	4.83	0.1	-	-	5.09	0.13	-	-	-	-
	D	6.36	0.09	6.04	0.18	6.45	0.20	5.55	0.49	5.29	0.02
	E	8.38	0.07	8.49	0.08	9.11	0.21	8.56	0.13	8.15	0.01
	F	9.83	0.1	10.16	0.07	10.92	0.28	10.56	0.12	10.48	0.01
	G	11.66	0.08	11.34	0.08	12.57	0.20	12.03	0.14	12.49	0.01
	H	14.41	0.10	15.06	0.16	15.11	0.18	14.17	0.18	14.01	0.01

[1] Willems et al. 2001

[2] Chaillet and Demirjian 2004

[3] Teivens and Mörnstad 2001

[4] McKenna et al. 2002

3.4 Discussion

A well-defined scale for measuring and quantifying tooth formation is a very reliable method for the assessment of growth patterns. It may also serve as a reference for inter- and intra-population comparisons from anthropological, forensic and clinical dentistry perspectives (Scheuer and Black 2000a; Scheuer and Black 2000b; Scott and Turner 2000; Scheuer and Black 2007a; Cunha et al. 2009). In developing such a scale for Black Southern Africans, this study documented advanced dental maturity compared to the French Canadian, South Korean and Belgian populations.

Few published studies on dental maturity exist from Africa. Two studies on Southern African Black children focused on predicting chronological age from dental maturity (Phillips and van Wyk Kotze 2009; Uys et al. 2014). Tompkins (1996) compared maturity scores of Black Southern African children with French Canadians and prehistoric Native Americans. He used modified Demirjian maturation stages that are not directly comparable to this study, but he documented that Black Southern African children had advanced tooth formation compared to the French Canadians and Native Americans. Uys et al. (2014), in another study on Black South Africans showed that the Demirjian method overestimated males by 0.8 years and females by 0.5 years. Uys et al. (2014) did not find a consistent pattern among the males, but their results documented the greatest range of difference in the early age groups, as was the case in the present study. Furthermore, the dental age estimates in the present study are quite similar to the results of Uys et al. (2014) for ages 10-12 years in males. Similar results were also found for the females, except for age groups 8 and 9 years.

A major limitation of the present study is the small sample sizes in age groups 6 and 18-20. Additional data from these age groups could have strengthened our findings. Ethical issues and health regulations on exposure of children to x-rays in the younger age groups, along with the small number of pupils in the age group 18-20 years due to low number of this age group in secondary schools contributed to the low sample sizes in these age groups.

3.4.1 Population variations in dental maturity

There is general consensus that the Demirjian method overestimates dental age in many populations. Several studies (Willems et al. 2001; Maber et al. 2006; Chen et al. 2010; Lee et al. 2011; Nik-Hussein et al. 2011; Ogodescu et al. 2011; Ifesanya and Adeyemi 2012; Uys et al. 2014; Cavrić et al. 2016) and two systematic reviews (Jayaraman et al. 2013; Yan et al. 2013) found different levels of overestimation using the Demirjian method. Some authors suggest that this could be due to population differences in growth and maturity (Tunc and Koyuturk 2008; Willems et al. 2001). However, Liversidge et al. (1999) proposed that this overestimation in dental age could be due to positive secular trends in growth and development during the last three decades.

Black Southern African children show advanced dental maturity in the age of attainment of specific developmental stages in most teeth compared to Asian and European ancestry populations (Chaillet and Demirjian 2004; Liversidge et al. 1999; McKenna et al. 2002; Nykänen et al. 1998; Teivens and Mörnstad 2001; Willems et al. 2001). However, the Australians have earlier ages of attainment of the root formation stages for central incisors, first premolars and second molars. Interestingly, Cavrić et al. (2016) found slightly earlier ages of attainment of tooth maturation stages in Botswana children compared to children from Southern Africa. One would have expected similar results from these countries because they border each other and have general populational affinities. A study conducted among people of similar genetic origin within Turkey (Cantekin et al. 2014) found variation in the dental maturity, with children living in a warmer climate and low altitude attaining dental maturity earlier. Hence climatic differences could account for the differences between these two Southern African countries. Genetic and environmental factors have also been implicated as the reason for population variability (Townsend et al. 2009). Pertaining to that, changes in population dynamics resulting from political developments over the last centuries may have contributed to the observed differences. Other possible sources of population variation in tooth formation include methodological differences among researchers. The present study used probit regression analysis after converting the data to a dichotomous variable based on whether or not a developmental stage is achieved. The methodology used by Cavrić et al. (2016) to derive the mean age of attainment is not clear. Also, the age structure of the sample and the precision of measurement could account for differences. A major implication of our findings is that population-specific dental maturity reference values should be developed for all populations especially for the accuracy of age estimation for forensic and anthropological purposes.

3.4.2 Sex variation in dental maturity

The present study found females to be more advanced in dental maturation than their male counterparts. This is in keeping with previous studies on dental maturity (Demirjian et al. 1973; Demirjian and Levesque 1980; Fanning 1961; Mörnstad et al. 1999; Liversidge et al. 1999; Uys et al. 2014; Cavrić et al. 2016). There is substantial evidence to show that females are correspondingly ahead of males in other developmental indicators including sexual maturity and skeletal development (Stang and Story 2005), but catch-up by males occurs after puberty. The onset of puberty signals the commencement of biological growth and development during adolescence and this begins earlier in females than in males (Giedd et al. 1999). These biological changes associated with puberty include not only sexual maturation, but also increases in height and weight, completion of skeletal growth accompanied by a marked increase in skeletal mass, changes in body composition and brain development (Bogin 1999). The earlier maturation of teeth in females and later catch-up by males mirrors the pattern seen for other growth events surrounding puberty.

3.5 Conclusions

The standards of dental maturation developed by Demirjian et al. (1973) overestimate the chronological ages of Southern African Black males and females, who are significantly advanced in dental maturity compared to Demirjian's French Canadian sample. Similarly, the age of attainment of specific developmental stages shows that the Southern Africans attain maturity earlier than South Korean, Canadian and Belgian children. As found in other studies, females were more advanced in dental maturity than males. These differences in dental maturity on the populational level, and in terms of sexual dimorphism, are statistically significant and thus clearly illustrate the need for population-specific reference values, rather than global, dental maturity standards.

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Contents

Chapter 4

Tooth Formation: Accuracy of the Willems method and Demirjian's seven tooth methods in a Black Southern African population

Abstract

Background: The accuracy of methods of age estimation is important in forensic and anthropological applications. The validity and accuracies of most methods used in estimating dental age have not been validated in Southern Africa.

Aim: The aim of this paper is to determine the accuracy of the Original Demirjian method, the Modified Demirjian method and the Willems method in a Black Southern African population to determine their usefulness for forensic and anthropological purposes.

Method: This was a cross-sectional study involving 540 (233 males and 307 females) school children aged 5 to 15.99 years. Panoramic radiographs from these children with the seven left mandibular teeth were scored using the Original Demirjian, Modified Demirjian and Willems methods. Estimated ages obtained using these methods were compared to the chronological ages. The differences in the estimated ages produced by the three methods and chronological ages were compared. Mean absolute errors were also calculated for each method. Statistical significance was inferred at $p<0.05$.

Results: The Original Demirjian method overestimated the age of the males by 0.85 years and the females by 1.0 years. A one sample t-test showed significant difference in the mean difference of the dental age and chronological age with the test value of zero. The mean absolute error was 1.1 years for both males and females. The Modified Demirjian method similarly shows significant overestimation, with 0.90 years for males and 1.21 years for females. The Modified Demirjian method has the highest mean absolute error for both males (1.1 years) and females (1.4 years). The Willems method showed the lowest, but still a significant mean difference (0.2 years for males and 0.3 years for females) between the dental age and chronological age. It also shows the least mean absolute errors (for males 0.70 years and 0.68 years for females) compared to the Original Demirjian and Modified Demirjian methods.

Conclusion: The Willems method produced significantly less overestimation compared to the two Demirjian methods. The Original Demirjian method produces better accuracy than the Modified Demirjian method for Black Southern African children.

4.1 Introduction

Knowledge of the accuracy of methods used in the assessment of growth and developmental age is very important in biological anthropology and health research. It is also critical for forensic purposes, especially with the increasing global incidences of mass deaths and disasters (Kieser et al. 2006; Perrier et al. 2006). Estimation of age with certainty is necessary where birth records are unreliable or lost, where people seek asylum (Schmeling et al. 2007), where specific aging is needed to prevent cheating in age-graded sports competitions, or where individuals seek favorable outcomes in civil or criminal cases (Schulze et al. 2006; Meijerman et al. 2007; Ríos et al. 2008; Baumann et al. 2009; Rios and Cardoso 2009). The age at death is usually the only biological parameter that can be determined for unidentified juvenile remains with any degree of accuracy (Scheuer and Black 2000).

4.1.1 Age estimation

Methods developed for estimating physiological age utilize one or combinations of the four main indices of growth and development: stature, secondary sex characteristics, bone growth and dental development (Moorrees et al. 1963; Schulze et al. 2006; Rios et al. 2008: Rios and Cardoso 2009; Bauman et al. 2009). Tooth formation is less variable when compared to other growth defining events such as the appearance of bone ossification centers, tooth emergence and root apical closure (Lewis and Garn 1960; Kurita et al. 2007). Additionally, a stronger correlation was found between chronological age and dental age than between skeletal age and dental age (Green 1961). Hence, many authors agree that dental development, especially tooth formation, is the most reliable method of estimating age (Kurita et al. 2007; Roberts et al. 2008) and that it should be adopted as the standard of estimating biological age (Kurita et al. 2007).

4.1.2 Methods of estimating dental age

Several methods are available for estimation of dental age in children and adults. Morphological techniques are based on cementum apposition and root resorption (Liversidge et al. 1998), occlusal wear (Kim et al. 2000; Yun et al. 2007), periodontosis, or translucency of the apical zone (Gustafson and Koch 1974; Nowell 1978). Other methods include the evaluation of aspartic acid racemization in the dentin (Helfman and Bada 1975, 1976), telomere shortening in the DNA of the dental pulp (Takasaki et al. 2003), the amount of radiocarbon in the enamel (Cook et al. 2006), attrition levels, secondary dentin formation and periodontal attachment (Jain 2012). All these methods necessitate extraction and require preparation of microscopic sections of at least one tooth per each individual. These methods cannot be used in living individuals for ethical reasons. Another method involves visual identification of the type and number of teeth emerged in an individual and extrapolating the age based on the known age range for the appearance of such teeth (tooth emergence). This method may not estimate age accurately, but it may be a useful guide in age estimation where facilities such as radiography do not exist. Radiological assessment of tooth development has gained worldwide acceptance because of its accuracy and use in clinical practice. It is non-destructive and other clinical information can be obtained.

4.1.3 Radiological methods of age estimation

There is a lack of consensus on the accuracy and applicability of the existing radiographic methods used in estimating age. The most widely used method, presented by Demirjian et al. (1973), produces significant overestimation for many populations. However, a newer method that refines the Demirjian method (Willems et al. 2001) is reported to have better accuracy for many populations. A previous study on Black South African children found Demirjian’s original method overestimated the chronological age (Uys et al. 2014). The accuracy and validity of other methods, such the revised Demirjian method (Demirjian and Goldstein 1976) and the Willems method (Willems et al. 2001), have never been tested on a Southern African population.

4.1.4 Demirjian's method

Demirjian and colleagues developed an age estimation methodology based on eight (A-H) developmental stages of the seven left mandibular teeth (Demirjian et al. 1973). The reference population was French-Canadian children. This method has gained wide acceptance and is globally utilized (Nik-Hussein et al. 2011). Biological weights, which are numerical scores derived using the same method for measuring skeletal maturity (Tanner et al. 1962), are assigned to the developmental stages for each of the seven teeth. The weights are added together to provide the total maturity score, which is converted to dental age using tables and percentile charts. The Demirjian method is described as simple, easy and highly reproducible (Litsas and Lucchese 2016). A study applying it to German children concluded that the Demirjian method yielded appropriate age estimates (Wolf et al. 2016). However, numerous studies (Willems et al. 2001; Tunc and Koyuturk 2008; Chen et al. 2010; Baghdadi and Pani 2012; Ifesanya and Adeyemi 2012; Uys et al. 2014), including two systematic reviews using different populations (Jayaraman et al. 2013; Yan et al. 2013), found overestimation of age by Demirjian's method. Hence some authors have questioned its applicability in forensic science where highly predictable and accurate results are required (Amberkove et al. 2014; Carneiro et al. 2015).

4.1.5 Modifications of the Demirjian method

Demirjian revised his original method because of two shortcomings (Demirjian and Goldstein 1976). Firstly, all teeth may not be present in the mouth and it may not be possible to use corresponding antimeres. So he devised two modifications using only four teeth. The first evaluated the molars and premolars (M_2 M_1 PM_2 and PM_1), while the second considered the second molar, the premolars, and the central incisors (M_2 PM_2 PM_1 and I_1) (Demirjian and Goldstein 1976). Secondly, there was a lack of sufficient numbers of very young and the oldest children in the first sample (Demirjian and Goldstein 1976). The inclusion of more children led to a change in the biological weighted score in the Modified Demirjian method. The Modified method was tested in many populations, with overestimation being reported for most groups (Lee et al. 2011; Akkaya et al. 2015; Ambarkova et al. 2014).

4.1.6 Willems method

Willems et al. (2001) adapted the maturity score format of Demirjian et al. (1973), but discarded the use of the biological weights for each stage. Instead, new biological weights were generated so that when the weights are summed the estimated age is given. This eliminates the cumbersome step of converting the maturity scores to dental age. The use of this method on a Belgian reference population found no significant difference between the mean dental age and the chronological age of the population (Willems et al. 2001).

Studies utilizing the Willems method in Egypt (El-Bakary et al. 2010), Malaysia (Mani et al. 2008; Nik-Hussein et al. 2011), Serbia (Djukic et al. 2013), France (Urzel and Bruzek 2013), China (Ye et al. 2014), Macedonia (Ambarkova et al. 2014) and India (Hegde et al. 2016) reported considerable accuracy in the estimation of chronological age of individuals in their populations. Akkaya et al. (2015), in a study of Turkish children, concluded that Willems' method can be recommended for dental age estimation for forensic purposes. However, another study from China (Zhai et al. 2016) found the Demirjian method to more accurate than the Willems method.

Studies utilizing the Willems method have not been conducted in sub-Saharan Africa. It is important to determine the accuracy of this method, in view of the overestimation of dental age by the Original Demirjian method previously reported for a Black South African population (Uys et al. 2014). Therefore, the aim of this paper is to determine the accuracy of the Original Demirjian method, the Modified Demirjian method and the Willems method in a Black Southern African population to determine their usefulness for forensic and anthropological purposes.

4.2 Materials and Methods

4.2.1 Study design

This was a quantitative cross-sectional study of 540 Southern African children comprising of 233 (43.1%) males and 307 (56.85%) females. The sample population was drawn from Black children whose parents and grandparents are indigenous Southern Africans.

4.2.2 Study population

The sample population was randomly selected from primary and secondary schools in Johannesburg Municipality, South Africa. Children screened for dental diseases by the Community Oral Health Outreach Program (COHOP) of the Department of Community Dentistry of the School of Oral Health Sciences, University of the Witwatersrand were evaluated for participation in the study. Permission to carry out the study was obtained from the local education authority and respective school heads. Written consent was obtained from the parent/guardian and assent from the child was required before participation. Ethical clearance (NO M141001) was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand.

4.2.3 Data collection

Panoramic radiographs of children screened for treatment during visits of the Community Oral Health Outreach Program were collected and analyzed.

4.2.4 Inclusion and exclusion criteria

Radiographs showing gross pathology or low image quality were excluded. Children with systemic diseases that can affect the development of teeth, mandibular hypodontia (except third molars), and those who had lost their teeth on both sides of the mandible were excluded. Also, children from age 16 years and above were also excluded because the Demirjian maturity scores do not include children over 16 years.

4.2.5 Sample size

A total of 11 age cohorts were sampled from ages 5 to 15.99 years. The sample size formula is $N = 4z_{\alpha}^{2}S^{2} \div W^{2}$, where the S= standard deviation, W= desired total width and Z_{α} is the standard normal deviate for the 95% confidence level. Cameriere et al. (2008), in a study of the reproducibility and accuracy of Demirjian’s method, found a mean of 1.076 and a standard deviation of 0.824 derived from a standard error of 0.030. Using a width of 0.2, the minimum sample size total required for the 11 cohorts is 280. However, to improve the power of the study, a total of 540 children were recruited. The chronological age (CA) was calculated by subtracting the date of the radiograph from the date of birth obtained from the school register and was recorded as decimal years.

4.2.6 Pilot test

Prior to data collection, a reliability study was conducted to determine the magnitude of the intra-observer error of interpretation and detection. The investigator assessed the maturation stage of the seven-left mandibular permanent teeth without knowledge of chronological age or sex. To evaluate reproducibility, 25 radiographs (with 175 individual-tooth ratings) were randomly selected and scored by the investigator at day one and day three. The investigator was the only rater for the developmental stages of the teeth. Intra-examiner reliability of rating the development stages was calculated using Cohen’s Kappa (Landis & Koch 1977) and was found to be 0.97.

4.2.7 Dental age assessment using the Demirjian seven tooth methods

The digital panoramic radiographs of each child were enhanced using Microsoft Office Picture Manager, properly labeled with a unique identity number and digitally archived. Each radiograph was assessed for the development of the left 7 permanent mandibular teeth and was rated on an 8-stage scale from A to H, based on the stages of tooth formation identified by Demirjian et al. (1973) with stage 0 for non-appearance. Each stage of the teeth was allocated a sex-specific biologically weighted score for the two Demirjian methods and the sum of the scores for each participant was used to determine the dental maturity on a scale of 0 to 100. The dental maturity score of each child was converted to dental age (DA) using standard tables and percentile curves for both sexes (Demirjian et al. 1973; Demirjian and Goldstein 1976).

4.2.8 Dental age assessment by the Willems method

Tooth development was divided into eight stages (A-H), as proposed by Demirjian et al. (1973), and a score for the developmental stage of each tooth was obtained from the score chart given by Willems et al. (2001). The sum of scores for the seven teeth provided the estimated dental age (DA) of the individual. When a tooth on the left side was missing, the corresponding tooth on the right side was substituted and scored. The scoring was done by the investigator.

4.2.9 Data analysis

The data were analyzed using IBM SPSS (version 22) software for Windows. Analyses were done for the entire group as well as for each sex and age cohort. Dental age (DA) was compared to chronological age (CA) for males and females separately. The difference between the DA and CA was tested using paired Student's *t*-tests at a significance level of $p<0.05$. The absolute mean difference between the DA and CA was calculated to express accuracy independent of bias.

A Bland Altman procedure was used to determine the agreement between dental age (DA) estimated by the different age estimation methods and the chronological age (CA) of the children. The presence of fixed bias is indicated when the mean of the mean difference differs significantly from zero, based on a one-sample t-test. In that case, there is an assumption that no agreement exists between the measurements and no Bland Altman plot is produced. However, when the one-sample t-test is not significant, the Bland Altman plot is generated and a linear regression analysis is done with the mean difference being the outcome variable and the average of the chronological age and the estimated dental age being the predictor variable. Statistical significance shows that there is a proportional bias between the chronological age and the estimated age. Statistical significance was inferred at the level of $p\leq0.05$.

4.3 Results

The distribution of the participants (233 males and 307 females) by age cohorts is shown in Tables 4.1 and 4.2. The mean ages are 10.69 ± 3.08 years and 11.15± 2.89 years for males and females, respectively. There is no significant difference between the mean ages of males and females ($p=0.078$).

4.3.1 Original Demirjian method

The mean differences between chronological age (CA) and estimated dental age (DA) using the Original Demirjian method are tabulated in Tables 4.1 and 4.2. The average mean difference (overestimation) for males is 0.85 years (p=0.00), while the average mean difference (overestimation) for the females is 1.0 years (p=0.00). The one sample t-test shows a significant difference between the mean of the mean differences and the test value of zero (Table 4.3). Therefore no Bland Altman plot was produced. The mean absolute error is 1.1 years for both males and females. Also, the mean absolute errors obtained for individual age cohorts are consistently close to 1.0 years in both males and females except for the lower error found for age cohort 15 years (Tables 4.1 and 4.2).

Table 4.1. Mean differences in dental and chronological ages in males using the Original Demirjian method

Age cohort (years)	N	Dental age (DA)		Chronological age (CA)		DA-CA		t	P	95% CI		Mean Absolute Error (MAE)
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**			**Lower**	**Upper**	
5 – 5.99	10	7.03	0.23	5.71	0.15	1.32	0.31	13.58	**0.00**	1.10	1.54	1.19
6 – 6.99	26	7.32	0.49	6.19	0.19	1.13	0.43	13.33	**0.00**	0.96	1.31	1.20
7 – 7.99	6	8.85	1.04	7.63	0.34	1.23	0.92	3.26	**0.02**	0.26	2.19	1.51
8 – 8.99	33	9.75	1.11	8.54	0.28	1.21	1.05	6.64	**0.00**	0.84	1.58	1.54
9 – 9.99	36	10.26	1.44	9.38	0.31	0.89	1.33	3.98	**0.00**	0.43	1.34	1.49
10 – 10.99	16	10.82	0.72	10.29	0.29	0.53	0.76	2.76	**0.01**	0.12	0.93	0.68
11 – 11.99	17	12.04	0.91	11.47	0.29	0.57	0.88	2.69	**0.02**	0.12	1.02	0.63
12 – 12.99	21	12.39	0.91	12.27	0.24	0.11	0.84	0.63	0.53	-0.27	0.50	0.73
13 – 13.99	15	14.73	1.20	13.31	0.25	1.42	1.14	4.81	**0.00**	0.79	2.06	1.73
14 – 14.99	28	15.01	1.14	14.45	0.32	0.56	1.12	2.67	**0.01**	0.13	1.10	1.01
15 – 15.99	25	15.86	0.48	15.47	0.26	0.39	0.56	3.47	**0.00**	0.16	0.62	0.36

Significant differences in bold.

Table 4.2. Mean differences in dental and chronological ages in females using the Original Demirjian method

Age cohort (years)	N	Dental age (DA)		Chronological age (CA)		DA-CA		t	P	95% CI		Mean Absolute Error (MAE)
		Mean	SD	Mean	SD	Mean	SD			Lower	Upper	
5 – 5.99	13	7.03	0.28	5.77	0.17	1.26	0.32	13.95	**0.00**	1.06	1.45	1.25
6 – 6.99	28	7.37	0.61	6.31	0.24	1.05	0.67	8.38	**0.00**	0.80	1.31	0.86
7 – 7.99	9	8.69	1.25	7.64	0.35	1.05	1.11	2.85	**0.02**	0.20	1.90	0.96
8 – 8.99	27	9.85	0.99	8.54	0.28	1.31	0.98	6.95	**0.00**	0.92	1.70	1.46
9 – 9.99	30	10.83	1.02	9.46	0.26	1.37	0.95	7.88	**0.00**	1.01	1.72	1.50
10 – 10.99	22	11.35	1.16	10.42	0.33	0.93	1.09	4.01	**0.00**	0.45	1.41	1.24
11 – 11.99	36	12.33	0.93	11.41	0.28	0.92	0.94	5.80	**0.00**	0.60	1.24	0.86
12 – 12.99	44	13.49	1.08	12.46	0.26	1.03	1.13	6.02	**0.00**	0.69	1.37	1.01
13 – 13.99	39	14.76	1.30	13.41	0.28	1.35	1.30	6.49	**0.00**	0.93	1.77	1.55
14 – 14.99	32	14.99	0.87	14.40	0.20	0.59	0.79	4.26	**0.00**	0.31	0.88	0.97
15 – 15.99	27	15.95	0.27	15.41	0.29	0.54	0.35	7.97	**0.00**	0.40	0.68	0.34

Significant differences in bold.

Table 4.3. One-sample t-test for the Original Demirjian method

Sex	Test Value = 0					
	T	Df	p	Mean difference	95% CI of the difference	
					Lower	Upper
Male	12.35	232	**0.00**	0.85	0.69	0.95
Female	18.41	306	**0.00**	1.04	0.92	1.14

Significant differences in bold.

4.3.2 Modified Demirjian method

Tables 4.4 and 4.5 compare dental age estimates using the Modified Demirjian method with the chronological age. The average mean difference (overestimation) for males is 0.9 years (p=0.00), while it is 1.21 years for females (p=0.00). The one-sample t-test shows a significant difference between the mean of the mean differences and the test value of zero (Table 4.6). Therefore a Bland Altman plot was not generated. The mean absolute error for males is 1.1 years, while it is 1.4 years for females. An absolute error of above 1 year is seen for age cohorts 7-14 years in males while high values above 2.0 years are shown for the age cohorts 10-13 years in females. Lower values are found at the extremes of the age cohort ranges in both males and females.

Table 4.4. Differences between mean dental age and chronological age in males using the Modified Demirjian method

Age group (years)	n	Dental Age (DA)		Chronological Age (CA)		DA – CA		T	P	95% CI		Mean Absolute Error (MAE)
		Mean	SD	Mean	SD	Mean	SD			Lower	Upper	
5 – 5.99	10	6.63	0.23	5.71	0.15	0.92	0.31	9.46	**0.00**	0.70	1.14	0.78
6 – 6.99	26	6.78	1.88	6.19	0.19	0.69	0.73	4.46	**0.00**	0.35	0.95	0.89
7 – 7.99	6	9.08	1.28	7.63	0.34	1.46	1.13	3.16	**0.00**	0.27	2.64	1.75
8 – 8.99	33	9.92	1.07	8.54	0.27	1.38	1.03	7.66	**0.00**	7.01	1.74	1.47
9 – 9.99	36	10.11	1.32	9.38	0.31	0.73	1.22	3.59	**0.01**	0.32	1.15	1.28
10 – 10.99	16	11.30	0.86	10.29	0.29	1.01	0.89	4.54	**0.00**	0.53	1.48	1.06
11 – 11.99	17	12.65	0.78	11.47	0.29	1.18	0.76	6.39	**0.00**	0.79	1.57	1.08
12 – 12.99	21	13.15	0.81	12.27	0.24	0.87	0.73	5,52	**0.00**	0.54	1.20	1.02
13 – 13.99	15	14.67	1.03	13.31	0.25	1.36	0.95	8.53	**0.00**	0.83	1.88	1.56
14 – 14.99	28	14.99	0.92	14.45	0.32	0.54	0.91	3.18	**0.04**	0.19	0.90	0.70
15 – 15.99	25	15.65	0.38	15.47	0.26	0.18	0.46	1.95	0.06	-0.01	0.37	0.21

Significant differences in bold.

Table 4.5. Differences between mean chronological and dental ages in females using the Modified Demirjian method

Age cohort (years)	n	Dental age (CA)		Chronological Age (DA)		DA – CA		T	P	95% CI		Mean Absolute Error (MAE)
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**			**Lower**	**Upper**	
5 – 5.99	13	6.18	0.45	5.77	0.17	0.40	0.48	3.02	**0.01**	0.11	0.69	0.33
6 – 6.99	28	6.78	0.85	6.31	0.24	0.47	0.89	2.76	**0.01**	0.12	0.82	0.63
7 – 7.99	9	8.45	1.24	7.64	0.35	-0.81	1.05	2.32	**0.04**	0.01	1.61	0.76
8 – 8.99	27	10.05	1.15	8.54	0.28	1.50	1.15	6.78	**0.00**	1.05	1.96	1.43
9 – 9.99	30	11.26	1.35	9.46	0.26	1.79	1.29	7.66	**0.00**	1.32	2.28	1.94
10 – 10.99	22	12.26	1.83	10.42	0.33	1.84	1.69	5.09	**0.00**	1.09	2.60	2.46
11 – 11.99	36	13.88	1.27	11.41	0.28	2.48	1.26	11.78	**0.00**	2.05	2.90	2.47
12 – 12.99	44	14.71	0.89	12.46	0.26	2.25	0.94	15.83	**0.00**	1.97	2.54	2.00
13 – 13.99	39	15.34	0.62	13.41	0.28	1.93	0.65	18.59	**0.00**	1.72	2.14	1.81
14 – 14.99	32	15.54	0.33	14.39	0.20	1.11	0.28	22.22	**0.00**	1.01	1,21	1.08
15 – 15.99	27	15.79	0.56	15.41	0.29	0.38	0.29	6.89	**0.00**	0.27	0.50	0.22

Significant differences in bold.

Table 4.6. One-sample t-test of the mean difference between chronological age and estimated dental age using the Modified Demirjian method

Sex	Test Value = 0					
	T	**df**	**p**	**Mean difference**	**95% CI of the difference**	
					Lower	**Upper**
Male	13.91	232	**0.00**	0.94	0.75	0.99
Female	22.10	306	**0.00**	1.21	1.40	1.67

Significant differences in bold.

4.3.3 Willems’ method

The estimated dental age and chronological age of the males and females are compared in Tables 4.7 and 4.8. The mean difference between dental age estimated by Willems’ method and chronological age (overestimation) for males is 0.2 years, while it is 0.3 years for females (Table 4.9). The one-sample t-test shows that the mean of the difference between the Willems method estimate and the chronological age is significant hence the Bland-Altman plot was not done. The mean absolute error is 0.70 years for males and 0.68 years for females. Only the age cohort 14 years in males and the age cohorts 13 and 14 years in females have mean absolute errors above 1.0 years (Tables 4.7 and 4.8).

4.3.4 Comparison between the Willems method and the Demirjian methods

The estimated dental age using Willems’ method is closer to the chronological age in all age groups. Similarly, the Willems method has the least mean absolute error value compared to the two Demirjian methods. All the three methods overestimate the chronological age in the early age cohorts; however at age 9 years the Willems’ method shows both positive and negative distribution patterns along the chronological age. In contrast, the two Demirjian methods consistently overestimate the chronological age for all age groups. The Modified Demirjian method overestimates the chronological age more than the Original Demirjian method in both males and females (Figures 4.1 and 4.2).

Table 4.7. Differences between mean dental and chronological age in males using the Willems method

Age cohort (years)	N	Dental age (DA)		Chronological age (CA)		DA-CA		t	p	95% CI		Mean Absolute Error (MAE)
		Mean	SD	Mean	SD	Mean	SD			Lower	Upper	
5 – 5.99	10	6.05	0.35	5.71	0.15	0.34	0.41	2.60	**0.03**	0.04	0.64	0.38
6 – 6.99	26	6.95	1.88	6.19	0.19	0.76	1.83	2.14	**0.04**	0.03	1.47	0.90
7 – 7.99	6	8.44	1.11	7.63	0.34	0.82	0.98	2.05	0.09	-0.21	1.84	0.99
8 – 8.99	33	9.34	0.84	8.54	0.28	0.81	0.77	6.04	**0.00**	0.54	1.09	0.91
9 – 9.99	36	9.70	1.07	9.38	0.31	0.32	0.97	2.01	0.05	-0.00	0.66	0.83
10 – 10.99	16	10.14	0.52	10.29	0.29	-0.15	0.57	-1.05	0.31	-0.45	0.15	0.57
11 – 11.99	17	11.32	0.84	11.47	0.29	-0.15	0.83	-0.74	0.47	-0.57	0.27	0.56
12 – 12.99	21	11.96	0.81	12.27	0.24	-0.31	0.72	-1.98	0.06	-0.64	0.02	0.47
13 – 13.99	15	13.55	0.87	13.31	0.25	0.24	0.81	1.13	0.28	-0.21	0.68	0.65
14 – 14.99	28	13.99	1.15	14.45	0.32	-0.45	1.09	-2.23	**0.03**	-0.88	-0.09	1.06
15 – 15.99	25	15.32	0.85	15.47	0.26	-0.15	0.87	-0.86	0.39	-0.51	0.21	0.41

Significant differences in bold.

Table 4.8. Differences between mean dental and chronological age in females using the Willems method

Age cohorts (years)	N	Dental age (DA)		Chronological age (CA)		DA-CA		T	p	95% CI		Mean Absolute Error (MAE)
		Mean	SD	Mean	SD	Mean	SD			Lower	Upper	
5 – 5.99	13	5.94	0.52	5.77	0.17	0.17	0.53	1.15	0.27	-0.15	0.49	0.44
6 – 6.99	28	6.63	0.85	6.31	0.24	0.32	0.92	1.83	0.08	-0.04	0.68	0.64
7 – 7.99	9	7.97	0.97	7.64	0.35	0.34	0.76	1.32	0.22	-0.25	0.92	0.34
8 – 8.99	27	9.19	0.65	8.54	0.28	0.65	0.65	5.22	**0.00**	0.39	0.90	0.64
9 – 9.99	30	9.79	0.86	9.46	0.26	0.34	0.78	2.36	**0.03**	0.04	0.63	0.61
10 – 10.99	22	10.37	0.89	10.42	0.33	-0.05	0.76	-0.32	0.75	-0.39	0.28	0.65
11 – 11.99	36	11.38	0.85	11.41	0.28	-0.02	0.85	-0.16	0.88	-0.31	0.26	0.67
12 – 12.99	44	12.65	1.26	12.46	0.26	0.19	1.29	0.96	0.34	-0.21	0.58	0.98
13 – 13.99	39	14.20	1.59	13.41	0.28	0.79	1.58	3.12	**0.00**	0.28	1.30	1.28
14 – 14.99	32	14.37	1.20	14.39	0.20	-0.03	1.10	-0.13	0.89	-0.42	0.37	1.00
15 – 15.99	27	15.72	0.38	15.41	0.29	0.31	0.42	3.81	**0.00**	0.14	0.48	0.22

Significant differences in bold.

Table 4.9. One-sample t-test of the mean difference between chronological age and estimated dental age for males and females using the Willems method

Sex	Test Value = 0					
	T	df	p	Mean difference	95% CI of the difference	
					Lower	Upper
Male	2.38	232	**0.02**	0.19	0.02	0.26
Female	4.75	306	**0.00**	0.27	0.16	0.40

Significant differences in bold.

Figure 4.1. Comparison between the Willems, Original Demirjian and Modified Demirjian methods in males

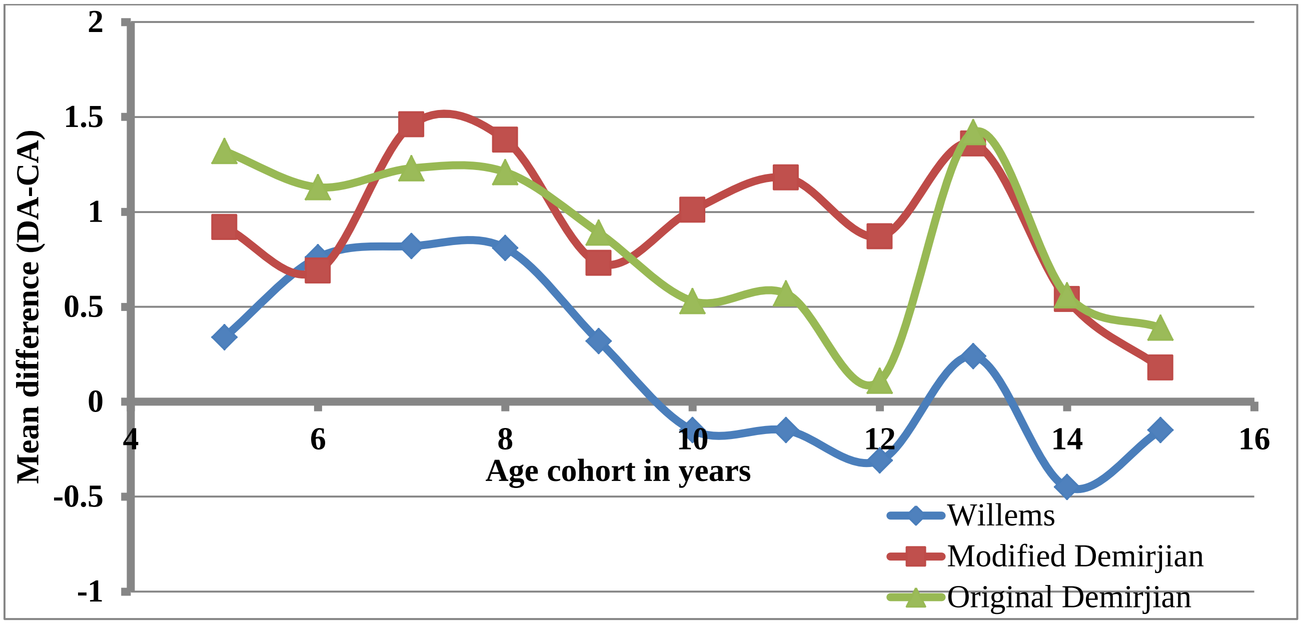
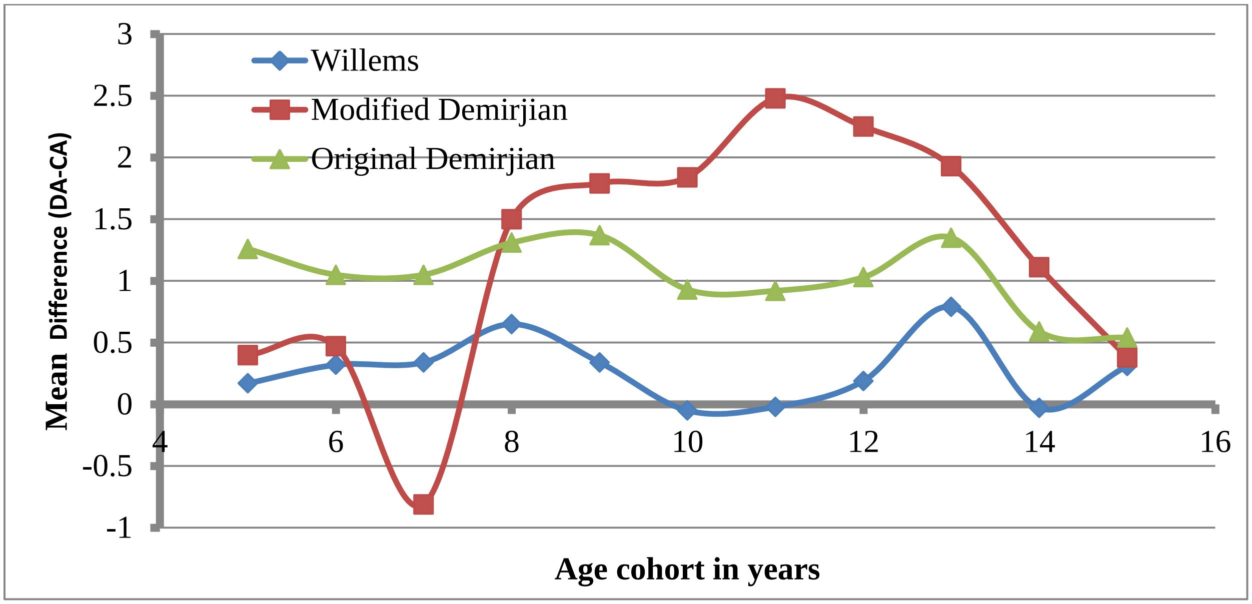


Figure 4.2. Comparison between the Willems, Original Demirjian and Modified Demirjian methods in females



4.4 Discussion

The reliability and reproducibility of the Demirjian methods of dental age estimation are not in doubt, but their accuracy for all populations is highly variable, especially for forensic purposes. Other radiographic methods such as the Willems method have been developed and studies are being conducted to ascertain their accuracies in different populations. The present study assessed the accuracy of the two Demirjian seven tooth methods and the Willems method in a Southern African Black population. Willems' method was found to have better accuracy than the two Demirjian methods. Both Demirjian methods significantly overestimated the chronological age of Southern African children.

4.4.1 Original Demirjian method

Numerous studies using this method had overestimation of chronological age (Willems et al. 2001; Hegde and Sood 2002; Baghdadi and Pani 2012; Ifesanya and Adeyemi 2012; Jayaraman et al. 2013; Uys et al. 2014; Carneiro et al. 2015; Cavrić et al. 2016). Like many other studies, the present study found overestimation of the chronological ages in both males and females when using the Original Demirjian method. The mean difference found in males and females was 0.85 and 1.04 years, respectively. The reason for the higher overestimation for females may be due to the advanced dental maturity in the females compared to males. The higher rate of stunting found in Black South African males compared to females (Kruger et al. 2014) may have also accounted for the difference. However, the mean absolute error was 1.1 years for both males and females. An earlier study conducted on the Black Southern African population found lower overestimations of 0.8 and 0.5 years for males and females respectively (Uys et al. 2014). No reason was given for their findings and the mean absolute errors were not provided. The reason for the difference between their study and ours could be the nature of the sample populations.

The present study was a cross-sectional study of healthy children and not a retrospective study of radiographs archived in a hospital.

Several reasons have been adduced for the overestimation seen with the Original Demirjian method. Liversidge (2012) argued that the method was devised to measure biological maturity where chronological age and dental maturity were expressed as a sigmoid curve. This curve excludes very young children and it also applies to individual teeth and not the sequence of development. Furthermore, Demirjian's work was based on a mixed longitudinal sample. It is likely that an individual in a longitudinal sample is seen to enter a developmental stage earlier than what is observed in a cross-sectional sample (Carneiro et al. 2015). Apart from these reasons, other sources of discrepancy may be due to population differences in terms of genetics, geography and climatic, sample size and methods of analysis (Wolf et al. 2016). Demirjian et al. (1973)cautioned although the maturity scoring system is probably universal in application, the conversation to dental age or the location of centiles for maturity at given ages depends on the population. This illustrates their recognition that the conversion to dental age is less accurate when applied to different population.is Despite these shortcomings, the Original Demirjian method remains one of the most widely used method for assessment of dental maturity (Koshy and Tandon 1998; Bagherpour et al. 2010). In general, the overestimation found in most studies using the Original Demirjian method led several authors to argue for population-specific maturity curves (Willems et al. 2001; Tunc and Koyuturk 2008; Chen et al. 2010; Baghdadi and Pani 2012; Erdem et al. 2013; Uys et al. 2014).

4.4.2 Modified Demirjian method

The Modified method uses a different scoring system, based on an extended age range of participants. Additional formation stages were included, namely stage A of the first premolar and stage C of the central incisor. The present study found statistically significant overestimation of the chronological age in both males and females when applying the Modified Demirjian method. On average, an overestimation of 0.9 years and 1.2 years was found in males and females respectfully. However, the mean absolute error was 1.1 years for males and 1.4 years for females. The reason for the overestimation for Black Southern African children may be due to advanced dental maturity in those children compared to the French-Canadian sample used to develop the dental maturity score. Several studies have shown African children to be dentally advanced compared to their European and Asian counterparts. The greater overestimation of chronological age of females compared to males may be due to advanced dental maturity found in the females. Our results confirm those of other studies that used the Modified Demirjian method (Willems et al. 2001; Lee et al. 2011; Flood et al. 2013; Akkaya et al. 2015) and found overestimation of the chronological age. Prior to this study the Modified Demirjian method has not been validated in a Southern African population. The present study found that the method is unsuitable for age estimation and for forensic investigations involving Black Southern African children.

4.3.3 The Willems method

Willems et al. (2001) proposed a new age estimation method after observing overestimation with the Original Demirjian method in a Belgium sample. The method is simple and does not use maturity tables, so no conversions of scores into age estimates need to be performed. The adapted method was validated and resulted in more accurate dental age estimations in the Belgian population (Willems et al. 2001). Other researchers confirmed the accuracy of this method in their respective populations from Asia, Europe and North Africa (Mani et al. 2008; El-

Bakary et al. 2010; Nik-Hussein et al. 2011; Urzel and Bruzek 2013; Djukic et al. 2013; Ye et al. 2014; Ambarkova et al. 2014; Hegde et al. 2016).

The present study found no significant overestimation or underestimation for Black South African children in seven of the age cohorts in males and another seven of the age cohorts in females. This reflects the accuracy of this method in estimating dental age and hence it may be used for clinical and forensic purposes among Black South African children.

The present study is in agreement with the studies listed above. Although a significant difference was found with a one sample t-test, a reduction was seen in overestimation of the chronological age of males and females compared to the two Demirjian methods. The mean overestimation found in this study was 0.2 and 0.3 years for males and females respectively. Again, the similar pattern of higher overestimation of the chronological age in the females compared to the males as with other methods may be due to advanced dental maturity in females. Most of the mean differences were lower than 0.5 years in the age cohorts investigated except for the earlier years in males. This is similar to the findings of a study conducted with Bangladeshi and British children (Maber et al. 2006). The accuracy expressed as mean absolute error for the Willems method was 0.7 years and 0.68 for males and females respectively. Akkaya et al. (2015) found a good accuracy of the Willem method and proposed that it could be used for forensic purposes among Turkish children. However, a study conducted in China by Zhai et al. (2016) found Willems to have high absolute mean errors and overestimation of age by over 1.0 years.

The better accuracy of Willems' method may be due to temporal differences. Willems' method was published more than twenty-five years after Demirjian's method and secular trends in dental maturation due to food, immobility, and exposure to sunlight (Liversidge et al. 2006; Altan et al. 2016) may be factors to consider. Further studies are needed to test this assertion.

4.4.4 Comparison between the three methods

For a measurement to be considered accurate in forensic anthropology, the mean difference between the predicted and actual measures should be within ±1 year (Chaillet et al. 2004) although others specify the acceptable value as ±0.5 years (McKenna et al. 2002; Flood et al. 2011). This study found that the Willems method has better accuracy than either of the Demirjian seven tooth methods. The mean difference was about 1 month for males and 3 months for females. This is very low compared to the results from the Demirjian methods. Our finding is supported by a systematic review comparing the methods (Chapter three of this thesis) and other studies on different populations (Willems et al. 2001; El-Bakary et al. 2010; Flood et al. 2013; Urzel and Bruzek 2013; Ambarkova et al. 2014; Akkaya et al. 2015; Ye et al. 2014). Although the Willems method was derived from a sample of Belgian children, it provides better accuracy and is better at age estimation than the Demirjian methods. Willems' method can be used in age estimation for males and females from Southern Africa due to the low mean absolute error obtained in the present study.

4.5 Conclusion

Willems' method could be used for anthropological and forensic purposes in Black Southern African children. Although significant overestimation of chronological age was observed with this method in only four age groups out of eleven in males and another 4 age groups in the females, the level of overestimation is within the acceptable limit for forensic purposes. The two Demirjian methods significantly overestimate chronological age in addition to very high mean absolute errors. Hence the two methods should not be used for age estimation in Black Southern African populations.

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Contents

Chapter 5

Effect of nutrition on tooth formation

Abstract

Background: There is abundant evidence that systemic stresses during the period of tooth formation lead to enamel hypoplasia and molar incisor hypomineralization. However, the role of nutritional status in delaying or accelerating tooth formation is not well understood.

Aim: This study investigated the effect of nutrition (measured as BMI, height, weight, mid-upper arm circumference and head circumference) on permanent tooth formation in a sample of Black Southern African children.

Method: The study design was a cross-sectional investigation involving 642 (270 males, 372 females) healthy children aged 5-20 years. The height and BMI were converted to z-scores using the WHO z-scores for age tables (WHO 1995). A cut-off z-score of <-2 for both BMI and Height for age (HAZ) was used to place children into the categories of underweight/short for age, normal weight/height, and overweight/obese/tall for age. Panoramic radiographs were analysed using eight (A-H) stages of tooth formation and the dental maturity score of the mandibular left permanent teeth was obtained according to the Demirjian et al. (1973) method. The dental maturity score of each child was converted to dental age using standard tables and percentiles curves for both boys and girls (Demirjian et al. 1973). The mean age of attainment of the H stage of tooth formation was calculated using probit regression analysis. Analysis of variance (ANOVA) and Student's *t*-tests were used where appropriate to determine if any differences exist between the subdivisions of the data.

Results: Games-Howell multiple comparisons of means showed significant advancement in the age of attainment of the H stage for all the permanent teeth in the overweight group compared to

the underweight group ($p<0.05$). Negative binomial regression analysis indicated that age, height, and BMI were significant predictors of the dental maturity score for males ($p<0.05$), while age, height, weight, BMI and also HC were significant predictors of the dental maturity score for females.

Conclusion: This study demonstrates that nutritional status does have a significant effect on the timing of tooth formation in males and females. The effect was mainly noticeable for children in the extremes of the spectrum of the BMI z-scores.

5.1 Introduction

Tooth formation is regarded as highly heritable and immune from environmental stresses relative to other aspects of growth and development (Demirjian 1986; Demirjian et al. 1985). The timing of tooth formation and emergence were found to be highly correlated within monozygotic twins compared to dizygotic twins, suggesting a heritability of 0.85-0.90 (Garn et al. 1960). Tooth size and morphology are also considered to be largely determined by the genome. This assumption is the primary basis for modern dental anthropological research that uses dental metrics and non-metric trait distributions to assess population relationships (Hillson 2014). In a study of Australian Aboriginal tooth dimensions, Townsend and Brown (1978) found a heritability of 64%, with a further 6% attributed to common environment. Furthermore, some authors (Krogman 1967; Towne et al. 2006) suggested molar cusp number and fissure patterns are under genetic control, although Biggerstaff (1975) argued for only a small component of hereditary variability for those same features. Garn and Bailey (1978) showed that environmental factors such as socioeconomic status may be partly responsible for variation in tooth morphology. Although genetic effects are undoubtedly important throughout dental development, environmental factors cannot be neglected, and the extent of their influence is still under discussion.

There is abundant evidence that systemic stresses during the period of tooth formation lead to enamel hypoplasia and molar incisor hypomineralization (Goodman et al. 1980). The severity of enamel hypoplasia is dose and time dependent. Populations that are exposed to a high degree of undernutrition and disease, from prehistoric to contemporary times, have higher rates of linear enamel hypoplasia (Goodman et al. 1980; Goodman and Rose 1990). Thus, it could be argued that if an extreme stressor leads to a complete disruption of the secretory phase of ameloblasts and results in a quantitative tooth defect, then lower stressors should limit the secretory ability of ameloblasts leading to a delay in the attainment of a phase. However, the specific relationship of these stressors to changes in the process of tooth formation is not known.

Malnutrition is a common stressor affecting growth and development. It can result in stunting and delayed maturity in situations of nutritional shortages, or advanced sexual and skeletal maturity in cases of obesity. The effect of nutrition on tooth formation remains controversial, with some studies finding no effect (Eid et al. 2002; Cameriere et al. 2007; Elamin and Liversidge 2013) while others observed a delay in formation (Hilgers et al. 2006; Mani et al. 2008). Well-designed studies on severely malnourished children are lacking and may be difficult to undertake because of ethical reasons. Previous studies focused on selected proxies of nutritional status (height, weight and body mass index (BMI) and tooth formation, but none investigated head circumference and mid-upper arm circumference as proxies of nutritional status. Hence, this study investigates the influence of nutritional status on tooth formation using these measures that are documented to correlate with nutritional stress.

5.1.1 Effect of body mass index (BMI) on tooth formation

There is no consensus on the effect of BMI on tooth formation. Studies utilizing BMI as a proxy for nutritional status from Peru, Brazil and Southern Sudan found no significant influence of BMI on tooth formation (Eid et al. 2002; Cameriere et al. 2007; Bagherian and Sadeghi 2011; Elamin and Liversidge 2013). In contrast, other studies (Hilgers et al. 2006; Zangouei-Booshehri et al. 2011; Mack et al. 2013; DuPlessis et al. 2016) showed that the timing of tooth formation was significantly accelerated with higher BMI, even after adjusting for age and sex. This developmental advancement was attributed to accelerated linear growth and early sexual maturation, which is usually associated with obesity (Slyper 1998; Sánchez-Pérez et al. 2010)).

One reason for the controversy over BMI may be the imprecision of the measures of nutritional status (Frisancho 1990; Myatt et al. 2009). For example, standardized BMI in children varies with body shape (Garn et al. 1986) and ethnicity (Lear et al. 2007), and may overestimate the prevalence of acute malnutrition in some populations (Garn et al. 1986; Frisancho 1990; Lin et al. 2004). BMI is poor in sensitivity and specificity, and does not necessarily reflect the changes that occur with age (Rothman 2008). Additionally, the relationship between BMI and percentage of body fat is not linear and differs for males and females (Rothman 2008).

Although the BMI cutoff point for malnourished children is still being debated, it is suggested that to be able to identify high-risk malnourished children, the cut-off point of standardized BMI should not be less than –3 (Briend et al. 2012). Frisancho (1990) reported that the assessment of nutritional status based only on BMI, especially when the degree of undernutrition or overweight is moderate, is unlikely to be effective in distinguishing the truly wasted or truly overweight individual from a normal or low weight child. This may have influenced the outcome of previous studies that used the cutoff point of -2 to categorize the study population into two groups without exploring the extremes of the BMI z-scores.

The method of analysis might also be responsible for conflicting results regarding the role of BMI in tooth formation variation. Eid et al. (2002), utilizing the extremes of BMI z-scores, generated mean ages of attainment and used correlational analysis to determine the relationship between timing of tooth formation and nutritional status. Growth curves are not always linear since acceleration, deceleration and stunting occur at different stages resulting in great variation in growth velocity (Pinhasi and Mays 2008), Thus, conclusions should not be solely derived from a linear perspective. In addition, the use of bivariate analysis may not account for potential confounders, especially in growth studies. One of the limitations of bivariate analysis is that it does not factor in how variables influence each other. Bivariate analysis therefore cannot give an explanation for the relationship between two variables; it only provides a description. Explanatory analysis is needed to infer cause (Spicer 2005). In summary, tooth formation studies utilizing at least one or both extremes of BMI classification and, in addition, employing robust and explanatory statistics, are needed.

5.1.2 Influence of height and weight on tooth formation

The relationship of height and weight with permanent tooth formation is complex. Short height for age reflects a failure of linear growth due to diseases or malnutrition (WHO 1997). Studies on permanent tooth emergence found a relationship with the height of an individual (Filipsson and Hall 1975; Green 1961; Kutesa et al. 2013). Vallejo-Bolanos and Espana-Lopez (1997) found a delay in tooth emergence in familial short stature children, while Keller et al. (1970) found similar results among children with metabolic and endocrine disorders. All these findings point to a relationship between growth hormones and tooth formation and emergence. Malnutrition is a known cause of growth hormone deficiency. Interestingly, Sarnat et al. (1988) did not find accelerated tooth emergence, but only enhanced bone development, in children being treated with growth hormones. Similarly, Takano et al. (1986) found an insignificant delay in the dental age but a significant delay in the skeletal age of children with growth hormone deficiency compared to normal children.

Body weight is also one of the most important variables for nutritional assessment. It is very useful in predicting macronutrient and fluid requirements and also acute malnutrition (ADSA 2009). Its limitation is that it is poor for distinguishing between fat, protein, bone and water levels. It can also be influenced by fluid status, organomegaly and tumor growth. Currently, there is no information on the relationship between weight and permanent tooth formation, although some studies on tooth emergence found a positive relationship (Garn et al. 1965; Billewicz and McGregor 1975), and others found weight and emergence to be uncorrelated (Khan et al. 2006; Kutesa et al. 2013).

5.1.3 Mid-upper arm circumference (MUAC) and tooth development

MUAC is a better index of nutritional status or malnutrition than BMI in children and adolescents (Jelliffe and Jelliffe 1969; Shakir 1975; Velzeboer et al. 1983; WHO 1986; Briend et al. 1986; Fernandez et al. 2010). It is less affected by the localized accumulation of excess fluid (pedal edema, periorbital edema, ascites) commonly seen in famine. MUAC is likely to be a more sensitive index of tissue atrophy than low body weight and it is relatively independent of height (Olukoya, 1990). MUAC correlates well with BMI in adult populations, yet a globally recognized cut-off point has not been established to classify malnutrition among adolescents and adults (Chakraborty et al. 2009; Mazıcıoğlu et al. 2010). Though many countries have established cut-off points for their populations, there is no evidence to support using any particular value. There is no study on the relationship between MUAC and tooth formation, hence there is a need to investigate the efficacy of MUAC versus BMI when comparing somatic growth with tooth formation

5.1.4 Head circumference (HC) and tooth formation

Is there a link between tooth formation and brain development? In a study carried out by Godfrey et al. (2001) on a large range of living primates, it was found that brain development was a better predictor of dental development than somatic development. The correspondence of head circumference and the emergence of deciduous teeth has been investigated in humans. Vejdani et al. (2015) found a relationship between primary tooth emergence and head circumference. An earlier study also demonstrated significant associations between the number of primary teeth present and head circumference in males but not in females (Infante and Owen 1973). These studies demonstrate that a relationship exists between brain development and primary tooth development in the very young. Head circumference is a proven surrogate for brain development and it is proportional to brain weight and volume in infants and children. Hence, head circumference helps to monitor cognitive function in post-natal brain growth (Thureen 2012). Its usefulness as a measure of nutrition is however limited. It is poorly sensitive to malnutrition since the brain is spared at marginal calorie intakes that do not support skeletal growth and weight gain. Therefore, it is important to investigate if a relationship exists between head circumference and permanent tooth formation, particularly in the early years of tooth formation.

5.2 Methods

This is a cross-sectional study of 642 clinically healthy Black Southern African children aged 5-20 years, whose parents and grandparents are indigenous Southern Africans. The participants were selected randomly from primary and secondary schools in Johannesburg during the operation of the Community Oral Health Outreach Program (COHOP) of the Department of Community Dentistry of the School of Oral Health Sciences, University of the Witwatersrand. Children with systemic diseases, mandibular hypodontia (except third molars), and those who had lost teeth on both sides of the mandible were excluded. Ethical approval (NO. M141001) was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. Permission to carry out the study was obtained from the local education authority and respective school heads. Consent was obtained from parents while assent was obtained from the children.

5.2.1 Sample size

A total of 14 one-year age cohorts (except for a combined 18-20year cohort) were sampled from ages 5-20 years. The sample size formula is N = $4z_{\alpha}^{2}S^{2}-W^{2}$, where S= standard deviation, W= desired total width and z_{α} is the standard normal deviate for the 95% confidence level. Cameriere et al. (2008), in a similar radiographic study, found a mean of 1.076 and a standard deviation of 0.824 derived from a standard error of 0.030. Using a width of 0.2, the minimum total sample size required for the 14 cohorts is 280. However, to improve the power of the study, a total of 642 children were recruited.

Information collected from the school records included the date of birth and sex. All selected participants were examined on a dental chair in a mobile dental van. The intraoral examination was done with a sterile wooden spatula. Teeth present in the mouth were recorded using Fédération Dentaire Internationale (FDI) notation. Panoramic radiographs were taken and those showing gross pathology or poor image quality were excluded.

Participants were weighed in the standing position on a platform scale (Hana Power), calibrated to a precision of 100g. Height was measured with an anthropometric stadiometer (Weylux model 424). The height was recorded to the nearest 0.1 cm. Mid-upper arm circumference (MUAC) of the left upper arm was obtained with a tape measure at the midpoint between the tip of the shoulder and the tip of the elbow and recorded (to the nearest 0.1 cm). The head circumference (HC) was measured by placing a tape measure (in cm) across the forehead and the greatest circumference was recorded. The study was pilot tested on 40 randomly selected students. Intra-examiner reliability of measurement was calculated using Lin's Concordance Correlation Coefficient (height = 0.99, HC= 0.92, MUAC= 0.96).

5.2.2 Pilot test

Prior to data collection, a reliability study was conducted to assess the magnitude of the intra-observer error. Firstly, two trained examiners assessed the maturation stage of the 7 left mandibular permanent teeth without the knowledge of chronological age or sex. To evaluate reproducibility, 25 radiographs (with 175 individual tooth ratings) were randomly selected and assessed by both examiners at day one and day three. The investigator was the only rater for the developmental stages of the teeth. Intra-examiner reliability of dental age assessment for the Demirjian et al. (1973) method was calculated using Cohen's Kappa (Landis and Koch 1977) and was found to be 0.97.

5.2.3 Dental maturity score and age assessment

Dental age assessment was performed according to the original version of Demirjian's method (Demirjian et al. 1973). The investigator did not have access to the chronological age of the participants. The panoramic radiographs of each child were enhanced using Microsoft Office Picture Manager, labeled with a unique identity number and digitally archived. Each radiograph was assessed for the development of the left 7 permanent mandibular teeth rated on an 8-stage scale from A to H, based on the stages of tooth formation proposed by Demirjian et al. (1973) with stage 0 for non-appearance. Each stage was allocated a sex-specific biologically weighted score and the sum of the scores for each participant was used to determine the dental maturity measured on a scale of 0 to 100. The dental maturity score of each child was converted to dental age using standard tables and percentiles curves for both sexes (Demirjian et al. 1973). The mean age of attainment of the H stage of tooth formation was calculated using probit regression analysis (Hayes and Mantel 1958).

5.2.4 Data analysis

The data were analyzed with Stata 12 for Windows. The analyses included frequencies and cross-tabulations. Association between categorical variables was tested with chi-square while those between continuous variables were tested with the Student's t-test. Non-parametric equivalents were used as appropriate. Body mass index (BMI) was calculated from the height in meters and weight in kilograms. The height and BMI were converted to z-scores using the WHO z-scores for age tables (WHO 1995). A cut-off z-score of <−2 for both BMI and Height for age (HAZ) was used to place children into the categories of <-2 underweight/short for age, ≥-2 to 2.0 for normal weight/height, and ≥2 for overweight/obese/tall for age.

The mean age at the time of attainment of tooth formation stages and standard deviations were computed using probit analysis after Liversidge (2003). Analysis of variance (ANOVA) was used to determine if any variation exists between the subdivisions of the data. Games-Howell multiple comparisons of means were used to compare the mean age of attainment of H status for the nutritional status subgroups with one another. Student's *t*-tests were used where necessary to compare the mean age of attainment of the H stage.

Since there are no WHO standard categories of mid-upper arm circumference or head circumference for older child age cohorts, they were modeled with other predictor variables in a regression analysis. A Shapiro-Wilk test showed that the dependent variable (maturity score) and the predictor variables were not normally distributed. Therefore, a negative binomial regression model was used with the maturity scores modeled as the dependent variable and the anthropometric variables as predictors. Adequacy of fit was checked using the deviance residuals as recommended by McCullagh and Nelder (1989). The deviance residuals showed normal distribution and the plot of the residuals against each of the covariates also showed model fit. As expected, the collinearity test showed that BMI, height, and weight were significantly collinear. When these variables were excluded from the model, there was no difference in the values of the output. Hence, the variables were included in the final model for generalized linear regression analysis. The model was built using forward selection. Statistical significance was inferred at $p<0.05$.

5.3 Results

5.3.1 Comparison of mean values of anthropometric variables and nutritional status

The age and sex distribution of the sample are shown in Tables 5.1 and 5.2. Only two females (0.006%) are categorized as underweight while 22 (8.1%) of males fall in the underweight category.

Table 5.1. BMI and height distribution of males

Age cohort	BMI z-scores category			Total 100%	Height z-scores category			Total 100%
	Under weight n(%)	Normal n(%)	Over Weight n(%)		Short for age n(%)	Normal n(%)	Tall n(%)	
5 – 5.99	0(0.0)	10 (100.0)	0(0.0)	10	0(0.0)	10(100.0)	0(0.0)	10
6 – 6.99	0(0.0)	24 (92.3)	2(7.7)	26	1(3.8)	25(96.2)	0(0.0)	26
7 – 7.99	0(0.0)	6 (100)	0(0.0)	6	1(26.7)	5(83.3)	0(0.0)	6
8 – 8.99	2(6.1)	25 (75.8)	6(18.1)	33	1(3.0)	32(97.0)	0(0.0)	33
9 – 9.99	1(2.8)	30 (83.3)	5(13.9)	36	0(0.0)	35(97.2)	1(0.8)	36
10 – 10.99	0(0.0)	16 (100.0)	0(0.0)	16	2(13.5)	14(87.5)	0(0.0)	16
11 – 11.99	0(0.0)	16 (94.1)	1(5.9)	17	2(11.8)	15(88.2)	0(0.0)	17
12 – 12.99	1(4.8)	19 (90.4)	1(4.8)	21	5(23.8)	16(76.2)	0(0.0)	21
13 – 13.99	2(13.3)	12 (80.0)	1(6.7)	15	4(26.7)	11(73.3)	0(0.0)	15
14 – 14.99	6(21.4)	18(64.3)	4(14.3)	28	6(21.4)	22(78.6)	0(0.0)	28
15 – 15.99	4(16.0))	20(80.0)	1(4.0)	25	4(16.0)	21(84.0)	0(0.0)	25
16 – 16.99	2(16.7)	9(75.0)	1(8.3)	12	1(8.3)	11(91.7)	0(0.0)	12
17 – 17.99	3(15.0)	16(80.0)	1(5.0)	20	3(15.0)	17(85)	0(0.0)	20
18 – 20.00	1(20.0)	4(80.0)	0(0.0)	5	2(40.0)	3(60.0)	0(0.0)	5
Total	**22((8.1)**	**225(83.3)**	**23(8.6)**	**270**	**32(11.9)**	**237(87.8)**	**1(0.3)**	**270**

Table 5.2. BMI and height distribution of females

Age cohort	BMI z-scores category			Total 100%	Height z-scores category			Total 100%
	Under weight n(%)	**Normal** n(%)	**Over Weight** n(%)		**Short for age** n(%)	**Normal** n(%)	**Tall** n(%)	
5 – 5.99	0 (0.0)	13(100.0)	0(0.0)	13	0(0.0)	13(100.0	0(0.0)	13
6 – 6.99	0(0.0)	26(92.9)	2(7.1)	28	1(3.6)	27(96.4)	0(0.0)	28
7 – 7.99	0(0.0)	8(88.9)	1(11,1)	9	1(11.1)	7(77.8)	1(11.1	9
8 – 8.99	0(0.0)	26(96.3)	1(3.7)	27	1(3.7)	24(88.9)	2(7.4)	27
9 – 9.99	0(0.0)	27(90.0)	3(10.0)	30	2(6.7)	28(93.3)	0(0.0)	30
10 – 10.99	0(0.0)	21(95.4)	1(4.6)	22	3(13.6)	19(86.4)	0(0.0)	22
11 – 11.99	192.8)	32(88.9)	3(8.3)	36	1(2.8)	35(97.2)	0(0.0)	36
12 – 12.99	0(0.0)	36(81.8)	8(18.2)	44	2(4.5)	42(95.5)	0(0.0)	44
13 – 13.99	0(0.0)	36(92.3)	3(7.7)	39	0(0.0)	39(100.0)	0(0.0)	39
14 – 14.99	0(0.0)	30(93.8)	2(6.2)	32	1(3.1)	31(96.9)	0(0.0)	32
15 – 15.99	0(0.0)	20(74.1)	7(25.9)	27	0(0.0)	27(100)	0(0.0)	27
16 – 16.99	0(0.0)	22(78.6)	6(21.4)	28	1(5.6)	27(96.4)	0(0.0)	28
17 – 17.99	1(4.0)	18(72.0)	6(24.0)	25	3(22.0)	22(88.0)	0(0.0)	25
18 – 20.00	0(0.0)	10(83.3)	2(16.7)	12	3(25.0)	9(75.0)	0(0.0)	12
Total	**2(0.5)**	**325(87.4)**	**45(12.1)**	**372**	**19(5.1)**	**350(94.1)**	**3(0.8)**	**372**

Table 5.3 provides the mean values for the anthropometric variables. The mean age and height of the underweight group are significantly greater than the normal and overweight groups for both males and females ($p=0.000$). In contrast, weight, BMI and MUAC values increase from the underweight children to the overweight ($p<0.05$). Similarly, the z-scores for height and BMI significantly increase from the underweight to the overweight children ($p<0.05$). In contrast, there is no significant increase in the mean head circumference from the underweight children to the overweight children ($p>0.05$).

Table 5.3. Anthropometric variables by BMI z-score categories in males and females

	MALES (N=270)							FEMALES (N=372)						
Variable	**Underweight (N=22)**		**Normal (N=225)**		**Overweight (N=23)**		**p**	**Underweight (N=2)**		**Normal (N=325)**		**Overweight (N=45)**		**p**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Age	14.40	2.79	11.40	3.70	11.03	3.45	0.00	14.92	4.24	11.99	3.52	13.75	3.17	0.00
Height	153.63	15.70	140.68	19.33	139.47	16.98	0.01	153.25	25.81	143.26	16.36	149.89	12.86	0.03
Weight	34.90	9.72	35.14	13.66	46.24	18.46	0.00	27.00	19.80	38.44	13.41	63.93	16.16	0.00
BMI	14.41	1.50	17.02	2.43	22.90	3.45	0.00	10.54	4.81	18.03	3.18	27.94	4.15	0.00
MUAC	18.11	1.96	18.60	1.86	20.59	3.04	0.00	19.35	0.92	19.14	1.81	23.29	4.07	0.00
HC	50.11	2.15	50.67	2.46	51.30	3.22	**0.28**	51.40	1.27	50.28	2.58	50.77	2.07	**0.48**
Height z-score	1.72	0.46	1.90	0.31	1.87	0.34	0.05	-2.39	0.29	-0.39	0.83	2.52	0.15	0.00
BMI z-score	-2.48	0.30	-0.31	0.95	3.12	1.03	0.00	-3.47	1.10	-0.04	0.92	3.43	1.18	0.00

Non-significant values in red.

5.3.2 Age of attainment of H stage by nutritional status

In males, age of attainment of the H developmental stage was calculated for each of the BMI z-score (underweight, normal, and overweight) categories using probit analysis after dichotomizing the appearance of the H developmental stage as present or absent. Other developmental stages were not considered because of the small sample size of the underweight group (Table 5.4). Analysis of variance (ANOVA) shows significant variations in the means of the BMI z-score categories for all the teeth except the lateral incisors among males (Table 5.4). Further analysis using Games-Howell multiple comparisons of means reveals significant advancement in the age of attainment of H stage for all the permanent teeth in the overweight group compared to the underweight group ($p<0.05$) (Table 5.4).

Table 5.4. Analysis of variance comparison of age of attainment of H stage by BMI z-score category in males

Tooth type	**BMI z-score**						**F**	**p**
	Underweight (N=22)		**Normal (N=225)**		**Overweight (N=23)**			
	Mean age	**SD**	**Mean age**	**SD**	**Mean age**	**SD**		
I2	9.05	0.66	8.65	1.33	8.38	0.85	1.69	0.196
C1	13.06	0.48	12.64	0.77	12.54	1.08	3.22	**0.042**
P1	14.17	1.69	12.86	0.91	11.17	0.89	52.34	**0.000**
P2	14.87	0.81	13.63	1.11	13.37	1.37	13.59	**0.000**
M1	9.05	0.66	8.73	1.06	8.16	0.17	4.90	**0.008**
M2	15.39	0.62	14.83	0.79	14.77	0.41	5.76	**0.004**

Significant values in bold.

For females, probit analysis was used to calculate the mean age of attainment of the H developmental stage in the normal and overweight groups only, due to very few participants in the underweight subgroup. The mean ages of attainment of H stage of all the teeth, except for the canine, were significantly advanced in the overweight group compared to the normal BMI group ($p<0.05$) (Table 5.5).

Table 5.5. Comparison of age of attainment of the dental H stage by BMI z-score groups in females*

Tooth type	**BMI z score**				**t**	**p**
	Normal (N=325)		**Overweight (N=45)**			
	Mean age	**SD**	**Mean age**	**SD**		
I2	8.44	1.14	6.58	1.16	10.24	**0.00**
C1	11.59	1.23	11.46	0.82	0.69	0.49
P1	12.21	1.30	11.75	1.72	2.13	**0.03**
P2	12.58	1.09	11.99	0.50	3.58	**0.00**
M1	8.12	1.26	6.58	1.27	7.68	**0.00**
M2	14.11	1.14	13.07	0.53	6.02	**0.00**

*The 2 females categorized as underweight were removed from the analysis.
Significant values in bold.

5.3.3 Age of attainment of the H stage by height for age (HAZ)

The height for age (z-scores for height or HAZ) was dichotomized into “short for age” and “normal” groups in the males. The only male in the tall category was removed from the analysis. The age of attainment of the H developmental stage was calculated using probit regression analysis and the mean ages of attainment of H stage were compared using Student’s *t*-tests. Significant differences ($p<0.05$) are found for the canine and the second molar in males (Table 5.6). In comparison, a significant difference ($p<0.05$) is only found between the mean ages of attainment of H stage of the first molars in females (Table 5.7).

Table 5.6. Comparison of age of attainment by height for age (HAZ) groups in males*

Tooth type	HAZ				t	p
	Short for age (N=22)		Normal (N=237)			
	Mean age	SD	Mean age	SD		
I2	-	-	-	-	-	-
C1	13.30	1.10	12.89	0.84	1.88	**0.03**
P1	13.27	1.96	12.90	1.06	1.64	0.15
P2	14.08	0.81	13.89	1.19	0.88	0.19
M1	8.17	0.66	8.06	1.06	0.48	0.63
M2	15.17	0.24	14.84	0.84	2.21	**0.01**

*The only male in the tall category was not included in the analysis.
Significant values are in bold.

Table 5.7. Comparison of age of attainment by height for age (HAZ) groups in females*

Tooth type	HAZ				t	p
	Short for age (N=19)		Normal (N=350)			
	Mean age	SD	Mean age	SD		
I2	8.75	0.95	8.37	1.16	1.40	0.16
C1	11.95	0.44	11.63	1.23	1.13	0.26
P1	-	-	12.17	1.28	-	-
P2	-	-	12.91	1.05	-	-
M1	8.75	1.00	8.01	1.27	2.50	**0.01**
M2	14.17	-	14.05	1.11	-	-

*The three females in the tall category were not included in the analysis.
Probit analysis did not return values for the empty cells

5.3.4 Correlation analysis between maturity score and anthropometric variables

Spearman rho’s correlational analysis was done to determine if linear relationships exist between the predictor variables and the maturity score. A significant strong correlation is observed between maturity score and height (r=0.92) and weight (r=0.88) in males and height (r=0.86) and weight (r=0.83) in females. A significant moderate correlation is found between maturity score and MUAC and BMI in both males (MUAC r=0.63, BMI r=0.49) and females (MUAC r=0.60, BMI r=0.65). A significant but weak correlation is found between HC and the maturity score in males and females (Table 5.8).

Table 5.8. Spearman's rho correlation between maturity score and anthropometric variables

Variable	**Male (N=270)**		**Female (N=372)**	
	Maturity score		**Maturity score**	
	r	**p**	**r**	**p**
Age	0.96	0.00	0.94	0.00
Height	0.92	0.00	0.86	0.00
Weight	0.88	0.00	0.83	0.00
HC	0.19	0.00	0.34	0.00
MUAC	0.63	0.00	0.60	0.00
BMI	0.49	0.00	0.65	0.00

5.3.5 Relationship between maturity score and anthropometric variables

Multivariate analysis was done to determine the relationship between the predictor variables (age, height, weight, BMI, HC and MUAC) and the outcome variable (maturity score). Negative binomial regression analysis was the preferred model because it had the best fit compared to Poisson or generalized linear models. Modeling was done separately for males and females. As expected, the collinearity test showed that BMI, height and weight were significantly collinear. When these variables were excluded from the model, there was no difference in the values of the output and model did not fit. Hence, the variables were included in the final model for generalized linear regression analysis. The model was built using forward selection. The results show that age, height, and BMI are significant predictors of the dental maturity for males ($p<0.05$) (Table 5.9). Age, height, weight, BMI and also HC are significant predictors of the dental maturity score for females ($p<0.05$) (Table 5.10).

Table 5.9. Negative binomial regression model of predictors of dental maturity in males

Maturity score	Odds ratio	Std. err	z	p	[95% CI]
Age	1.49	0.04	16.94	**0.000**	1.42, 1.56
Height (cm)	1.03	0.01	4.24	**0.000**	1.02. 1.05
Weight (kg)	0.97	0.01	-1.87	0.062	0.94, 1.00
HC (cm)	1.01	0.01	1.32	0.188	0.99, 1.03
MUAC (cm)	0.99	0.02	-0.21	0.834	0.96, 1.03
BMI	1.06	0.03	2.17	**0.030**	1.01, 0.12
_cons	0.00	0.00	-7.30	0.000	0.00, 0.01

Significant values in bold.

Table 5.10. Negative binomial regression model of predictors of dental maturity in females

Maturity score	Odds ratio	Std. err	z	p	[95% CI]
Age	1.65	0.04	20.77	**0.000**	1.58, 1.73
Height (cm)	1.04	0.01	5.29	**0.000**	1.03, 1.06
Weight (kg)	0.96	0.01	-2.67	**0.008**	0.93, 0.99
HC (cm)	0.98	0.01	-2.21	**0.027**	0.97, 0.99
MUAC (cm)	0.98	0.02	-1.13	0.257	0.94, 1.02
BMI	1.08	0.03	2.65	**0.008**	1.02, 1.15
_cons	0.00	0.00	-7.00	0.000	0.00, 0.01

Significant values in bold.

5.3.6 Sexual dimorphism and dental maturity

Sexual dimorphism is seen in the dental maturity of the participants, with the females showing advanced dental maturity scores compared to males when controlled for age. The male-female difference in the dental maturity score increases at around age 9 until age 16, when the males catch up with the females (Figure 5.1). Similarly, females have higher maturity scores than males after controlling for height and weight (Figures 5.2 and 5.3). However, a gradual narrowing of the gap occurs as the weight of the children increases (Figure 5.3).

Figure 5.1. Maturity score of males and females while controlling for age

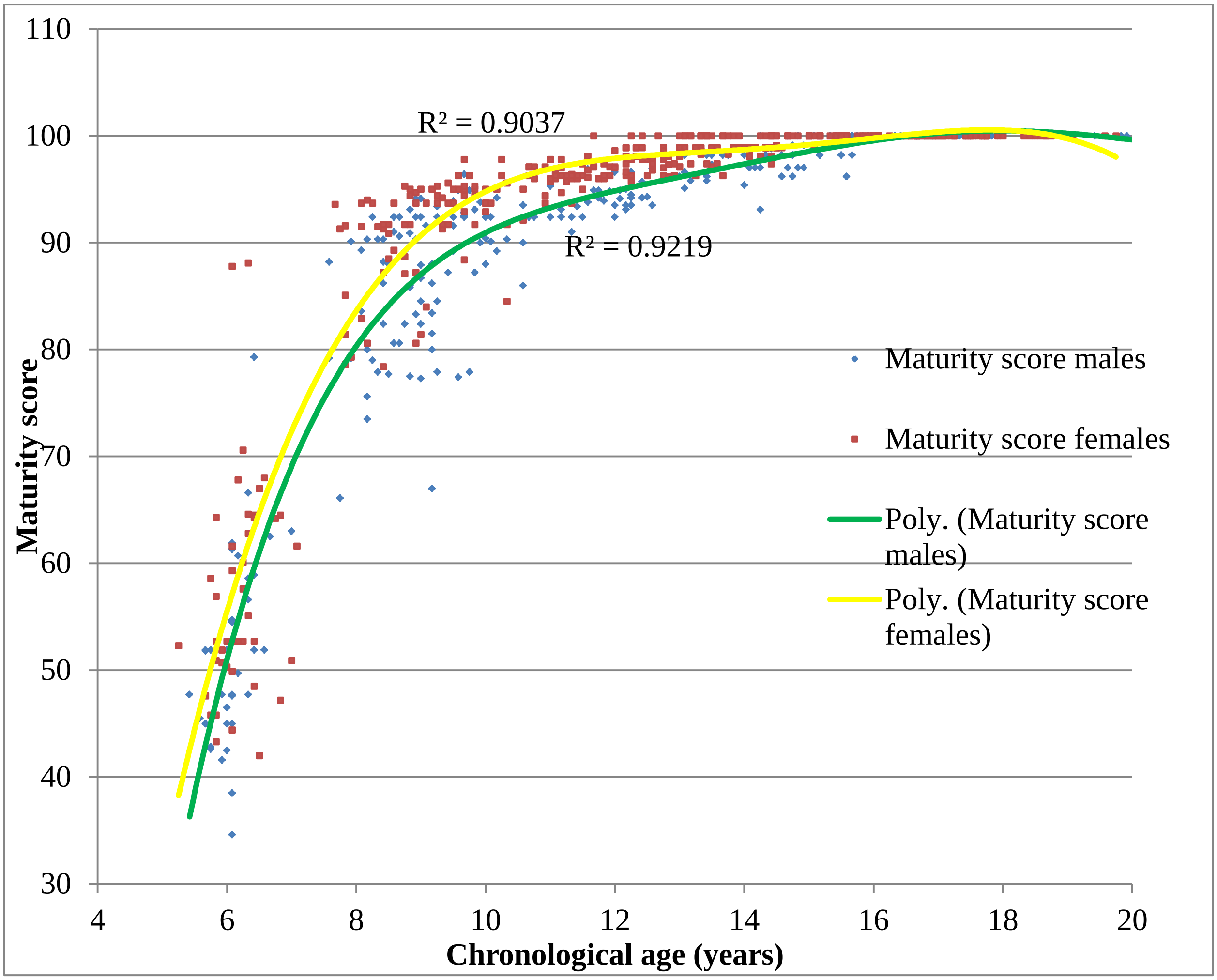


Figure 5.2. Maturity score by sex while controlling for height

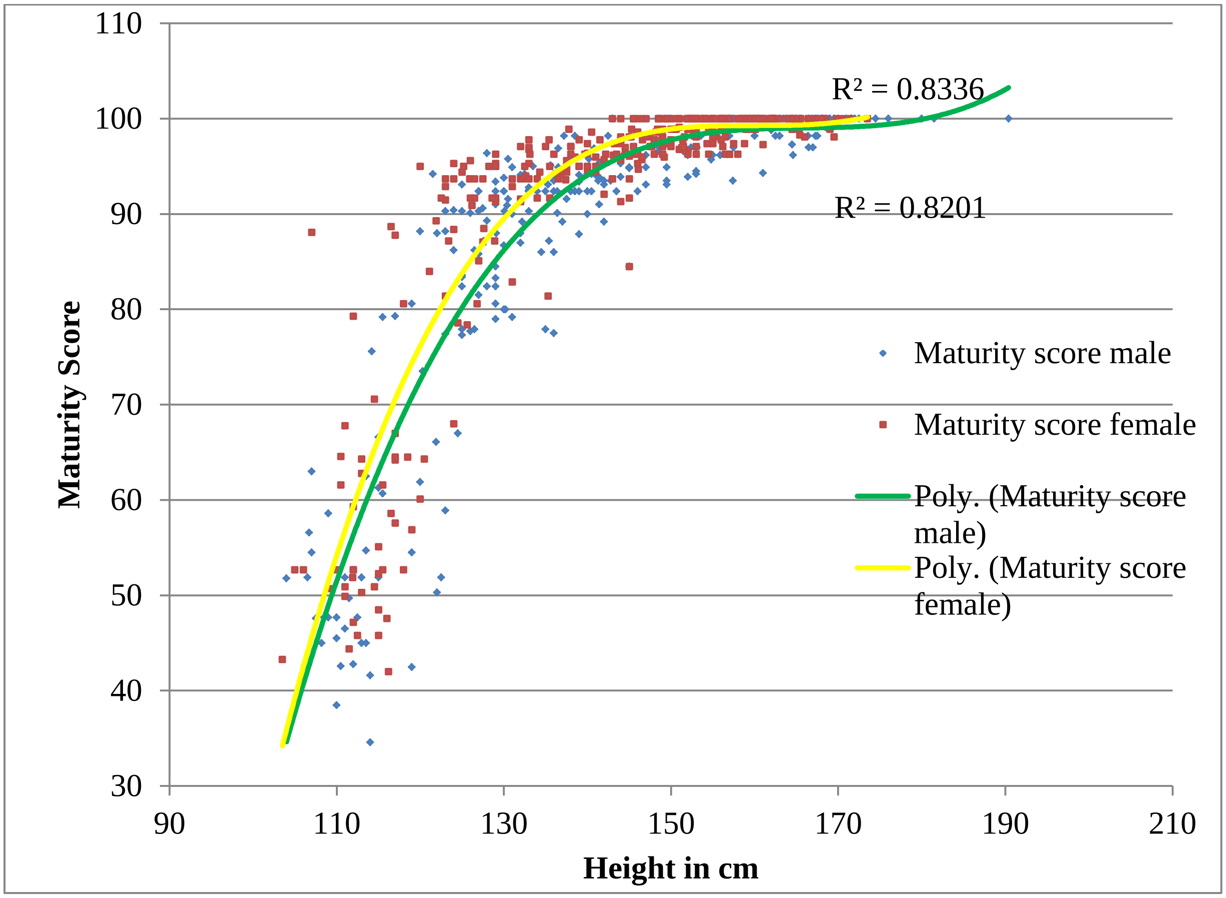
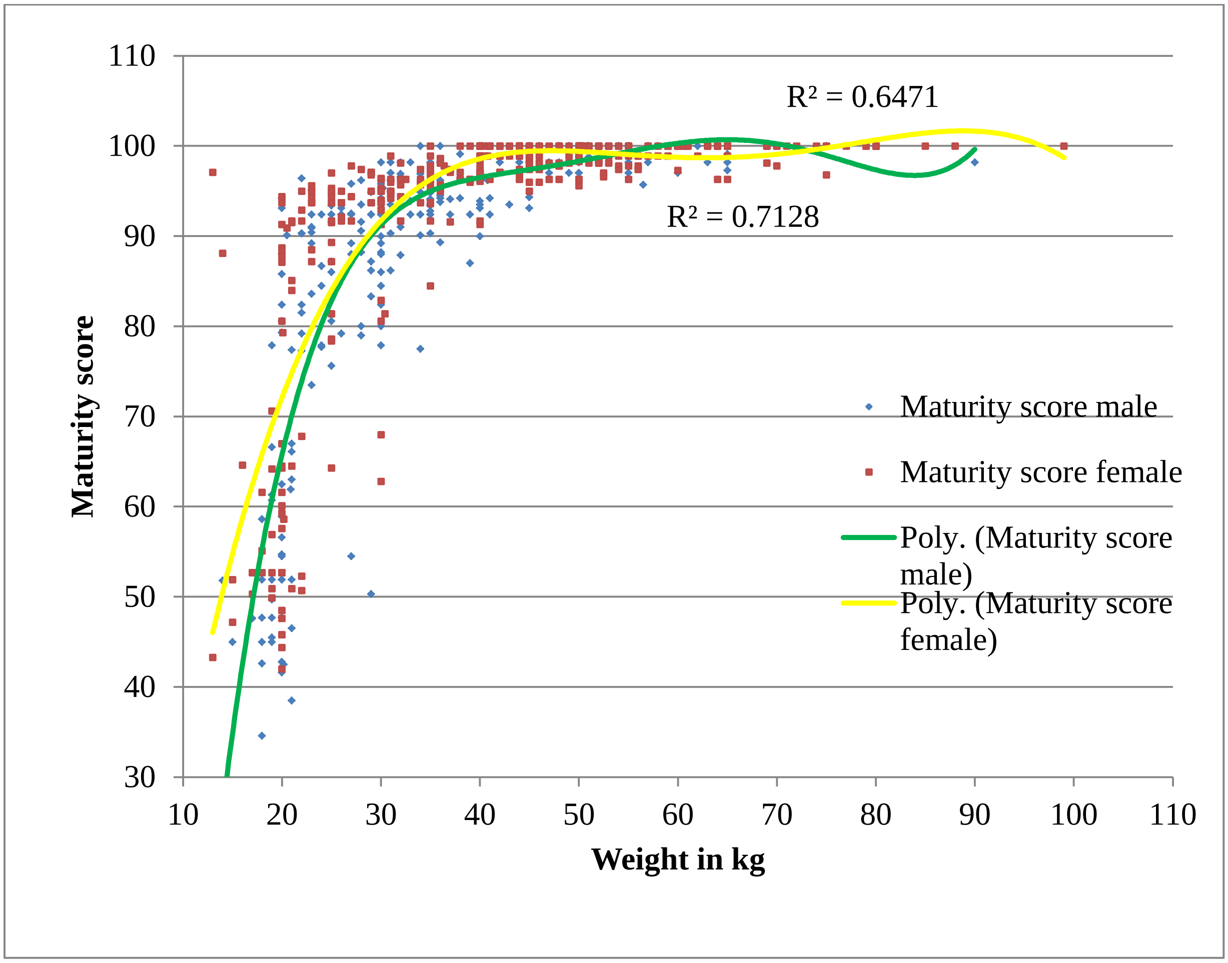


Figure 5.3. Maturity score by sex while controlling for the weight



Females show higher tooth maturity scores than males after controlling for the BMI (Figure 5.4), and at the same HC, females show advanced tooth maturity than their male counterparts (Figure 5.5). At lower values of the MUAC, females showed huge advancement in the maturity scores when compared to males. However, the gap narrows as MUAC in the children increases (Figure 5.6).

Figure 5.4. Maturity score by sex while controlling for the BMI

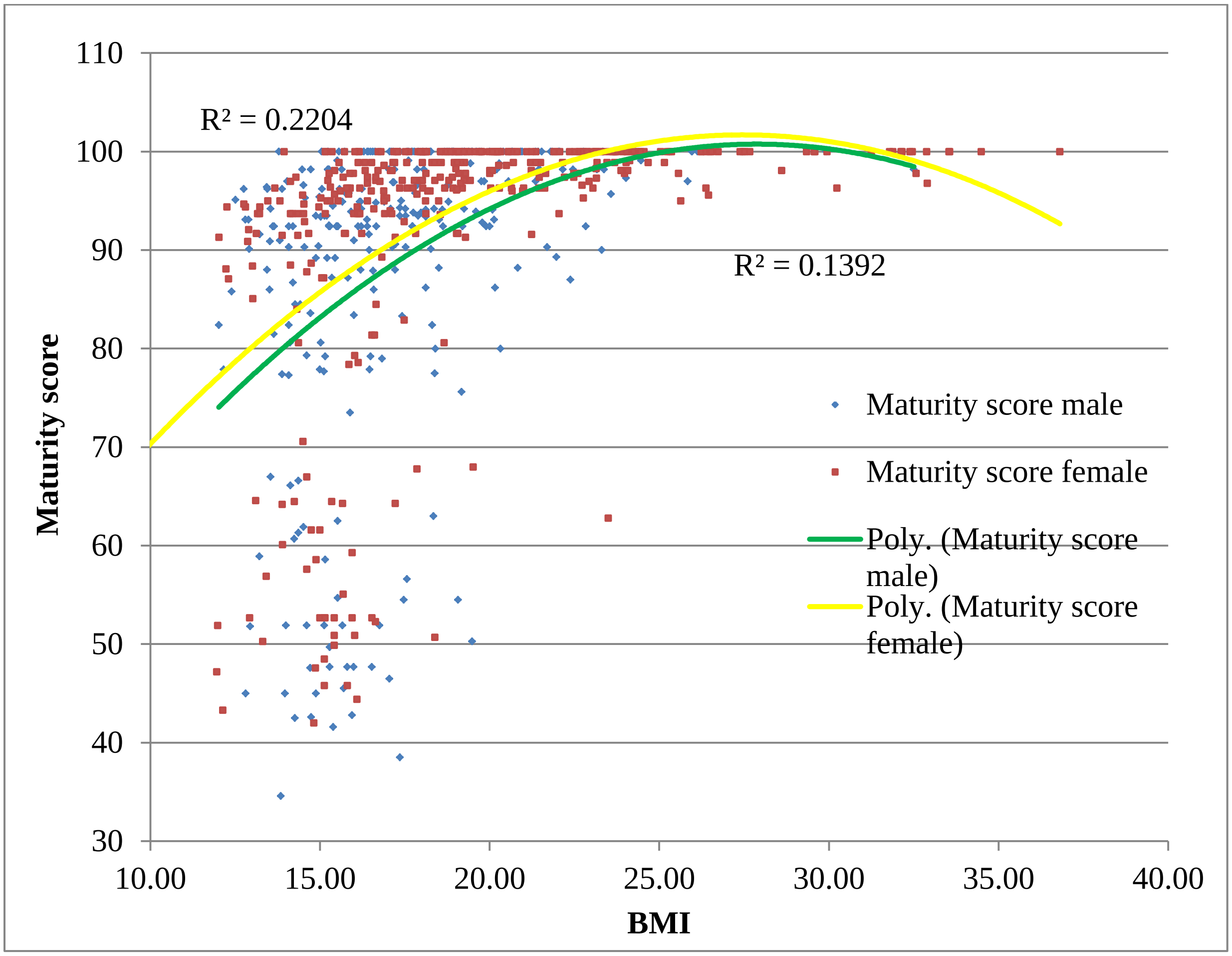


Figure 5.5. Maturity score by sex while controlling for head circumference

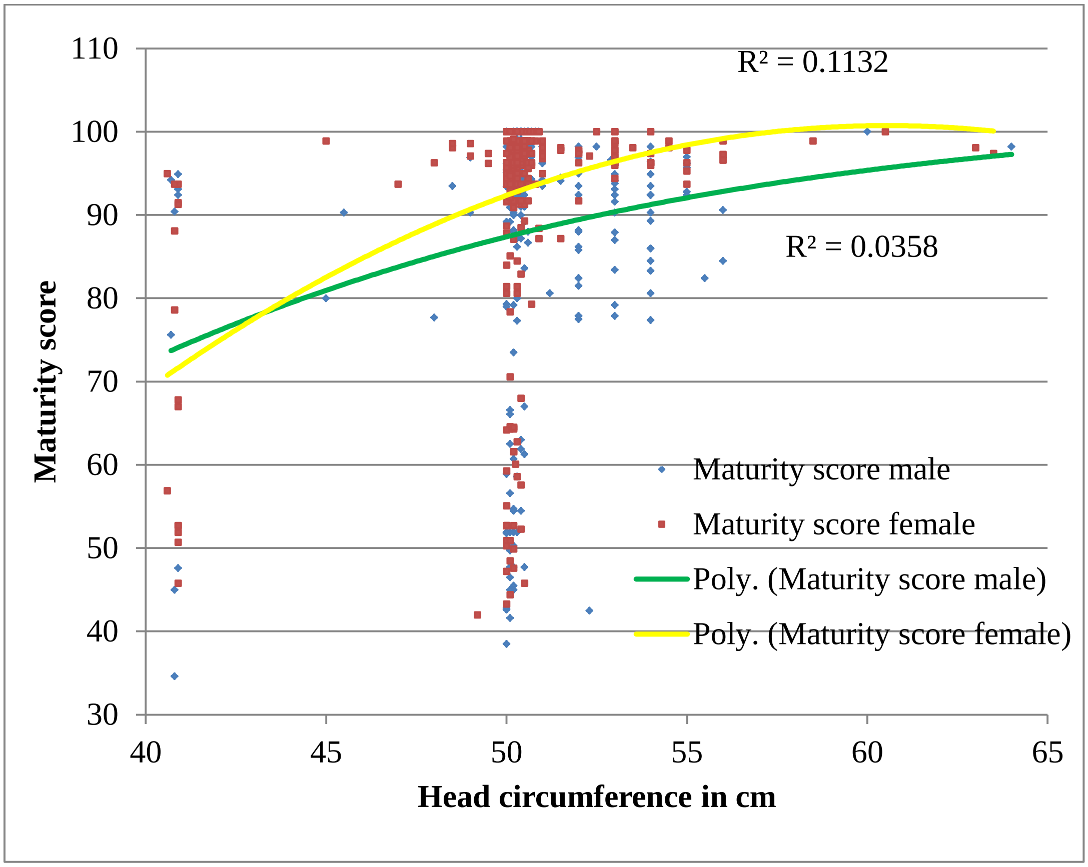
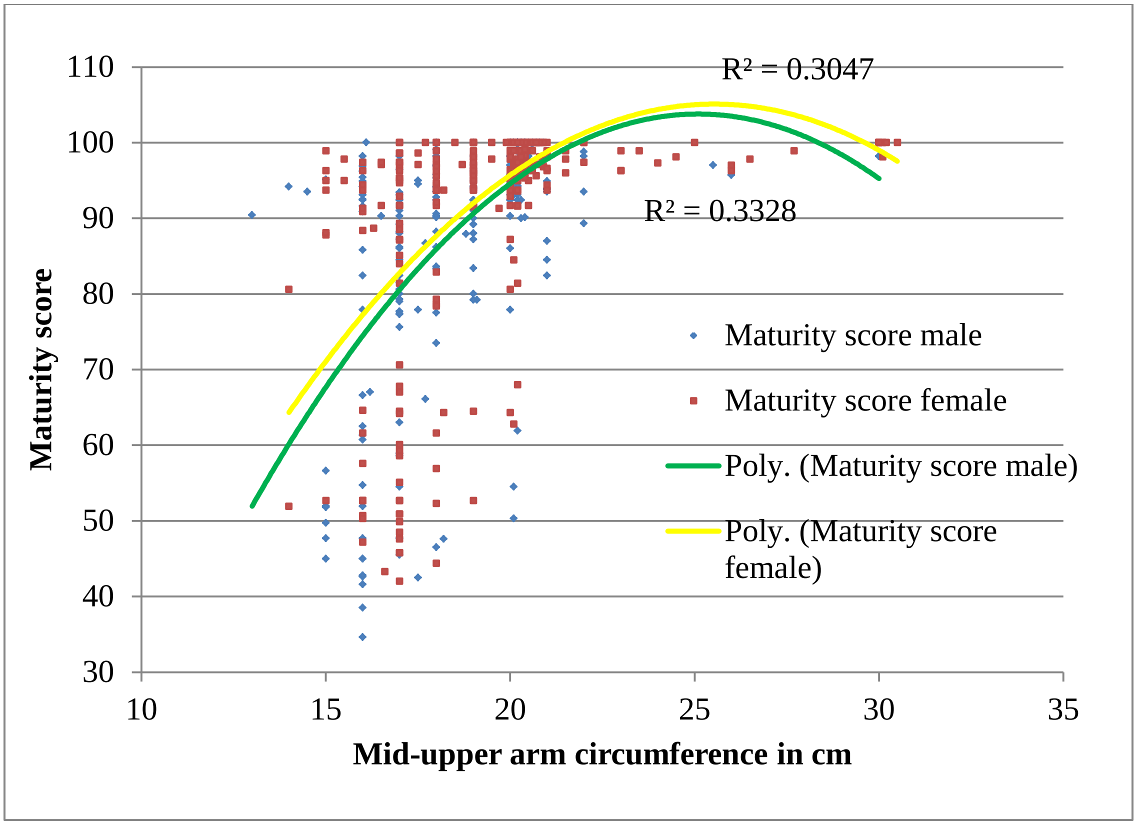


Figure 5.6. Maturity score by sex while controlling for the mid-upper arm circumference



5.4 Discussion

The investigation of the assumed relative immunity of dental development (especially tooth formation) to environmental perturbations is very important for anthropological and forensic purposes. Wide variation between skeletal age and chronological age makes the use of references based on somatic growth very unreliable. If the immunity of tooth formation is verified, timing of tooth formation could be used as a global standard for the assessment of growth and development.

The major environmental stressors that can potentially alter tooth formation are childhood diseases/infections and malnutrition. These factors have synergistic effects as diseases and infections often suppress appetite or reduce absorption of nutrients—creating or augmenting existing malnutrition. Thus, by studying proxies for nutritional status in this study, the two major sources of potential dental developmental perturbations can be evaluated.

5.4.1 Relationship between BMI and tooth formation

The results of this study confirm significant influence of nutritional status on tooth formation. In both males and females, lower BMI is associated with delay in the age of attainment of the H tooth formation stage. This is in agreement with previous studies that found a significant influence of nutrition on tooth formation in children from the United States (Hilgers et al. 2006; Mack et al. 2013) and Iranians (Zangouei-Booshehri et al. 2011).

The findings of the present study are at variance with three previous reports that found no significant difference in the timing of tooth formation of underweight or overweight children (Eid et al. 2002; Bagherian and Sadeghi 2011; Elamin and Liversidge 2013). Careful consideration of these studies raises some methodological questions that may impact upon their conclusions. Although Elamin and Liversidge (2013) interpreted their results as showing no difference between underweight and normal weight groups, their results document a uniform and consistent trend of advancement in all stages of tooth formation in the normal children compared to the malnourished children. Aside from using a combined sex sample that might obscure detection of the magnitude of the variation (as would have been the case for the Black Southern African children in this study), a more important reason for the lack of significant difference may be that they did not investigate the extremes of the BMI z-scores. Pertaining to this point, Garn et al. (1965) found nutritional status was only slightly related to tooth formation timing in the Fels Growth Study, but they concluded that the relationship was expected to be more marked if the nutritional range was broader and in situations where protein deficits were the key underlying nutritional difference. Bagherian and Sadeghi (2011) also reported a trend of advancing dental maturity from the underweight group to the overweight group in their study, but the correlational analysis results were not statistically significant. Correlation analysis usually shows a linear relationship even though growth patterns are not always linear (Scheuer and Black 2004). Any patterns should be further explored with a robust multivariate analysis. Eid et al. (2002) used Demirjian's age estimation method but the age of entry into the developmental stages was not calculated and the BMI scores were not standardized for age. Therefore the individual ages of the participants might have skewed their final outcome.

A limitation of this study was the small number of participants, especially in females, in the underweight (low BMI) category. The government of South Africa started providing free meals in all public schools in 1994 (Rendall-Mkosi et al. 2013), and this practice might account for the low numbers of underweight participants. The sexual dimorphism found in this study pertaining to BMI may be explained by the theory that females are better buffered from environmental stresses than males (Stini 1975, 1982; Stinson 1985). Additionally, school attendance is higher for females in this study and the access to school meals may vary considerably as a consequence.

5.4.2 Influence of height and weight on tooth formation

This study found that height has an influence on tooth formation, similar to what is seen for BMI. Unfortunately the relationship with height could not be fully explored because of limited sample sizes at the extremes of height (tall for age and short for age) for both sexes. However, regression and correlational analyses showed that height significantly influenced the dental maturity scores in both males and females. This is similar to the findings of Green (1961) and Dermirjian et al. (1985), who found a strong correlation between height and dental maturity.

There are very few studies on the relationship between height and tooth formation, but previous studies on tooth emergence did identify a positive relationship (Oziegbe et al. 2009; Kutesa et al. 2013). It is expected that height and tooth formation are correlated because they are both measures of growth. In Black Southern African children, there is a significant difference in the timing of canine and second molar formation in males that are short-for-age and of normal height. For females, the significant differences are seen in the lateral incisors and first molars.

This study found no relationship between weight and tooth formation in the males whereas a significant relationship was found in females. This may be due to the different pattern of weight gain in the two sexes (Geer and Shen 2009). The weight gain in females occurs earlier and continues till puberty while that of males occurs much later. These differences in the pattern of weight gain characterises the Black Southern African children. Although our study found a strong correlation between weight and maturity scores in males and females, it is possible that the correlation may be due to weight gain as age increases. Green (1961) found a moderate correlation between weight and dental age although he did not correct for sex. While there is presently no study to directly compare with the result of our multivariate analysis, previous studies on tooth emergence found no relationship with weight (Oziegbe et al. 2009; Kutesa et al. 2013). Weight has poor sensitivity and specificity to malnutrition. Furthermore, to determine its effect, it should not be a “once off” measurement as done in this study. A well-designed longitudinal study may validate our findings.

5.4.3 Association between mid-upper arm circumference and tooth formation

The relationship between MUAC and tooth formation has not been studied prior to this study. No significant relationship was found between MUAC and dental maturity. This may be due to the fact that most of the children are within the normal range of MUAC. MUAC is good at detecting children at risk of mortality as extremely low values indicate muscular and adipose tissue loss (Chen et al. 1980; Vella et al. 1994). Another consideration is that the distribution of adipose tissue differs from one population to another (Gasperino 1996). Other measures of body composition, such as waist-to-hip ratios, may be more informative about nutritional status in Southern Africa. Furthermore, MUAC is affected by exercise, type of work or household chores and this may make the use of it solely for nutritional assessment unpredictable. Future studies are needed to explore this research area.

5.4.4 Influence of head circumference on dental maturity

This study found a significant relationship between head circumference and tooth formation in females but not in males. Although the correlation was low to moderate, the relationship among females was significant for the multivariate analysis. The reason for the difference could be explained by a pattern of increase in the head circumference over a longer period of time in females compared to males. The males had increased head circumference from age 5 to 9 years while it was from age 5 to 12 years in females. The findings may be an indicator of a relationship between permanent tooth formation and brain growth. Presently there are no other studies that investigated the correspondence of head circumference with permanent tooth development. However, a study on primary teeth showed that the number of erupted teeth has a relationship with head circumference (Vejdani et al. 2015). During perinatal and postnatal development, there is mobilization of resources to the brain at the expense of other tissues (known as the Expensive Tissue Hypothesis) (Aiello and Wheeler 1995). It is during this same period that the development of the primary and permanent dentition commences, with the emergence of all primary dentition occurring by approximately 30 months. The first phase of permanent tooth development continues until the emergence of the incisors and first molars at approximately 6 years, by which time brain size increase is almost completed. The second phase of permanent tooth development is associated with the pubertal growth spurt. Further studies should be done to unravel the precise relationship between tooth development and brain development to validate this assertion.

5.5 Conclusion

This study demonstrates that height, weight and BMI have a significant effect on the timing of tooth formation in males and females. The effect was mainly noticeable for children in the extremes of the spectrum of the BMI z-scores. A well-designed longitudinal study is needed to verify this finding.

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Table of Contents

Chapter 6

Permanent tooth emergence: Timing and sequence in Black Southern African children

Abstract

Background: Data on timing of tooth emergence are essential for gaining an understanding of morphological variation in children and can be very useful for identification and aging. Currently, there are no comprehensive data on permanent tooth emergence of Black Southern African children.

Aim: The study investigated the mean age of emergence and sequence of emergence of permanent teeth in Black Southern African children and compared the findings with other populations. The study also considered temporal trends in mean ages of emergence by comparing the present results with a limited study.

Methods: The cross-sectional study involved 639 (266 males and 373 females) black Southern African children aged 5-20 years. The teeth emerged and the number of permanent teeth present in the mouth was recorded. Probit analysis was used to derive the mean age at emergence of the permanent teeth. Sex and cross population comparisons were done to determine differences in timing of emergence. Statistical significance was inferred at $p<0.05$.

Results: Females emerged all the permanent teeth earlier than males except for the third molars ($p<0.05$). Children from other sub-Saharan African countries and Black Southern African children have similar ages of emergence of the permanent teeth. In general, Black Southern African children have earlier mean ages of emergence of permanent teeth compared to children from the USA, Europe, Australia and Asia. Sexual dimorphism was noted in the sequence of emergence of I1/M1 in the mandible with females having the M_1I_1 sequence as opposed to I_1M_I

in males. The sequence of emergence of Southern African males is similar in both jaws to males from other sub-Saharan African countries, the USA and Europe but differs from Iranians and Pakistanis who have similar sequences with $P^1$and P^2 emerging before C^1. Females show similar pattern of sequence with other sub Saharan Africans, Australians and US females in the maxilla. Southern African display M_I/I_1 variation in the mandible with M_I I_1 being the commonest sequence of emergence among the Southern African females. No temporal changes were noted in the timing of emergence of permanent teeth of Black Southern African children

Conclusion: The mean age of tooth emergence of Black Southern African children is similar to children from most other sub-Saharan African populations and earlier mean ages of emergence than children from Europe and Asia. There is variation in the M1/I1 sequence between males and females and between South African females and populations of European ancestry.

Despite major socioeconomic and political changes in South Africa over the past two decades, no temporal change was noted in the mean age of emergence for the Black Southern African children when compared to an earlier report on the same population. This suggests that earlier emergence of the permanent dentition in Black Southern Africans is part of a general sub-Saharan pattern of dental emergence that is distinct from European and Asian populations

6.1 Introduction

Comparative data on growth and development, such as timing of tooth emergence, are essential for gaining an understanding of morphological variation in children. The importance of population-specific growth references extends beyond their utility in biological anthropology and health research. For many populations in Africa, including South Africa, where birth registry and eliciting the date of birth are still a challenge, developmental information can be very useful for identification and aging (Kim et al. 2000; Yun et al. 2007).

Initial data on timing of tooth emergence in Black Southern Africans were published twenty-seven years ago (Blankenstein et al. 1990a). It is expected that there would be changes in the timing of tooth emergence since that time due to alterations in nutritional status, socioeconomic status, and possibly greater levels of intermarriages stemming from increasing urbanization. Therefore, new studies are needed to keep up with the expected evolution and to assess temporal changes in timing of tooth emergence. Furthermore, the previous study did not include timing of emergence for all the teeth (Blankenstein et al. 1990b). This study provides tables of emergence for all tooth classes of Black Southern African children, with a view to determine any temporal changes in the timing of emergence of permanent teeth and to also compare the findings with other populations.

Dental development and other developmental indicators such as stature, secondary sex characteristics and bone growth have been used in estimating physiological age (Moorrees et al. 1963). Dental development shows less variation in general and also low variability in relation to chronological age (Demirjian et al. 1985; Demirjian 1986) when compared to other growth defining events (Lewis and Garn 1960). Tooth emergence and formation are frequently used to assess dental development. Tooth emergence is the more variable of the two measures because it is affected by retention or early loss of primary teeth and odontogenic infections (Holt et al. 2001). However, it is a very simple, quick and reliable method to assess growth and development when there are no available radiographs.

6.1.1 Population variation in tooth emergence

Variation in tooth emergence within and among populations is reported in the literature (Akpata 1971; Billewicz and McGregor 1975; Hassanali and Odhiambo 1981; Triratana et al. 1990; Pahkala et al. 1991; Kutesa et al. 2013). The reason for the variation among different populations is unknown, although several explanations involving genetic or environmental factors have been proposed (Garn et al. 1960, 1965; Pahkala et al. 1991; Liversidge 2003; Chaillet et al. 2005). A number of studies highlighted genetic differences. For example, the study by Blankenstein et al. (1990a, 1990b) on children in Johannesburg showed that Black South African children had earlier times of emergence compared to their Indian counterparts. Similarly, Hassanali and Odhiambo (1981) showed that the teeth of African Kenyans emerged earlier than the Asian Kenyans. West African children (Richardson et al. 1975) and African American children were found to have a pattern of early emergence compared to their European ancestry counterparts (Garn et al. 1973a; Stewart et al. 1982; Harris and McKee 1990; Koch and Poulsen 2001). This study aims to compare age of tooth emergence in Black Southern African children with other populations to see if the early timing of emergence pattern is fully characteristic of the sub-Saharan populations.

Environmental factors such as temperature and humidity can influence growth through adaptive responses (Smithers and Smith 1997). Eveleth (1966) suggested that tropical climate has an accelerating effect on maturation. Her study showed that White American children living in

Brazil, who were brought up under similar conditions to those living in the United States, had earlier permanent tooth emergence times. Similarly, Friedlander and Bailit (1969) found that populations living in tropical climates tend to be more dentally advanced than those living in temperate regions. Another factor that has received great attention is nutrition and its influence on tooth emergence. The view of some authors is that dental development is immune to malnutrition (Demirjian et al. 1985; Demirjian 1986; Elamin and Liversidge 2013). In contrast, more recent researchers have found a significant influence of nutritional status on tooth emergence (Psoter et al. 2007; Sanchez-Pérez et al. 2010; Must et al. 2012).

6.1.2 Sex variation in tooth emergence

Universally, it appears that females in any given population are more advanced in tooth emergence than males (Kochhar and Richardson 1998; Eskeli et al. 1999; Moslemi 2004; Oziegbe et al. 2014). Kochhar and Richardson (1998) found significant differences for maxillary lateral incisor and canine emergence with the mandibular canine showing the largest sexual dimorphism in timing of emergence. Blankenstein et al. (1990a) found that Black South African females were ahead of males in the maxillary central and lateral incisors and the mandibular lateral incisors. However, Oziegbe et al. (2014) found that Nigerian girls had all permanent teeth emerged earlier than boys, with the greatest difference observed in the mandibular canine. Earlier emergence of permanent teeth in girls has been ascribed to an earlier commencement of maturation in general (Almonaitiene et al. 2010).

6.1.3 Variation in sequence of tooth emergence

The sequence of emergence of permanent teeth is of great importance in orthodontics, pediatric dentistry, anthropology, comparative odontology, and evolution of the dentition. The common sequence of emergence is said to be M^1 I^1 I^2 P^1 C^1 P^2 M^2 in the maxilla and M_1 I_1 I_2 C_1 P_1 P_2 M_2 in the mandible and any other sequence is regarded as a variant.

A comprehensive study of Black and White American children showed polymorphic sequences occur within, and not between, teeth in eruption Phase I (Ml, 11, 12) and Phase II (C, P1, P2, and M2) (Smith and Garn 1987). More sequence variations are found in the mandible than in the maxilla, and mostly occur between C_1 and P_1. C_I usually precedes P_1 in the mandible (Smith and Garn 1987). Conversely, P^1 precedes C^I in the maxilla in both males and females. Sexual dimorphism appears in canine sequences (Whites only) with P^2 preceding C^1 in the maxilla, and also in the sequence M_1I_1/I_1M_I in the mandible with males having the I_1M_I sequence more frequently in both Blacks and Whites (Smith and Garn 1987). Other studies conducted in the USA, Australia, Finland, Iran and Nigeria found variation in the sequence of tooth emergence by sex, jaw (mandible vs maxilla) and population (Savara and Stein 1978; Diamanti and Townsend 2003: Leroy et al. 2003; Moslemi 2004; Oziegbe et al. 2014), with the same variants described by Smith and Garn (1987).

Polymorphisms in the sequence of tooth emergence may be linked to general ancestry or more specific population-level genetic variation (Garn et al. 1973b). Furthermore, the variants of emergence sequence may result in crowding and even non- emergence. For example, the emergence of M_2 ahead of P_1 and P_2 may lead to the premolars being blocked out of the arch. Similarly, emergence of C^1 at about the same time as the P^1 may result in labial displacement of C^1 (Profit 2007). The sequence of emergence is said to be affected by endocrine factors, chronic childhood illnesses, rickets, acute infection and even the mental status of the child in the first six months of life. Other factors are the thickness of the oral mucosa, density of the bone, tooth ankyloses, dental caries and dental abscesses (Gordon and Kuskin 1935).

6.1.4 Temporal changes in permanent tooth emergence

In several human populations, temporal changes have been described for impaction and agenesis of the third molars as well as decreases in mandibular arcade dimensions (Garn et al. 1968; Kieser and Cameron 1987; Chorn and Hennenberg 1994; Quek et al. 2003). The results of studies comparing temporal changes in tooth emergence are not as straightforward. Rousset et al. (2003) found later emergence of the maxillary premolars and earlier emergence of permanent second molars in a French population compared to a similar study done over 40 years earlier. Similarly, Höuffding et al. (1984) found earlier emergence of teeth in a contemporary Japanese population when compared to data obtained in 1934. These changes were attributed to evolutionary reduction in the size of the maxilla, a progressive decrease in genetic control of permanent canines and premolars, or advances in preventive measures to preserve primary molars. Conversely, a study from Britain found delayed tooth emergence in recent populations compared to earlier populations (Elmes et al. 2010). However, in studies done in Uganda (Kutesa et al. 2013) and Nigeria (Oziegbe et al. 2014), no difference was found in the timing of tooth emergence in the recent populations compared to data from earlier studies.

The recent and rapid sociopolitical changes in South Africa due to the fall of apartheid rule provide an ideal setting to explore changes in the timing of tooth emergence and dental variation in a context of demographic, nutritional and economic changes.

6.2 Materials and methods

This is a cross-sectional study of clinically healthy Black Southern African children aged 5-20 years, whose parents and grandparents are indigenous Southern Africans. A total of 639 children (373 females, 266 males) out of 642 children who met the inclusion criteria were randomly selected from those pupils being screened for dental treatment by the Community Oral Health Outreach Program (COHOP), Department of Community Dentistry, University of the Witwatersrand. Ethical approval (NO. M141001) was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. Permission to carry out the study was obtained from the local education authority and respective school heads. Consent was obtained from parents while assent was obtained from the children.

Information collected from the dental records of the children included date of birth and sex. All selected students were examined in a mobile dental van equipped with a panoramic radiograph machine. Intra oral examination was done with sterile dental mirror and probe under a light source. Teeth present were recorded using *Fédération Dentaire Internationale (*FDI) notation. An emerged tooth was defined as a tooth with any part of its crown penetrating the gingiva and visible in the oral cavity (Al-Jasser and Bello 2003). In general oral surgery, some children have their emerged teeth extracted due to consequences of untreated dental caries, trauma or for orthodontic purposes. Extracted teeth were considered to have emerged for this study. After examination, panoramic radiographs were taken and children having agenesis of lateral incisors and third molar were excluded from the study. Three children were excluded because of incomplete data on tooth emergence.

The data were analyzed with Stata 12 for Windows. The analysis included frequencies and cross-tabulations. The mean age at the time of emergence and standard deviations for each tooth were computed separately for males and females using probit analysis (Hayes and Mantel 1958). Various methods have been used to determine ages of emergence including graphical methods that require no assumption regarding the underlying distribution of age at emergence. To calculate mean age of attainment of emergence, which is assessed as a discrete event, it is best to use the principles of cumulative frequency curves, which is best done with probit regression (Healy 1986). This allows for adequate sampling of age range from early to late developers (Smith 1991). Probit regression helps to derive the mean age and range most probable for 50% of the population to have emerged a tooth, a measure equal to the median. Differences between the mean age of emergence of males and females were tested using Student's *t*-tests, after Liversidge (2003). Statistical significance was inferred at $p \leq 0.05$.

6.3 Results

6.3.1 Variation in emergence times

Among the males, there is no significant difference in the mean emergence times between the left and right maxillary teeth or the right and left mandibular teeth (Table 6.1). There are no statistically significant differences in the mean ages at emergence of the right and left maxillary teeth in females, although there is a tendency for earlier emergence times for the maxillary left teeth with the exception of the second premolar and first molar (Table 6.2).

Table 6.1. Mean emergence age (years) of permanent teeth in males

Maxilla						
Tooth type	**Upper right**		**Upper left**		**Combined**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
I^1	6.94	0.93	6.93	0.80	6.93	0.87
I^2	7.78	1.06	7.78	1.08	7.78	1.07
C^1	11.15	1.81	11.04	1.58	11.09	1.69
P^1	10.27	1.84	10.34	1.59	10.31	1.72
P^2	11.18	1.70	11.14	1.79	11.16	1.74
M^1	6.27	0.29	6.26	0.29	6.27	0.29
M^2	12.69	1.80	12.81	1.75	12.75	1.78
M^3	18.93	1.39	18.71	1.50	18.82	1.45
Mandible						
Tooth type	**Lower right**		**Lower left**		**Combined**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
I_1	6.07	0.34	6.09	0.34	6.08	0.34
I_2	7.06	0.95	7.06	0.95	7.06	0.95
C_1	10.17	1.67	10.17	1.67	10.17	1.67
P_1	10.76	1.76	10.76	1.76	10.76	1.76
P_2	11.67	2.42	11.67	2.42	11.67	2.42
M_1	6.09	0.31	6.12	0.25	6.11	0.28
M_2	12.64	2.07	12.64	2.07	12.64	2.07
M_3	18.45	1.97	18.45	1.97	18.45	1.97

Note: No significant difference in the emergence times between the right and left teeth

Table 6.2. Mean emergence age (years) of permanent teeth in females

Maxilla						
Tooth type	**Upper right**		**Upper left**		**Combined**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
$\mathbf{I^1}$	6.44	0.61	6.41	0.71	6.43	0.66
$\mathbf{I^2}$	7.47	0.76	7.38	1.08	7.42	0.92
$\mathbf{C^1}$	10.51	1.39	10.44	1.53	10.47	1.46
$\mathbf{P^1}$	9.90	1.31	9.79	1.38	9.84	1.34
$\mathbf{P^2}$	10.59	1.88	10.67	1.42	10.63	1.65
$\mathbf{M^1}$	6.01	0.94	6.08	0.86	6.04	0.89
$\mathbf{M^2}$	12.14	1.28	11.99	1.32	12.07	1.30
$\mathbf{M^3}$	19.67	2.99	19.45	2.79	19.56	2.89
Mandible						
Tooth type	**Lower right**		**Lower left**		**Combined**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
$\mathbf{I_1}$	5.44	0.93	5.49	0.96	5.46	0.94
$\mathbf{I_2}$	6.70	1.71	6.79	1.65	6.74	1.68
$\mathbf{C_1}$	9.47	1.63	9.46	1.11	9.47	1.37
$\mathbf{P_1}$	9.94	1.32	10.07	1.24	10.01	1.28
$\mathbf{P_2}$	10.66	1.26	10.66	1.32	10.66	1.29
$\mathbf{M_1}$	5.33	1.17	5.41	1.09	5.37	1.13
$\mathbf{M_2}$	11.70	1.43	11.68	1.59	11.69	1.51
$\mathbf{M_3}$	18.36	2.37	18.80	2.61	18.58	2.49

Note: No significant difference in the emergence times between the right and left teeth

The females have a significantly earlier mean age of emergence of all the permanent teeth in the maxilla compared to the males (p=0.00), except for the maxillary third molar that emerges significantly earlier in males (p=0.00) (Table 6.3 and Figure 6.2). A similar pattern was observed in the mandible except that the mandibular third molars of males have an earlier, but not significantly earlier, mean age of emergence (p=0.48) (Table 6.3 and Figure 6.2). The greatest difference in the timing of emergence between males and females is in the mandibular second premolar (Table 6.3).

Table 6.3. Comparison of mean age (years) of permanent tooth emergence between males and females

Maxilla						
Tooth type	**Males=266**		**Females=373**		**t**	**p**
	Mean	**SD**	**Mean**	**SD**		
$\mathbf{I^1}$	6.93	0.87	6.43	0.66	8.26	**0.000**
$\mathbf{I^2}$	7.78	1.07	7.42	0.92	4.44	**0.000**
$\mathbf{C^1}$	11.09	1.69	10.47	1.46	4.95	**0.000**
$\mathbf{P^1}$	10.31	1.72	9.84	1.34	3.88	**0.000**
$\mathbf{P^2}$	11.16	1.74	10.63	1.65	3.91	**0.000**
$\mathbf{M^1}$	6.27	0.29	6.04	0.89	4.06	**0.000**
$\mathbf{M^2}$	12.75	1.78	12.07	1.30	5.58	**0.000**
$\mathbf{M^3}$	18.82	1.45	19.56	2.89	3.84	**0.000**
Mandible						
Tooth type	**Males**		**Females**		**t**	**p**
	Mean	**SD**	**Mean**	**SD**		
$\mathbf{I_1}$	6.08	0.34	5.46	0.94	10.29	**0.000**
$\mathbf{I_2}$	7.06	0.95	6.74	1.68	2.80	**0.005**
$\mathbf{C_1}$	10.17	1.67	9.47	1.37	5.81	**0.000**
$\mathbf{P_1}$	10.76	1.76	10.01	1.28	6.24	**0.000**
$\mathbf{P_2}$	11.67	2.42	10.66	1.29	6.82	**0.000**
$\mathbf{M_1}$	6.11	0.28	5.37	1.13	10.45	**0.000**
$\mathbf{M_2}$	12.64	2.07	11.69	1.51	6.71	**0.000**
$\mathbf{M_3}$	18.45	1.97	18.58	2.49	0.71	0.479

Figure 6.1. Mean emergence times of maxillary teeth by sex in Black Southern Africans

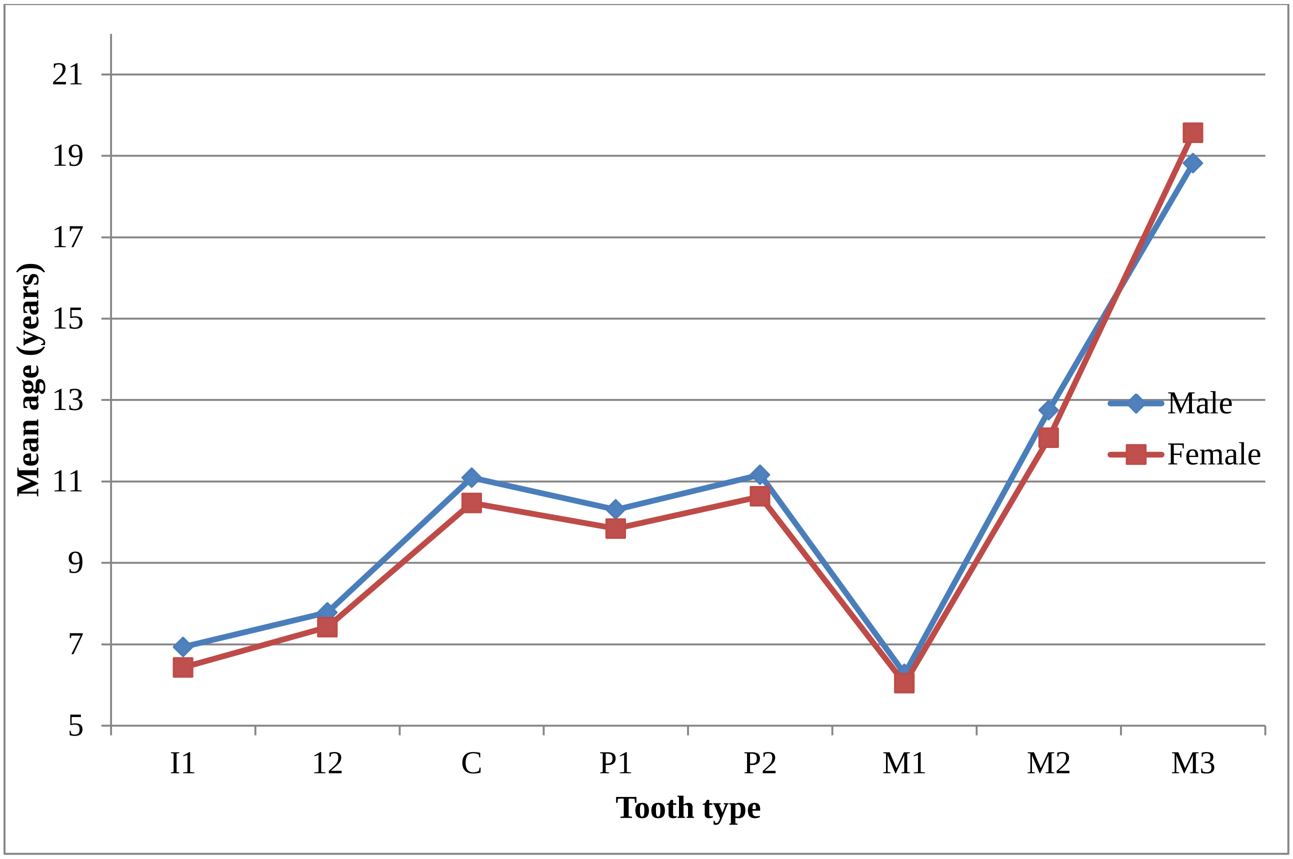
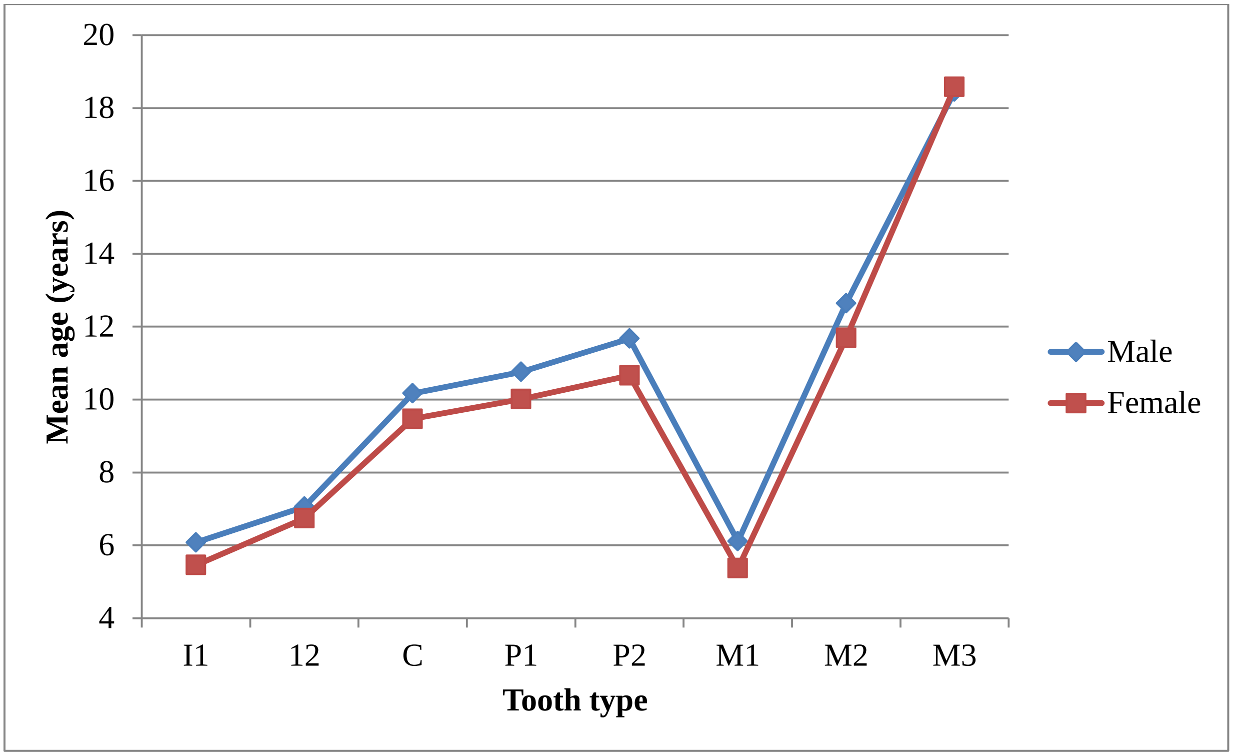


Figure 6.2. Mean emergence times of mandibular teeth by sex in Black Southern Africans



Comparative analysis of emergence times between the maxilla and the mandible shows that all mandibular teeth emerge significantly earlier, except for the first premolar (p=0.058) and the second premolar (p=0.568) (Table 6.4).

Table 6.4. Comparison of mean emergence age (years) of permanent tooth in the mandible and maxilla (males and females combined)

Maxilla			**Mandible**			**t**	**p**
Tooth type	**Mean**	**SD**	**Tooth type**	**Mean**	**SD**		
$\mathbf{I^1}$	6.68	0.85	$\mathbf{I_1}$	5.88	0.60	13.95	**0.000**
$\mathbf{I^2}$	7.61	1.03	$\mathbf{I_2}$	6.88	1.36	7.37	**0.000**
$\mathbf{C^1}$	10.71	1.56	$\mathbf{C_1}$	9.78	1.54	7.48	**0.000**
$\mathbf{P^1}$	10.02	1.51	$\mathbf{P_1}$	10.25	1.51	1.90	0.058
$\mathbf{P^2}$	10.84	1.68	$\mathbf{P_2}$	10.92	1.79	0.57	0.568
$\mathbf{M^1}$	6.22	0.63	$\mathbf{M_1}$	5.89	0.63	6.53	**0.000**
$\mathbf{M^2}$	12.29	1.55	$\mathbf{M_2}$	12.03	1.83	1.88	0.060
$\mathbf{M^3}$	19.42	2.50	M_3	18.73	2.37	3.55	**0.000**

6.3.2 Sexual dimorphism in the sequence of emergence

M_1 and I_1 variation in the sequence of emergence was noted. The sequence is M1I1 in both jaws in females, whereas in males it is I_1M_1 in the mandible and M^1I^1 in the maxilla. Other teeth emerged in the same sequence for both sexes (Tables 6.1 and 6.2). The overall emergence sequence for the whole population is similar to the pattern for the males (I_1M_1 $M^1I^1I_2$ I^2 C_1P^1 P_1 $C^1P^2P_2M_2$ M^2 M_3 M^3) (Table 6.5). However, the two teeth (I_1M_1) appear to emerge in close succession in the combined male and female data (Table 6.6).

Table 6.5. Sequence of tooth emergence in the mandibular* and maxillary teeth

Males			**Females**			**Combined**		
Tooth type	**Dental age**	**Sequence**	**Tooth type**	**Dental age**	**Sequence**	**Tooth type**	**Dental age**	**Sequence**
I_1	6.08	1	M_1	5.37	1	I_1	5.88	1
M_1	6.11	2	I_1	5.46	2	M_1	5.89	2
M^1	6.27	3	M^1	6.04	3	M^1	6.22	3
I^1	6.93	4	I^1	6.43	4	I^1	6.68	4
I_2	7.06	5	I_2	6.74	5	I_2	6.88	5
I^2	7.78	6	I^2	7.42	6	I^2	7.61	6
C_1	10.17	7	C_1	9.47	7	C_1	9.78	7
P^1	10.31	8	P^1	9.84	8	P^1	10.02	8
P_1	10.76	9	P_1	10.01	9	P_1	10.25	9
C^1	11.09	10	C^1	10.47	10	C^1	10.71	10
P^2	11.16	11	P^2	10.63	11	P^2	10.84	11
P_2	11.67	12	P_2	10.66	12	P_2	10.92	12
M_2	12.64	13	M_2	11.69	13	M_2	12.03	13
M^2	12.75	14	M^2	12.07	14	M^2	12.29	14
M_3	18.45	15	M_3	18.58	15	M_3	18.73	15
M^3	18.82	16	M^3	19.56	16	M^3	19.42	16

*Mandibular teeth in subscripts and italics

6.3.3 Sequence of emergence: Sub-Saharan African population comparisons

Maxilla C1/P1 polymorphism

Males from sub-Saharan African countries have similar sequences of maxillary tooth emergence with the exception of the C^1/P^1 polymorphism. For males from these countries, except those from Zambia, the P^1 precedes the C^1. In general, females from sub-Saharan African countries have a P^1C^1 sequence of tooth emergence in the maxilla. The exception to this pattern is the Baka females from Cameroon, who have simultaneous emergence of the P^1 and C^1 (Table 6.6).

Mandibular M1/I1 polymorphism

In the mandible, the I_1M_1 polymorphism is evidently widespread in sub-Saharan populations. The I_1 emerges earlier than the M_1 (I_1M_1) in Black Southern African males. This is similar to the pattern in males from Kenya and Nigeria, but contrary to the finding of the previous Southern African study where males are characterized by the M_1I_1 pattern. Males from other sub-Saharan countries have the M_1I_1 sequence, demonstrating that this is a very common polymorphism (Table 6.7).

Variations in the I_1/M_1 sequence of emergence are noted in the mandible among the sub-Saharan African females. Females from Southern Africa, Ghana, Gambia and Cameroon have M_1 emerge before the I_1, whereas other females emerge their I_1 first.

Mandibular C1/P1 polymorphism

Zambia females show C_1/P_1 variation with the P_1 preceding the C_1 whereas females from the other sub-Saharan countries have the C_1P_1 sequence (Tables 6.6 and 6.7). Variations in the mandibular C_1/P_1 sequence of emergence characterize the sub-Saharan African males. Males from Southern Africa, Kenya and Cameroon have similar tooth emergence sequences with the C_1 preceding the P_1. In contrast, males from Nigeria and Zambia emerged their P_1 before the C_1. Ghanaian and Ugandan males seem to have their C_1 and P_1 emerge simultaneously.

Table 6.6. Mean age of emergence of maxillary permanent teeth in different sub-Saharan African populations*

Country	Sex	I^1	I^2	C^1	P^1	P^2	M^1	M^2	M^3
Southern Africa (Black) (Present Study)	**M**	**6.93**	**7.78**	**11.09**	**10.31**	**11.16**	**6.27**	**12.75**	**18.82**
	F	**6.43**	**7.72**	**10.47**	**9.84**	**10.63**	**6.04**	**12.07**	**19.56**
South Africa (Black) (Blankenstein et al. 1990)	M	6.94	8.00				6.09		
	F	6.79	7.72				6.02		
Zambia (Gillett 1997)	M	6.63	7.89	10.06	10.42	10.94	5.77	11.46	
	F	6.47	7.32	9.81	9.30	10.45	5.06	11.18	
Uganda (Krumholt 1971)	M	6.60	7.80	10.50	9.60	11.00	5.60	11.00	
	F	6.60	7.40	9.70	9.30	10.10	6.00	10.30	
Kenya (Hassanali & Odhiambo 1989)	M	6.91	7.99	10.93	9.87	10.74	6.32	11.54	
	F	6.55	7.71	10.26	9.40	10.15	6.13	11.40	
Nigeria (Oziegbe et al. 2013)	M	6.89	8.05	10.96	10.25	11.08	6.15	12.01	
	F	6.45	7.68	10.45	9.76	10.75	5.95	11.61	
Ghana (Houpt et al. 1967)	M	6.80	8.00	10.90	10.00	11.00	5.50	11.40	
	F	6.50	7.80	10.00	9.50	10.50	5.50	11.40	
Gambia (Billewicz & McGregor 1975)	M	7.38	8.60	11.29	10.26	11.23	6.00	11.96	
	F	7.13	8.10	10.56	9.78	10.60	5.80	11.19	
Baka, Cameroon (Ramirez Rozzi 2016)	M	6.47	7.77	9.55	9.27	10.09	5.33	10.97	19.63
	F	6.14	7.43	8.93	8.94	9.57	5.15	9.98	16.66

*Table arranged by geographical proximity of countries to South Africa

Table 6.7. Mean age of emergence of mandibular permanent teeth in different sub-Saharan African populations by sex*

Country	Sex	I_1	I_2	C_1	P_1	P_2	M_1	M_2	M_3
Southern Africa (Present Study)	**M**	**6.08**	**7.06**	**10.17**	**10.76**	**11.67**	**6.11**	**12.64**	**18.45**
	F	**5.46**	**6.74**	**9.47**	**10.01**	**10.66**	**5.37**	**11.69**	**18.58**
South Africa Black (Blankenstein et al. 1990)	M	5.83	6.79				5.78		
	F	5.79	6.73				5.68		
Zambia (Gillett 1997)	M	5.79	6.62	9.95	9.90	11.23	5.19	11.30	
	F	5.31	6.55	9.51	8.87	10.59	5.35	10.74	
Uganda (Krumholt 1971)	M	6,00	6.70	10.10	10.10	11.00	5.80	10.60	
	F	5.80	6.40	8.50	9.50	10.30	5.80	10.10	
Kenya (Hassanali & Odhiambo 1989)	M	5.83	6.86	9.96	10.05	10.90	6.03	11.39	
	F	5.62	6.56	9.20	9.62	10.23	5.70	11.07	
Nigeria (Oziegbe et al. 2013)	M	5.52	7.01	10.33	10.29	10.85	5.78	11.58	
	F	5.43	6.58	9.65	9.80	10.56	5.59	11.25	
Ghana (Houpt et al. 1967)	M	5.80	6.60	10.50	10.50	11.10	5.40	11.30	
	F	5.60	7.00	9.40	9.70	10.80	5.00	11.00	
Gambia (Billewicz & McGregor 1975)	M	6.22	7.46	10.55	10.70	11.44	5.67	11.56	
	F	6.08	7.07	9.64	9.96	10.69	5.46	10.91	
Baka, Cameroon (Rameriz Rozzi 2016)	M	5.62	6.62	9.40	9.73	10.47	5.18	10.76	18.33
	F	5.58	6.46	8.46	8.97	9.60	4.95	9.88	16.13

*Table arranged by proximity of countries to South Africa

6.3.4 Sequence of emergence: comparison with European ancestry populations

The sequence of emergence of maxillary permanent teeth in Black Southern African males and females is similar to the sequence of European ancestry males and females from Australia, Belgium and the USA with the P^1 preceding the C^1 (Table 6.8). In contrast, the males from Iran and Pakistan show a different emergence sequence with the P^1 and P^2 preceding the C^1.

In the mandible, the males from Southern Africa and other populations have similar sequence of emergence of mandibular teeth (Table 6.8). The sequence of tooth emergence among Black Southern African females is different from females from other continents (Table 6.8). The

Southern African females show variation in the I_1/M_1 polymorphism with the mandibular M_1 emerging before the mandibular I_1 ($M_1I_1I_2CP_1M_2P_2$), whereas the mandibular I_1 emerged earlier than the mandibular M_1 in other populations ($I_1M_1I_2CP_1M_2P_2$) except for females from Pakistan (Table 6.8).

Southern African males have a shorter interval between the first tooth to emerge and the last tooth to emerge (6.67 years) compared with females (6.70 years), even though tooth emergence commenced earlier in females (Table 6.8). The third molar was not considered in this comparison because of its high variability.

6.3.5 Comparisons of mean emergence times

The results of this study are in agreement with the emergence times reported by an earlier study of Black Southern Africans (Blankenstein et al. 1990) (Tables 6.6 and 6.7). Similar patterns in the timing of tooth emergence occur among the males and females from sub-Saharan African populations, except for the variations in the mean emergence times of canines and first premolars that characterise both males and females (Figures 6.3-6.6). Southern Africans have later M2 emergence in both males and females compared to other sub-Saharan African countries. Notably, the Baka females of the Cameroon have the lowest mean age of emergence for most of the teeth compared to other sub-Saharan African samples (Figures 6.3-6.6).

Black Southern African children have earlier emergence times for all the permanent teeth, except the second molars, compared to European ancestry children from the USA (Savara and Steen 1978), Belgium (Leroy et al. 2003) and Australia (Diamanti and Townsend 2003). Children from Iran have all of their teeth emerging later than the Black Southern African children (Moslemi 2004) (Table 6.8). Compared to the Pakistani males, South Africans have earlier emergence of all the mandibular teeth and the central and lateral incisors and the first molars in the maxilla (Table 6.8). The Southern African females have earlier emergence times of all the teeth compared to Pakistani females (Table 6.8).

Table 6.8. Comparative table of emergence times of Southern African children with children from other continents

Tooth type	**Southern Africa (Present study)**		**IRAN (Moslemi 2004)**		**PAKISTAN (Khan 2011)**		**AUSTRALIA* (Diamanti & Townsend 2003)**		**USA* (Savara & Steen 1978)**		**BELGIUM* (Leroy et al. 2003)**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Maxilla												
I^1	**6.93**	**6.43**	8.04	7.54	7.50	7.50	7.40	7.20	7.20	7.00	7.10	6.89
I^2	**7.78**	**7.42**	9.26	8.79	8.45	8.35	8.60	8.20	8.30	8.00	8.25	7.88
C^1	**11.09**	**10.47**	12.86	12.12	10.95	10.70	11.80	11.20	11.50	11.00	11.50	10.99
P^1	**10.31**	**9.84**	11.41	11.08	10.10	10.10	11.30	10.80	11.10	10.50	10.70	10.37
P^2	**11.16**	**10.63**	12.38	12.58	10.10	10.75	12.10	11.70	11.70	11.20	11.60	11.35
M^1	**6.27**	**6.04**	6.83	6.71	6.65	6.65	6.70	6.60	6.50	6.40	6.30	6.17
M^2	**12.75**	**12.07**	12.96	12.58	11.65	12.00	12.70	12.30	12.20	12.10	12.25	11.98
Mandible												
I_1	**6.08**	**5.46**	6.75	6.50	6.70	7.05	6.60	6.40	6.20	6.10	6.30	6.14
I_2	**7.06**	**6.74**	8.42	7.91	8.40	7.85	7.80	7.50	7.50	7.20	7.40	7.13
C_1	**10.17**	**9.47**	11.75	10.25	11.80	9.95	11.00	10.10	10.70	9.90	10.60	9.74
P_1	**10.76**	**10.01**	11.92	11.08	12.20	10.35	11.20	10.60	10.90	10.40	10.70	10.25
P_2	**11.67**	**10.66**	12.96	12.63	12.80	10.75	12.10	11.70	11.60	11.10	11.70	11.37
M_1	**6.11**	**5.37**	6.83	6.67	6.80	6.45	6.60	6.40	6.50	6.30	6.30	6.17
M_2	**12.64**	**11.69**	12.67	12.42	12.90	11.35	12.20	11.80	12.00	11.80	11.80	11.55

*Samples include children of European ancestry.

Figure 6.3. Mean emergence times in males for different African groups (Maxilla)

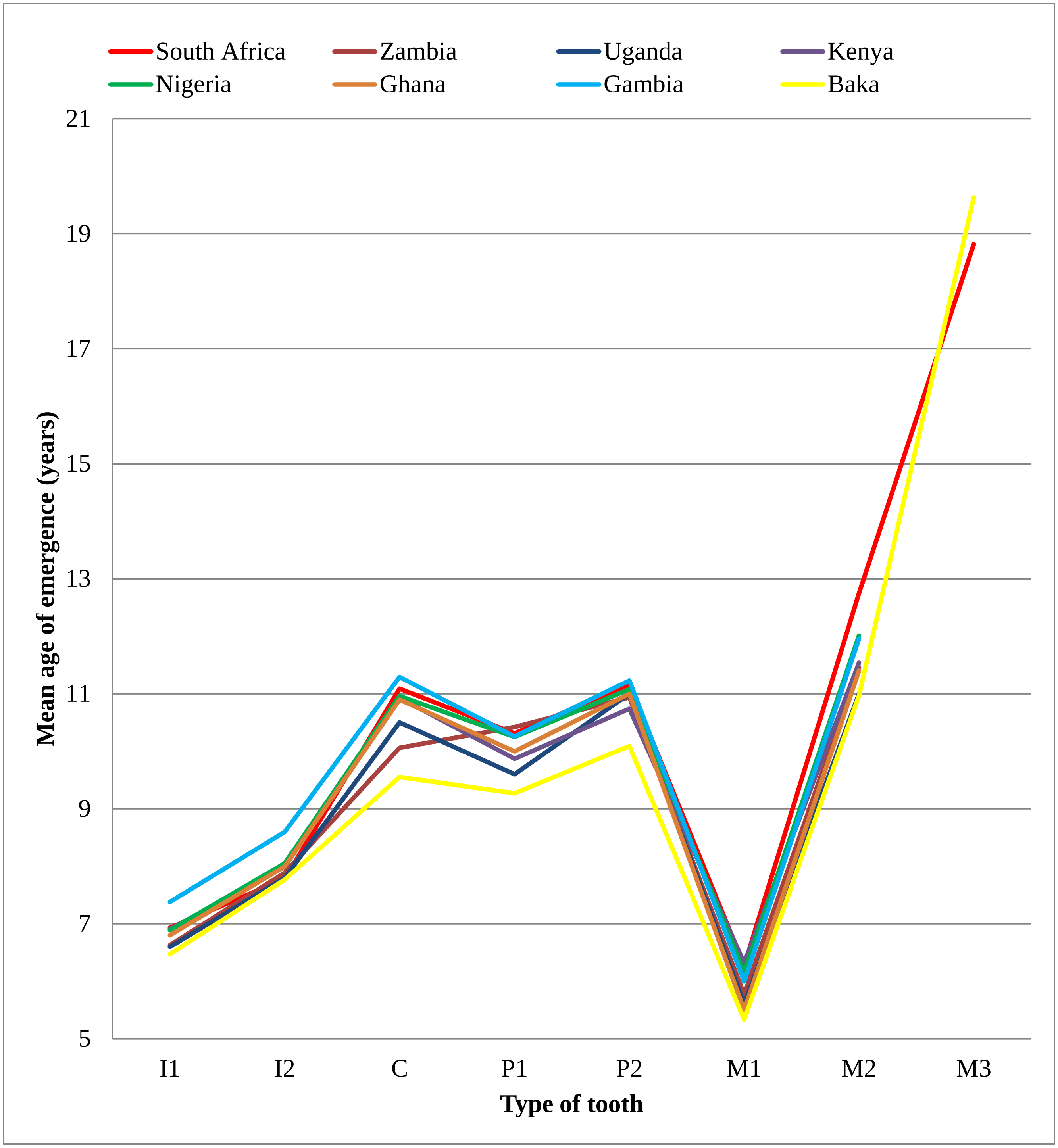


Figure 6.4. Mean emergence times in females for different African groups (Maxilla)

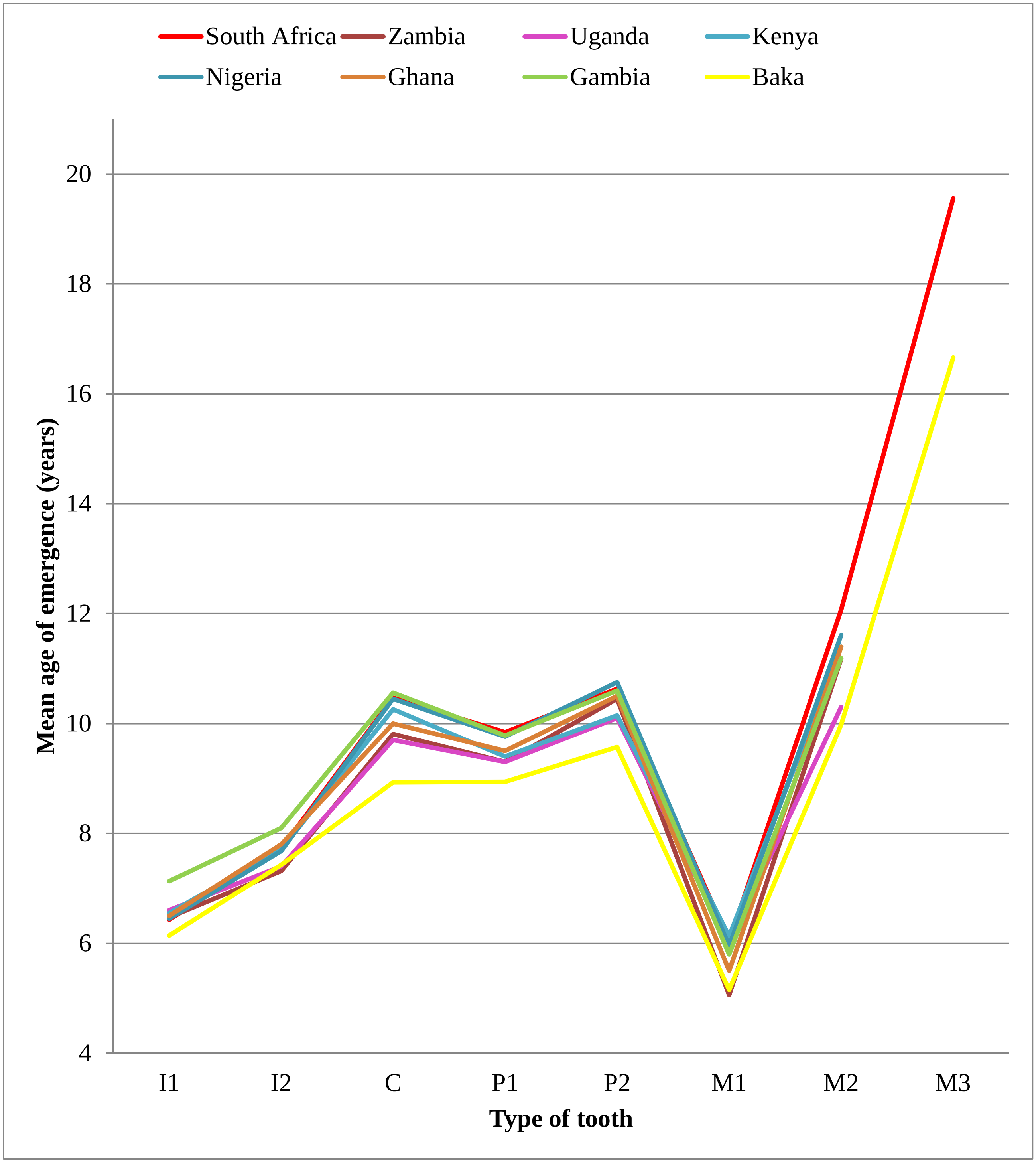


Figure 6.5. Mean emergence times in males for different African groups (Mandible)

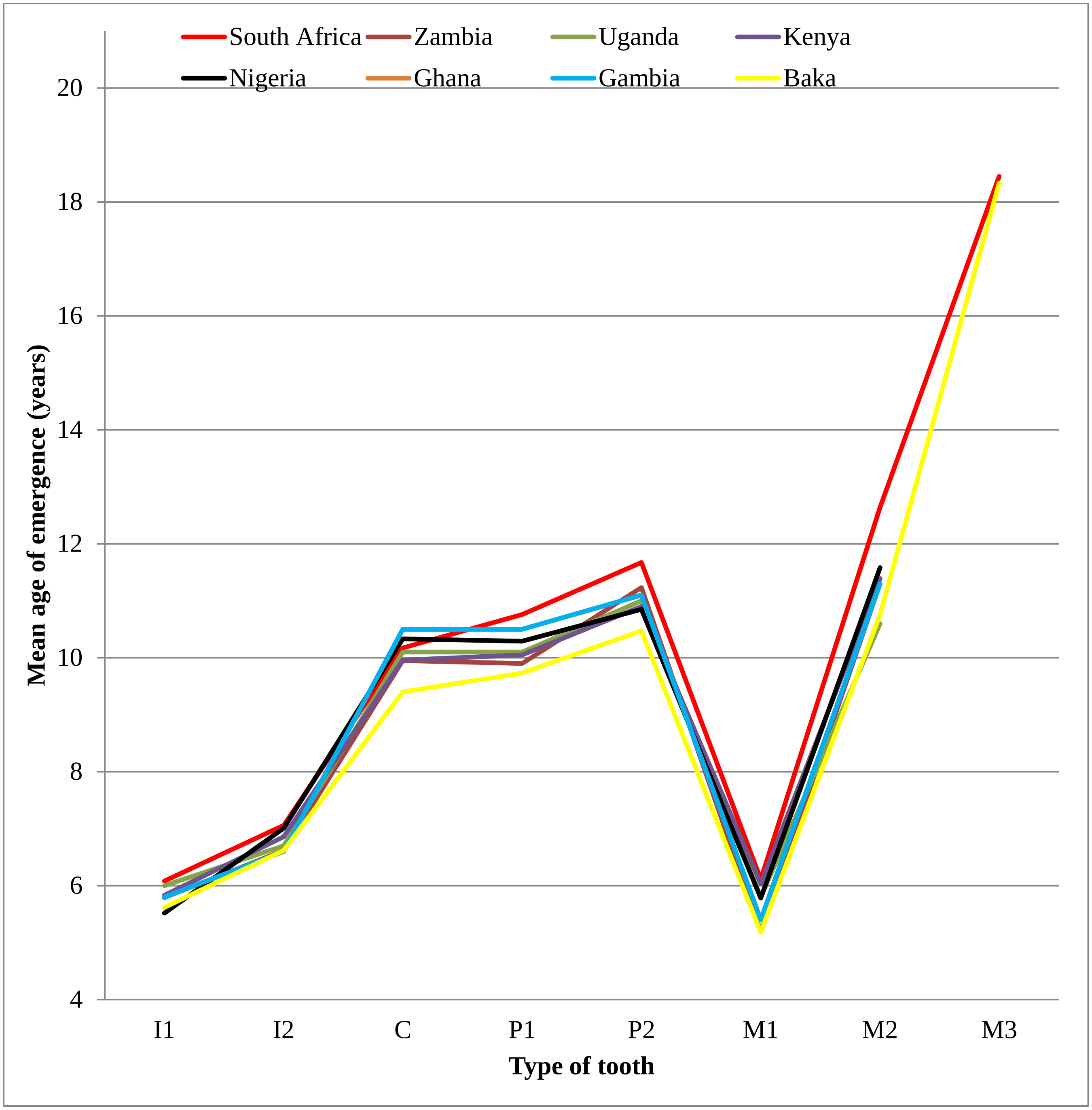
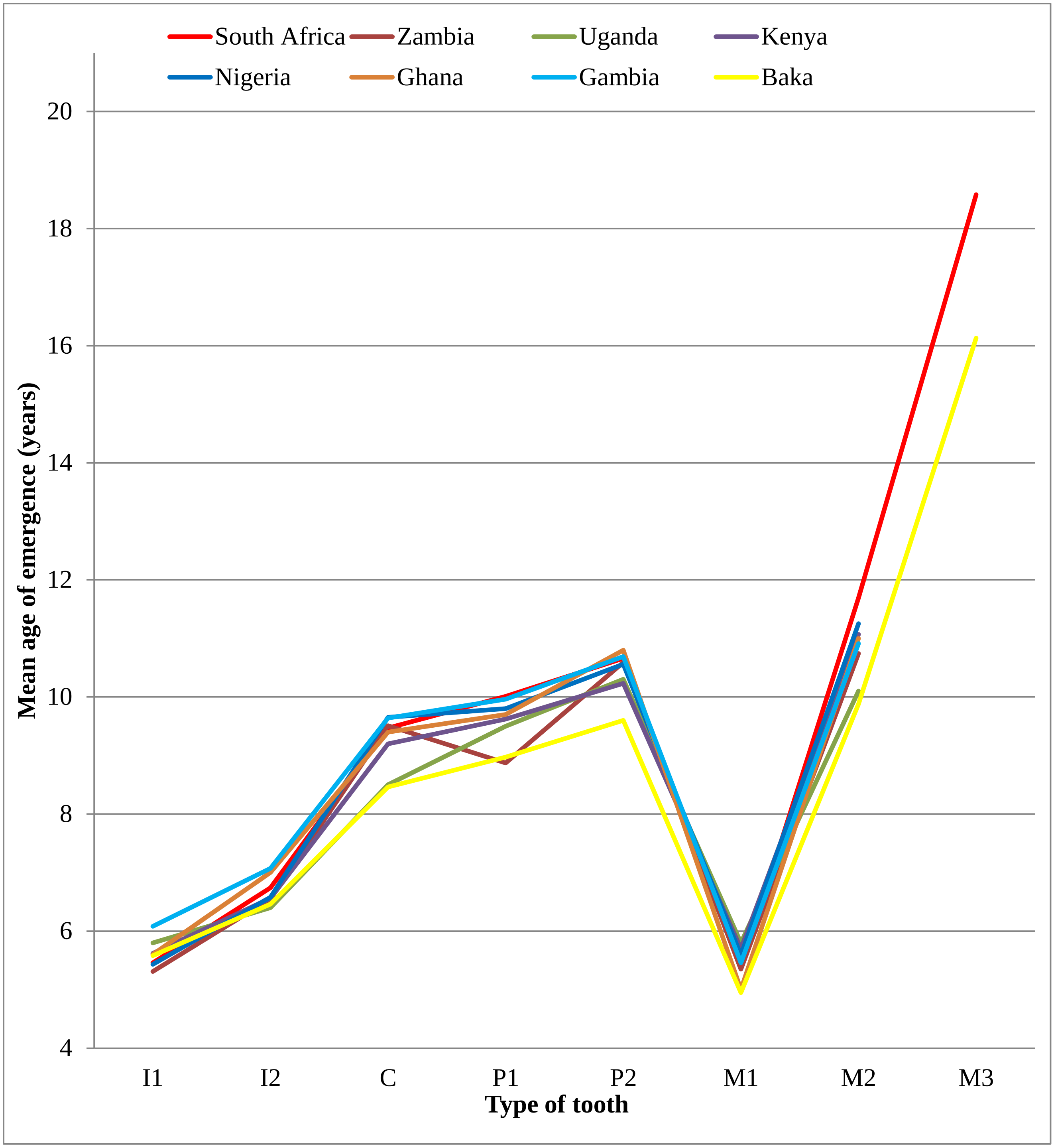


Figure 6.6. Mean emergence times in females for different African groups (Mandible)



6.4 Discussion

This is the first comprehensive study of tooth emergence in permanent teeth of Black Southern African children. An earlier study did not evaluate the full dentition and was conducted more than 25 years ago. The mean emergence ages for permanent teeth in Black Southern African children determined in this study are similar, in some respects, to a previous study of the same population (Blankenstein et al. 1990). Methodological differences could have accounted for the small differences between this study and the earlier study.

6.4.1 Right/left asymmetry in emergence

The question of asymmetrical timing of tooth emergence has generated some interest among researchers, but no clear patterns are documented. There is no specific pattern of asymmetry observed for mandibular or maxillary teeth in the Southern African children. This result is the same as previous reports (Billewicz and McGregor 1975; Leroy et al. 2003; Hernandez et al. 2008) where no propensity towards earlier emergence on one side was detected in Gambian, Flemish or Spanish children respectively. However, Kaur et al. (2010) noted that maxillary teeth emerged earlier on the right while mandibular teeth emerged earlier on the left in a study of Indian children. The differences were not significant. A Nigerian study (Oziegbe et al. 2014) found a propensity for earlier emergence of the permanent maxillary canine on the right in females but this was not statistically significant. No reason was adduced for this variation in their study.

Asymmetry in the timing of emergence therefore seems to be random and may reflect the effects of several confounding variables, such as tooth loss, tooth decay and occlusion variation, that are difficult to document in cross-sectional studies.

6.4.2 Maxillary versus mandibular emergence times

The present study found that the mandibular incisors, canines and first and second molars emerged before their maxillary counterparts while the two premolars emerged earlier in the maxilla. This is similar to studies done in the Gambia (Billewicz and McGregor, 1975), Ghana (Houpt et al. 1967), Kenya (Hassanali and Odhiambo 1989) and Congo (Rameriz Rozzi 2016). This may represent the general pattern for sub-Saharan Africa. However, in Nigerian females only the P^1 emerged earlier in the maxilla (Oziegbe et al. 2014) so some variation is present.

Other variants characterise the non-African populations. In the Iranian females, only the second premolar emerged earlier in the maxilla (Moslemi 2004). Data from the USA (Savara and Steen 1978) and Australia (Diamanti and Townsend 2003) European ancestry children show earlier or similar emergence times for the mandibular premolars compared with the maxilla. The reason for this variation might be due to higher decay and missing components of the DMFT in those populations (WHO 2000). Earlier loss of deciduous precursors of the premolars is more frequently due to dental caries in the mandible compared to the maxilla (Ahamed et al. 2012).

6.4.3 Sex variation in teeth emergence

Black Southern African females have all their teeth emerged earlier than males except for the third molars. The reason for this could be due to the variability of third molars because of impaction and agenesis. Although a similar trend was found in studies of other populations, (Kochhar and Richardson 1998; Eskeli et al. 1999; Moslemi 2004; Oziegbe et al. 2014), the difference was not always significant for all the teeth. The significantly earlier emergence times found in females could be due to the earlier maturation of females in general compared to males. The influence of environmental factors may also contribute to the difference. Females are more buffered from environmental influences compared to males and these is seen in the slightly higher incidence of stunting (Kruger et al. 2014) and delay in the emergence of permanent teeth seen in Black Southern African males than females.

We found that the largest difference in the timing of emergence between males and females is in the mandibular second premolar. In contrast, Kochhar and Richardson (1998) and Oziegbe et al. (2014) observed the greatest difference in the mandibular canine in the Northern Irish and Nigerian populations. Earlier emergence of permanent teeth in females is ascribed to an earlier commencement of maturation in general (Almonaitiene et al. 2010).

6.4.4 Temporal changes in the mean age of tooth emergence

The mean age of emergence from the present study is very similar to that obtained by Blankenstein et al. (1990a) for the same population**.** During the nearly 30-year interval between the two studies, South Africa underwent tremendous nutritional, socioeconomic and sociopolitical transformations that would presumably affect development. The lack of temporal changes in the timing of tooth emergence may be due to the relatively short time between the two studies. Similarly, Kutesa et al. (2013) and Oziegbe et al. (2014) did not find any secular trend in the mean age of permanent tooth emergence in Uganda and Nigerian populations. Others found secular trends in the mean age of tooth emergence in Japanese (Höuffding et al. 1984) and French (Rousset et al. 2003) populations. The differential prevalence of dental caries of primary teeth, and the quality of health care services between the generations (especially the generations before the advent of water fluoridation and fluoride containing dentifrices) could account for the secular trends reported.

6.4.5 Sequence of emergence times

The I_1/ M_I mandibular polymorphism between males and females in South Africa is in agreement with the findings by Smith and Garn (1987) for Black and White populations in the USA. They noted that the occurrence of the I_1M_I sequence in the mandible is more frequently encountered in males. This is contrary to the findings of this study, which shows the variation to be present among females. No reason can be readily adduced for this variation. A limitation of this study is the cross-sectional design, where individual sequence of emergence and types of polymorphism in sequence of emergence cannot be calculated. Notwithstanding, the commonest sequence of emergence is reported in this study.

The C1/P1 variation detected in the present study is consistent with the known variation in emergence of the canine teeth in the maxilla and mandible (Savara and Steen 1978; Diamanti and Townsend 2003: Khan 2011; Leroy et al. 2003). However, these classes of teeth (P_1 and C_1; P_2 and C^1) emerge in close succession in Southern African males and the females. This further supports the observation that variation in this aspect of the sequence is common both within and among populations. The reason for this is not clear; Harila-Kaera et al. (2003) suggest that the timing of tooth emergence is determined more by genetics than by the environment. Apart from this, children whose P1 precede their C1 in emergence are more likely to develop malocclusions related to tooth size arch size discrepancy (TSALD) later on (Moshkelgosha et al. 2014). The influence of the sequence of emergence on malocclusion in Southern African children is beyond the scope of this study. Therefore, more studies are needed to elaborate on this aspect of dental development.

The sequence of emergence in Southern African males is similar to that of the other sub-Saharan African countries compared here except Zambia. More research is needed to confirm whether the similarity is due to common ancestry of Bantu language speaking peoples. However, M1/I1 variation was seen in the Southern African females. The reason for the difference may be due to sample size and the method of analysis.

The sequence of emergence for the maxilla and mandible in males from Southern Africa is very similar to the sequence of emergence found in males of European ancestry in the USA (Savara and Steen 1978), Australia (Diamanti and Townsend 2003) and Belgium (Leroy et al. 2003) but differs from the Iranian and Pakistani males who have their maxillary P^1 and P^2 preceding the C^1. It is not clear why the same pattern appears in Southern Africans and the European ancestry populations. However, the reason for the similar sequence between European ancestry populations, as well as the similarity between Iran and Pakistan could be attributed to genetic, common ancestry and population affinity. Southern African, Australian and US females have similar sequences of emergence in the maxilla. Southern Africans display M_I/I_1 variation in the mandible with M_I I_1 being the commonest sequence of emergence among the Southern African females.

6.4.6 Population comparison of emergence times

Several reasons have been suggested for the variations in the timing of tooth emergence between and within populations, including genetic variation (Houpt et al. 1967; Garn et al. 1972, 1973a; Hassanali and Odhiambo 1981), socio-economic factors with their implicit connection to nutritional and health statuses (Lee et al. 1965; Kaul et al. 1975), fluoride use (Short 1944) and climate adaptation (Friedlaender and Bailit 1969). The general consensus is that genetic diversity is the main determinant of emergence age. When the mean emergence ages by tooth in Southern

Africa are compared with those of other populations, the mean emergence times in Southern Africa Black children are similar to the Nigerians (Oziegbe et al. 2014), the Gambians (Billewicz and McGregor 1975), Ghanaians (Houpt et al. 1967), Kenyans (Hassanali and Odhiambo 1989) and Ugandans (Krumholt 1971). The reason for the close emergence times is likely be due to genetic similarity because African populations experience diverse environments yet the timing is similar. Previous studies among populations of African ancestry have shown earlier timing of permanent tooth emergence compared to their European ancestry and Asian counterparts in the USA (Garn et al. 1972, 1973a) and England (Lavelle 1976; Stewart et al. 1982; Harris and McKee 1990; Koch and Poulsen 2001). This suggests genetic influence on variations in odontogenesis and eruption. Black Southern African children show earlier emergence ages for all permanent teeth than European ancestry children in the USA (Savara and Steen 1978), Iran (Moslemi 2004), Belgium (Leroy et al. 2003), and Australia (Diamanti and Townsend 2003). Southern African children are also advanced in the emergence of most permanent teeth compared to children from Pakistan (Khan 2011).

The permanent tooth emergence times in both Southern African males and females are later compared with the Baka population from Cameroon. Similarly, Baka people have earlier emergence times in most of their permanent teeth compared to other sub-Saharan African countries cited in the results although close emergence times in some teeth are found among these countries and the Baka people. The reason for the difference may be due to genetic divergence of the Bantu language speaking people from the Baka who are the descendants of the Late Stone Age hunter-gatherer peoples of the central African rainforest (Diamond and Bellwood 2003). They are the second most genetically diverse and tremendously divergent African population after the Khoisan peoples (Tishkoff et al. 2009). The initial divergence between ancestors of pygmies and non-pygmies occurred around 59,000 years ago (Verdu et al.2009). The closeness in the timing of emergence could be explained by genetic admixture between these hunter-gatherers and Bantu language speaking peoples. More research is needed to confirm this supposition.

6.5 Conclusion

The mean age of tooth emergence of Southern African children is similar to children from most other sub-Saharan African populations. Black Southern African children of both sexes show earlier mean ages of emergence than children from Europe and Asia. There is variation in the M1/I1 sequence between males and females and between South African females and populations of European ancestry.

Despite major socioeconomic and political changes in South Africa over the past two decades, no temporal change is seen in the mean age of emergence for Southern African Black children when compared to an earlier report on the same population. This suggests that earlier emergence of the permanent dentition in Black Southern Africans is part of a general sub-Saharan pattern of dental emergence, a pattern that is distinct from European and Asian populations.

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Contents

Chapter 7

Influence of nutrition on permanent tooth emergence

Abstract

Background: The relative immunity of tooth development from the effects of the environment, in comparison to skeletal development, is debated. While evidence shows that systemic stresses during the period of tooth formation lead to enamel hypoplasia and molar incisor hypomineralization, the effect of malnutrition in not clear.

Aim: This study investigated the effect of nutritional status (as measured by BMI, height, weight, mid-upper arm circumference and head circumference) on the timing of permanent tooth emergence and the number of emerged teeth in a sample of Black Southern African children.

Method: This was a cross-sectional study involving 639 (266 males, 373 females) healthy children aged 5-20 years. The height and BMI were converted to z-scores using the WHO z-scores for age tables (WHO 1995). A cut-off z-score of <-2 for both BMI and Height for age (HAZ) was used to place children into the categories of underweight/short for age, normal weight/height for age and overweight/obese/tall for age. ANOVA and a Games Howell procedure were used to determine the differences between the categories. Multivariate analysis was performed to determine the effect of the anthropometric variables on the number of teeth emerged. Statistical significance was inferred at $p<0.05$.

Results: Males who are overweight/obese generally show significantly earlier tooth emergence times than those who are severely underweight ($p<0.05$). Overweight/obese females generally show significantly earlier tooth emergence times than normal weight/ height females ($p<0.05$). Females have significantly more emerged teeth than males ($p<0.05$) and taller children have more emerged teeth than shorter children when corrected for age and sex ($p<0.05$). The

generalized linear regression model (negative binomial) shows that height, weight and BMI have significant associations ($p<0.05$) with the number of emerged teeth.

Conclusion: Height, weight and BMI significantly influence the timing of tooth emergence and the number of emerged permanent teeth in Southern African Black children. Obese and overweight children are more advanced in the timing of emergence and had more emerged teeth than underweight individuals. No relationship was found between head circumference or mid-upper arm circumference and the number of emerged teeth. These results challenge the notion that dental development is relatively immune to environmental stresses.

7.1 Introduction

Tooth emergence and tooth maturation are fundamental components of a child's growth pattern that are frequently used to assess dental development and physiological age (Al-Jasser and Bello 2003; Helm and Seidler 1974; Holman and Jones 2003). Of the two dental measures, tooth emergence is more variable because it is affected by retention or early loss of primary teeth as well as odontogenic infections (Holt et al. 2001). Furthermore, there is substantial evidence that dental development shows less variability and also low variability in relation to calendric age (Demirjian et al. 1985; Demirjian 1986; Green 1961; Holt et al. 2001) and relative to other growth events such as the appearance of bone ossification centers (Lewis and Garn 1960).

The relative immunity of dental development from the effects of the environment, in comparison to skeletal development, is debated. While some studies have shown that tooth formation is a very reliable measure of chronological age (Elamin and Liversidge 2013; Poureslami et al. 2015), there is overwhelming evidence for variability in the relationship between tooth emergence and age even within the same population (Clements et al. 1957; Garn et al. 1965; Garn et al. 1973). Researchers explain this plasticity in the timing of tooth emergence as due to an array of factors, including genetic-based population variation, hormonal influence, geographical location (Adler 1963; Lee et al. 1965; Tanguay et al. 1984), economic status (Enwonwu 1973), and gross malnutrition (Holman and Yamaguchi 2005). Previous studies investigating the relationship between tooth emergence and malnutrition used height, weight, Body Mass Index (BMI) and head circumference (HC) as proxies of nutritional status. None investigated whether another important indicator of malnutrition, mid-upper arm circumference (MUAC), correlates with tooth emergence. This study focuses on nutritional correlates (weight, height, BMI, head circumference (HC), and mid-upper arm circumference (MUAC)) and their relationship with tooth emergence.

7.1.1 Sex and tooth emergence

Sex-based variations have been observed in permanent tooth emergence, with most studies (Nanda 1960; Akpata 1971; Eskeli et al. 1999; Moslemi 2004) reporting female advancement. This variation has been credited to earlier onset of development in females (Boas 1927; Magnússon 1982; Demirjian 1986; Holman and Jones 2003). Pertaining specifically to sub-Saharan African children, Kutesa et al. (2013) found Ugandan females to be ahead of their male counterparts. In a Nigerian study, females were found to precede males in the emergence of all teeth with one notable exception the mandibular central incisor (Oziegbe et al. 2014). A previous study from South Africa showed females to be ahead in emergence (Blankeinstein et al. 1990), but not all of the permanent teeth were investigated. This study investigates sexual dimorphism in the timimg of emergence of all permanent teeth in Black Southern Africans.

7.1.2 Body Mass Index (BMI) and tooth emergence

Obesity has been shown to accelerate growth and it affects almost every system involved in growth (Must et al. 2006). For example, obese children attain puberty earlier than underweight children (Aksglaede et al. 2009) and are significantly taller than their underweight counterparts (He and Karlberg 2001). The data on BMI and tooth emergence generally follow this pattern. Earlier studies of African Americans and European Americans (Clements et al. 1957; Garn et al. 1965; Garn et al. 1973) showed that children from high socioeconomic backgrounds had earlier tooth emergence, which was attributed to differences in health care and nutrition. Furthermore, obesity and being overweight are significantly and positively associated with the number of emerged teeth (Sánchez-Pérez et al. 2010; Must et al. 2012). Sánchez-Pérez et al. (2010) found this association to have occurred specifically during the mixed dentition period before puberty; however, there was catch-up by the non-obese after reaching puberty. Yet these findings cannot be generalized to all populations. For example, Oziegbe et al. (2009) did not find a consistent difference in the timing of primary tooth emergence among three Nigerian socioeconomic classes.

7.1.3 Weight, height and tooth emergence

Height and weight are the physical manifestations of growth and development that are utilized most frequently in diagnostic procedures and in growth assessment. It is expected that any delay or disruption to these components of growth should have a similar effect on tooth emergence. Even so, the relationship between weight or height and tooth emergence remains unclear.

Overweight and obese children, in comparison to normal weight peers, have accelerated growth affecting the timing of puberty (Aksglaede et al. 2009) and their height curves (He and Karlberg 2001). Hence, it is expected that increased weight should lead to earlier timing of tooth emergence (and associated increased stature). A few studies describe a relationship between emergence times and the weight and height of children (Green 1961; Almonaitiene et al. 2010), while others found no such effects (Robinow et al. 1942; Lysell et al. 1962; Kutesa et al. 2013).

Considerable delay in dental age and bone age, as compared to chronological age, was reported in underweight children (Green 1961; Garn 1965; Keller et al. 1970; Infante and Owen 1973; Takano et al. 1986; Sarnat et al. 1988; Vallejo-Bolaños et al. 1999; Haddad and Pires Correa 2005; Kumar et al. 2013). Some studies found children with below average weight and height have later emergence times than those who are within the normal range (Billewicz and McGregor 1975; Triratana and Kiatiparjuk 1989). Khan (2011) reported that tall children exhibited delayed tooth emergence irrespective of their weight while heavy and short children had early emergence. Haddad and Pires Correa (2005) and Oziegbe et al. (2009), in studies of Brazilians and Nigerians respectively, found that height and age correlated with the number of erupted teeth.

7.1.4 Mid-upper arm circumference (MUAC) and tooth emergence

Adult MUAC is known to reflect changes in body weight (Ohlson et al. 1956), and the major contributors to MUAC variation, namely muscle and sub-cutaneous fat, are both important determinants of survival in starvation (Leiter and Marliss 1982; Leiter and Marliss 1983). MUAC is also useful for the assessment of nutritional status in children (Briend et al. 1986; Jelliffe and Jelliffe 1969; Shakir 1975; Velzeboer et al. 1983; WHO 1986). It is good at predicting mortality and in some studies MUAC alone (Alam et al. 1989; Briend and Zimicki 1986; Vella et al. 1994), or MUAC for age (Chen et al. 1980), predicted death in children better than any other anthropometric indicator.

As MUAC is less affected than BMI by the localized accumulation of the excess fluid (pedal edema, periorbital edema, ascites) commonly seen in famine conditions, it is likely to be a more sensitive index of tissue atrophy than low body weight. It is also relatively independent of height (Olukoya 1990). The influence of MUAC on tooth emergence has not been evaluated in Southern African children.

7.1.5 Head circumference (HC) and tooth emergence

Head circumference is a proven proxy for brain development and it is proportional to brain weight and volume in infants. Vejdani et al. (2015) found a relationship between primary tooth emergence and head circumference. Infante and Owen (1973) also demonstrated significant associations between the total number of primary teeth and head circumference in males but not in females. A study by Godfrey et al. (2001) on a large range of primates found brain development to be a better predictor of tooth emergence than somatic development. However, the relationship of HC with permanent tooth emergence in humans has not been evaluated. While brain size increase is greatest during the early years of development, there is continued growth throughout childhood. It is unknown how brain growth correlates with permanent tooth emergence during the formative years when children are learning new skills.

In view of the controversies surrounding the effect of the above factors on tooth emergence, there is a need to investigate the influence of these anthropometric measures in African children. Most of the literature on tooth formation and emergence is essentially from outside Africa, which may not be directly applicable to African populations due to environmental differences.

7.2 Materials and Methods

This is a cross-sectional study of 639 clinically healthy black Southern African children aged 5-20 years, whose parents and grandparents are indigenous Southern Africans. Children who met these inclusion criteria were randomly selected from those being treated by the Community Oral Health Outreach Program (COHOP) of the Department of Community Dentistry, University of the Witwatersrand. Ethical approval (NO.M141001) was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. Permission to carry out the study was obtained from the local education authority and respective school heads. Consent was obtained from the parents and assent was obtained from the children.

Date of birth and sex were collected from the dental records of the children. All selected participants were examined on a dental chair in a mobile dental van. Intra oral examination was done with a sterile wooden spatula. Teeth present in the mouth were recorded by the principal investigator using *Fédération Dentaire Internationale (*FDI) notation. An emerged tooth was defined as a tooth with any part of its crown penetrating the gingiva and visible in the oral cavity (Al-Jasser and Bello 2003). Extracted teeth were considered to have emerged. Panoramic radiographs of all participants were taken with a Carestream CS8100 Access digital x-ray fitted into the dental van. After viewing of the images, children with agenesis of lateral incisors or third molars were excluded from the study.

7.2.1 Data collection

Participants were weighed in the standing position on a Hana Power platform scale, calibrated to a precision of 100g. Height was measured with an anthropometric standiometer (Weylux model 424) with the horizontal headboard making contact with the uppermost point of the head. The height was recorded to the nearest 0.1 cm. Mid-upper arm circumference (MUAC) of the left upper arm was measured with a tape measure at the mid-point between the tip of the shoulder and the tip of the elbow (the acromion and olecranon process) and recorded to the nearest 0.1 cm. Head circumference was measured by placing the tape across the forehead and measuring around the fullest circumference of the head.

The study was pilot tested on 40 randomly selected students. Intra-examiner test and retest reliability of measurements were calculated using Lin's Concordance Correlation Coefficient (height= 0.99, head circumference= 0.92, mid-upper arm circumference= 0.96).

7.2.2 Statistical Analyses

Data were analyzed with Stata 12 for Windows. The analysis included frequencies and cross-tabulations. Associations between categorical variables were tested with chi square while those between continuous variables were tested with Student's *t*-tests. Non-parametric equivalents were used as appropriate. The mean age at the time of emergence and the standard deviation were computed using probit analysis and compared using Student’s *t*-tests, after Liversidge (2003). Body mass index (BMI) was calculated from the height in meters and weight in kilograms. The height and BMI were converted to z-scores using the WHO z-scores for age tables (WHO 1995). A cut-off z-score of ≤−2 for BMI was used to place children into underweight/short for age, ≥-2 to 2.0 for normal weight, and ≥2 for overweight/obese and tall for age. Mean age of emergence was calculated for each tooth using these BMI subdivisions. Analysis of variance (ANOVA) was used to determine if any variation exists between the subdivisions. Post hoc analysis was done using Games-Howell multiple comparison of means. A Student’s *t*-test was used to compare any two means whenever one of the three subdivisions of BMI did not yield a mean age of emergence. A Spearman’s rho correlation analysis between total number of teeth emerged and the anthropometric variables was done. A Shapiro-Wilk W test showed that the dependent variable (total number of teeth emerged) and the predictor variables were not normally distributed. Therefore, a generalized linear model (negative binomial) was used with the number of emerged teeth modelled as the dependent variable and anthropometric variables and age as predictors. Adequacy of fit was checked using the deviance residuals as recommended by M^{c}Cullagh and Nelder (1989). The deviance residuals showed that it was normally distributed and the plot of the residuals against each of the covariates also showed model fit. As expected, the collinearity test showed that BMI, height and weight were significantly collinear. When these variables were excluded from the model, there was no difference in the values of the output. Hence, the variables were included in the final model for generalized linear regression analysis. The model was built using forward selection. Statistical significance was inferred at $p<0.05$.

7.3 Results

There are more females than males in the study population (p=0.037) (Table 7.1). The overall mean age was 11.88±3.57 years. The mean age for males is 11.52±3.66 years and for females, 12.4±3.48 years. There is a significant difference between the mean ages of males and females (p=0.022).

The mean weight of the children is 39.29±15.57 kg while the mean height is 143.12±17.52 cm. There were significant sex differences in the mean weight, MUAC and BMI with females having higher values than males (p<0.05) (Table 7.2). Conversely, the mean height and HC do not differ significantly between males and females.

Table 7.1. Age and sex distribution of participants

Age group (years)	**Sex**				**Total**	
	Male		**Female**			
	n	**%**	**n**	**%**	**N**	**%**
5.00-5.99	10	1.6	13	2.0	23	3.6
6.00-6.99	27	4.2	28	4.4	55	8.6
7.00-7.99	6	0.9	9	1.4	15	2.3
8.00-8.99	32	5.0	27	4.2	59	9.2
9.00.9.99	34	5.3	30	4.7	64	10.0
10.00.10.99	15	2.3	22	3.4	37	5.8
11.00-11.99	17	2.7	36	5.6	53	8.3
12.00-12.99	20	3.1	44	6.9	64	10.0
13.00-13.00	15	2.3	39	6.1	54	8.5
14.00-14.00	29	4.5	33	5.2	62	9.7
15.00-15.99	25	3.9	27	4.2	52	8.1
16.00-16.99	11	1.7	28	4.4	39	6.1
17.00-17.00	20	3.1	25	3.9	45	7.0
18.00-20.00	5	0.8	12	1.9	17	2.7
Total	**266**	**41.6%**	**373**	**58.4%**	**639**	**100.0%**

Table 7.2. Mean anthropometric values for males and females

Variable	Male n=266				Female n=373				Total N=639	
	Min	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Mean**	**SD**
Age*	5.00	22.00	11.52	3.6	5.00	22.00	12.14	3.47	11.88	3.57
Height	104.00	190.40	141.77	19.28	103.50	173.50	144.08	16.11	143.12	17.52
Weight*	14.00	90.00	36.21	14.21	13.00	99.00	41.49	16.13	39.29	15.57
HC	40.70	64.00	50.71	2.51	40.60	63.50	50.36	2.53	50.51	2.53
MUAC*	13.00	30.00	18.72	2.08	14.00	30.50	19.66	2.60	19.27	2.44
BMI*	12.02	32.50	17.30	3.09	7.13	36.81	19.20	4.69	18.41	4.21
# teeth emerged*	0.00	32.00	19.05	9.31	0.00	32.00	22.02	8.40	20.78	8.90

*****Significant difference between males and females ($p<0.05$)

7.3.1 Influence of BMI on emergence

More males are in the underweight category compared to the females; only two females can be classified as underweight (Tables 7.3 and 7.4). Males who are overweight/obese generally show significantly earlier emergence times than those who are severely underweight ($p<0.05$). Furthermore, males of normal weight have significantly earlier emergence of most teeth compared to the underweight males. Males who are overweight displayed advanced timing of emergence of all teeth compared to normal weight children (Table 7.3).

In the females, timing of emergence was not calculated for the underweight cohort because of the limited sample. However, the Student's *t*-test showed that the overweight children have advanced dental emergence compared to normal weight children for the second premolar and second molar in the maxilla, and the canine, first premolar and second molar in the mandible ($p<0.05$) (Table 7.4).

Table 7.3. Mean age of emergence of teeth by BMI z-score category (Males)

Maxilla							**Mandible**						
Tooth	**BMI Category**	**n**	**Age**	**SD**	**F/t**	**p**	**Tooth**	**BMI Category**	**n**	**Age**	**SD**	**F/t**	**p**
$\mathbf{I^1}$	Underweight	22	-	-	t=4.88	**0.00**	$\mathbf{I_1}$	Underweight	22	-	-	-	-
	Normal	223	6.97	0.85				Normal	223	6.06	0.33		
	Overweight	21	6.03	0.78				Overweight	21	-	-		
$\mathbf{I^2}$	Underweight	22	8.33	1.04	t=1.61	0.11	$\mathbf{I_2}$	Underweight	22	-	-	t=2.74	**0.01**
	Normal	223	7.95	1.06				Normal	223	6.96	0.79		
	Overweight	21	-	-				Overweight	21	6.40	1.67		
$\mathbf{C^1}$	Underweight	22	11.73	1.69	F=4.80	**0.01**	$\mathbf{C_1}$	Underweight	22	-	-	t=2.36	**0.01**
	Normal	223	11.20	1.69				Normal	223	10.38	1.69		
	Overweight	21	10.22	0.99				Overweight	21	9.49	1.14		
$\mathbf{P^1}$	Underweight	22	11.73	1.69	F=12.71	**0.00**	$\mathbf{P_1}$	Underweight	22	12.88*	1.11	F=22.63	**0.00**
	Normal	223	10.29	1.20				Normal	223	10.77*	1.45		
	Overweight	21	10.18	1.75				Overweight	21	10.53*	1.52		
$\mathbf{P^2}$	Underweight	22	12.98	1.52	F=21.26	**0.00**	$\mathbf{P_2}$	Underweight	22	13.70	0.97	F=28.91	**0.00**
	Normal	223	11.08	1.35				Normal	223	11.05	1.64		
	Overweight	21	10.59	1.52				Overweight	21	10.85	1.41		
$\mathbf{M^1}$	Underweight	22	-	-	t=2.71	**0.01**	$\mathbf{M_1}$	Underweight	22	-	-	-	**-**
	Normal	223	6.27	0.33				Normal	223	6.16	0.24		
	Overweight	21	6.03	0.78				Overweight	21	-	-		
$\mathbf{M^2}$	Underweight	22	14.40	0.41	F=34.42	**0.00**	$\mathbf{M_2}$	Underweight	22	14.05	2.27	F=14.96	**0.00**
	Normal	223	12.68	1.33				Normal	223	12.57	1.67		
	Overweight	21	11.17	1.32				Overweight	21	11.17	1.70		

Significant values are in bold.

The Games-Howell test for post ANOVA pair-wise comparison of means is significant between overweight and underweight group for all teeth ($p<0.05$).

Student's *t*-tests calculated where two means are available for comparison.

Probit analysis did not return values for the empty cells.

Table 7.4, Mean age of emergence of teeth by BMI category (Females)

Maxilla							**Mandible**						
Tooth	**BMI Category**	**n**	**Age**	**SD**	**T**	**p**	**Tooth**	**BMI Category**	**n**	**Age**	**SD**	**t**	**p**
I^1	Underweight	2	-	-	-	-	**I_1**	Underweight	2	-	-	-	-
	Normal	324	6.46	0.73				Normal	324	5.48	1.00		
	Overweight	47	-	-				Overweight	47	-	-		
I^2	Underweight	2	-	-	-	-	**I_2**	Underweight	2	-	-	-	-
	Normal	324	7.52	-				Normal	324	6.83	1.67		
	Overweight	47	-	-				Overweight	47	-	-		
C^1	Underweight	2	-	-			**C_1**	Underweight	2	-	-		
	Normal	324	10.42	1.44	0.77	0.44		Normal	324	9.46	1.14	5.89	**0.00**
	Overweight	47	10.23	2.33				Overweight	47	8.00	3.33		
P^1	Underweight	2	-	-			**P_1**	Underweight	2	-	-		
	Normal	324	9.73	1.41	0.00	1.00		Normal	324	10.17	1.34	9.29	**0.00**
	Overweight	47	9.73	0.42				Overweight	47	8.25	1.21		
P^2	Underweight	2	-	-			**P_2**	Underweight	2	-	-		
	Normal	324	10.78	1.49	2.17	**0.03**		Normal	324	10.47	1.34	1.30	0.19
	Overweight	47	10.30	0.69				Overweight	47	10.20	1.21		
M^1	Underweight	2	-	-	**-**	**-**	**M_1**	Underweight	2	-	-	-	-
	Normal	324	6.13	0.89				Normal	324	5.44	1.12		
	Overweight	47	-	-				Overweight	47	-	-		
M^2	Underweight	2	-	-			**M_2**	Underweight	2	-	-		
	Normal	324	12.12	1.37	2.27	**0.02**		Normal	324	11.77	1.61	4.53	**0.00**
	Overweight	47	11.65	0.94				Overweight	47	10.68	0.94		

Significant values are in bold.
Probit analysis did not return values for the empty cells.

7.3.2 Number of emerged teeth by sex

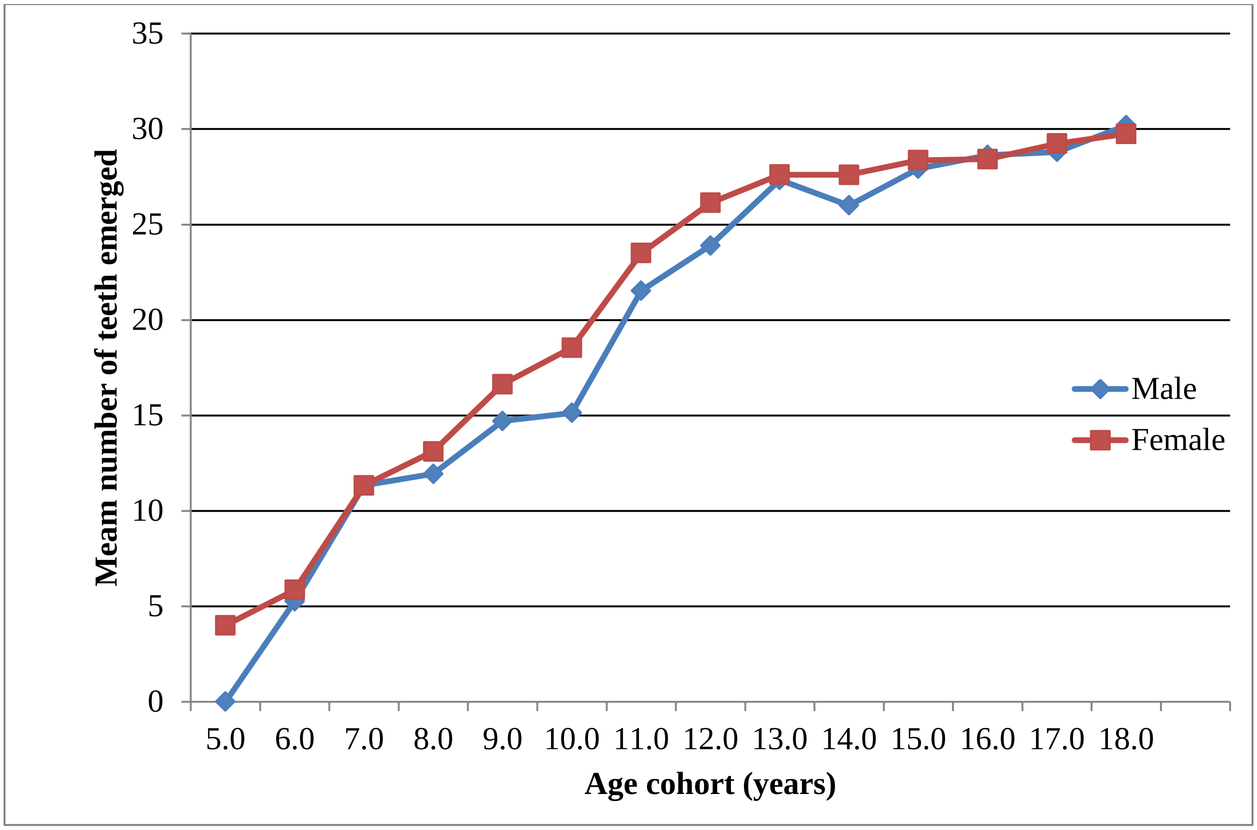
The mean number of emerged teeth in females is significantly greater than males ($p<0.05$) (Table 7.5). After correcting for age, females have more emerged teeth than males from age 5 to 15 years, after which no specific pattern occurs. Statistically significant differences were noted for ages 5, 10, 12 and 14 years ($p<0.05$) (Table 7.5). None of the male participants had any of their permanent teeth before the age of 5 years, whereas some of the females did (Table 7.5). A graphic representation of the number of emerged teeth by sex shows that females start emergence far earlier than their male counterparts. The males soon catch up at age 6 years, but the females are again ahead between ages 7 to 13 years, after which the males again catch up (Figure 7.1).

Table 7.5. Mean number of emerged teeth by sex and age

Age cohort (years)	Sex	n	Mean # teeth emerged	SD	p	95% CI
5 - 5.99	M	10	0.00	0.00	**0.00**	[-5.95, -2.02]
	F	13	4.00	2.94		
6 - 6.99	M	27	5.26	5.66	0.63	[-3.11, 1.91]
	F	28	5.86	3.36		
7 -7.99	M	6	11.33	3.08	1.00	[-2.81, 2.81]
	F	9	11.33	2.00		
8 - 8.99	M	32	11.94	2.55	0.06	[-2.40, 0.06]
	F	27	13.11	2.08		
9 - 9.99	M	34	14.71	3.64	0.06	[-3.93, 0.08]
	F	30	16.63	4.37		
10 -10.99	M	15	15.13	3.52	**0.03**	[-6.42, -0.40]
	F	22	18.55	4.94		
11 - 11.99	M	17	21.53	5.41	0.15	[-4.66, 0.72]
	F	36	23.50	4.11		
12 -12.99	M	20	23.90	4.01	**0.02**	[-4.15, -0.32]
	F	44	26.14	3.33		
13 - 13.99	M	15	27.33	1.29	0.60	[-1.34, 0.78]
	F	39	27.62	1.87		
14 - 14.99	M	29	26.00	3.11	**0.01**	[-2.78, -0.43]
	F	33	27.61	1.22		
15 - 15.99	M	25	27.92	0.57	0.11	[-1.01, 0.11]
	F	27	28.37	1.28		
16 -15.99	M	11	28.64	1.43	0.64	[-0.69, 1.11]
	F	28	28.43	1.17		
17 -17.99	M	20	28.80	1.61	0.41	[-1.50, 0.62]
	F	25	29.24	1.85		
18 -18.99	M	5	30.20	2.05	0.64	[-1.56, 2.46]
	F	12	29.75	1.66		

Significant values in bold.

Figure 7.1. Mean number of emerged teeth by age and sex



7.3.3 Height and number of emerged teeth

Table 7.6 provides the comparative analysis of the mean height between the males and females in different age cohorts. There is no consistent pattern of variation in the mean height of males and females until the age of 14 years, after which males are taller. Statistically significant differences occur between males and females in the age cohorts 12, 15, 16, and 17 years, with the males being taller than their female counterparts at all ages except age 12 ($p<0.05$). The number of emerged teeth has a significant relationship with height in both males and females ($R^2=0.89$ for each) (Table 7.7). The trend line in Figure 7.2 shows that females had more emerged teeth than their male counterparts of the same height.

Table 7.6. Comparison of height by sex, controlling for chronological age

Age (years)	Sex	n	Mean height (cm)	SD	p	95% CI
5 - 5.99	M	10	109.72	2.99	0.06	[-7.14, 0.15]
	F	13	113.22	4.87		[-6.93, -0.06]
6 - 6.99	M	27	116.04	11.38	0.35	[-2.44, 6.73]
	F	28	113.90	4.02		[-2.58, 6.86]
7 -7.99	M	6	120.73	8.43	0.50	[-12.83, 6.54]
	F	9	123.88	8.55		[-12.99, 6.70]
8 - 8.99	M	32	127.44	5.35	0.41	[-4.58, 1.88]
	F	27	128.79	7.04		[-4.67, 1.97]
9 - 9.99	M	34	131.74	5.32	0.96	[-2.83, 2.99]
	F	30	131.66	6.33		[-2.87, 3.03]
10 -10.99	M	15	134.30	6.19	0.18	[-7.71, 1.63]
	F	22	137.34	7.28		[-7.57, 1.49]
11 - 11.99	M	17	141.16	5.66	0.09	[-6.24, 0.41]
	F	36	144.08	5.62		[-6.31, 0.47]
12 -12.99	M	20	145.19	8.72	**0.01**	[-9.37, -1.59]
	F	44	150.67	6.45		[-9.93, 1.02]
13 - 13.99	M	15	149.77	8.78	0.07	[-9.10, 10.30]
	F	39	154.47	6.54		[-9.90, 0.51]
14 - 14.99	M	29	157.70	9.65	0.56	[-2.93, 5.38]
	F	33	156.48	6.61		[-3.05, 5.50]
15 - 15.99	M	25	162.12	7.99	**0.01**	[1.42, 8.87]
	F	27	156.97	5.18		[1.34, 8.95]
16 -15.99	M	11	166.41	5.78	**0.00**	[3.56, 12.39]
	F	28	158.43	6.25		[3.58, 12.37]
17 -17.99	M	20	167.27	8.28	**0.00**	[4.66, 13.93]
	F	25	157.97	7.13		[4.56, 14.03]
18 -18.99	M	5	166.50	8.54	0.09	[-0.87, 18.77]
	F	12	157.55	8.70		[-1.66, 19.56]

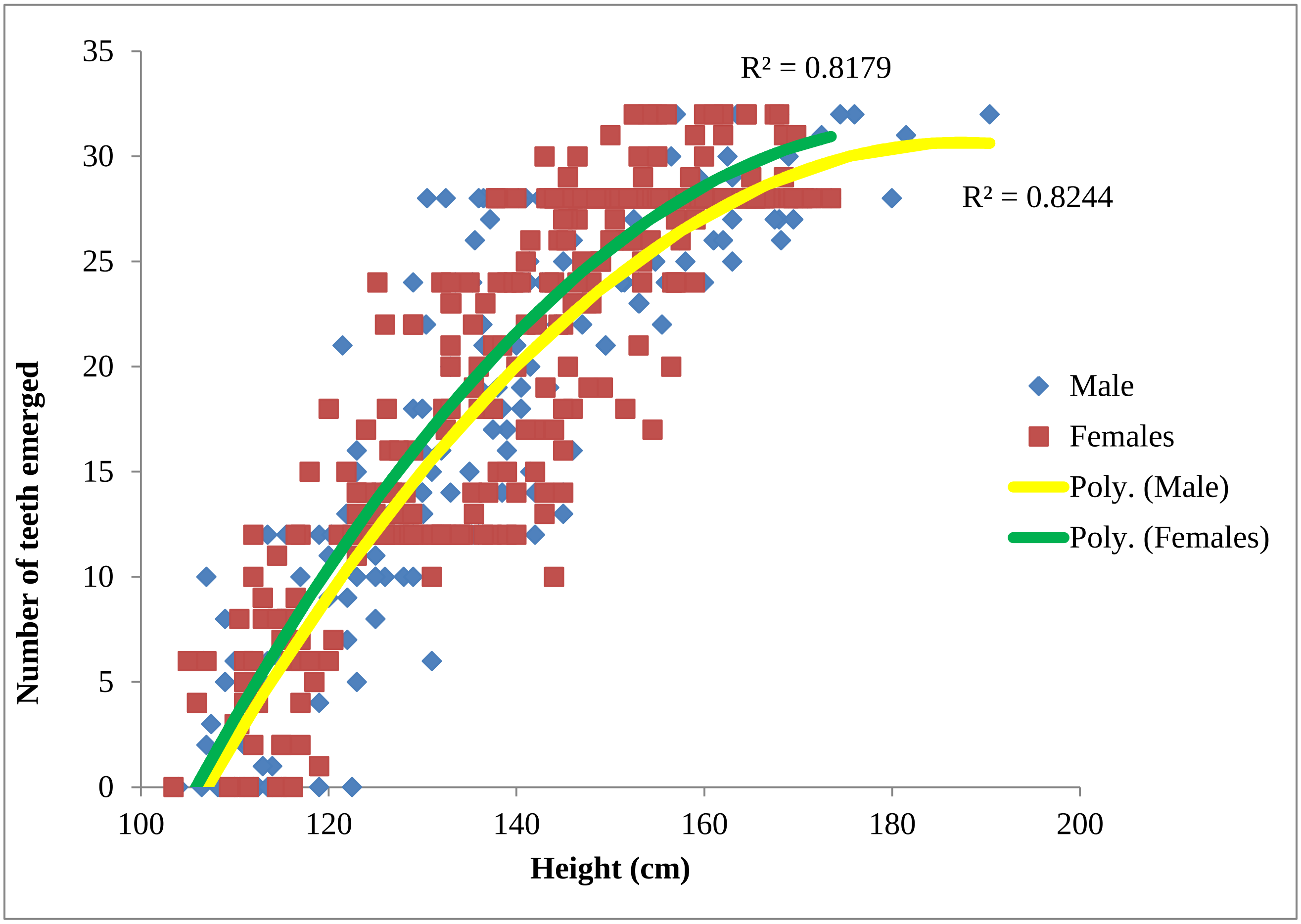
Significant values in bold.

Table 7.7. Correlation between anthropometric variables and number of emerged teeth

Variable	Males N=266		Females N=373	
	# emerged teeth		# emerged teeth	
	r	p	r	P
Height	0.89**	0.00	0.89**	0.00
Weight	0.79**	0.00	0.75**	0.00
HC	0.12*	0.05	0.38**	0.00
MUAC	0.57**	0.00	0.51**	0.00
BMI	0.44**	0.00	0.53**	0.00

**Significant difference at $p<0.001$
*Significant difference at $p<0.05$

Figure 7.2. Number of emerged teeth by height in males and females



7.3.4 Weight and number of emerged teeth

Generally, South African females weigh significantly more than males (Tables 7.2 and 7.8). Figure 7.3 shows that females have a greater number of emerged teeth than males of the same weight before they reach weights of 50 kg. Above 50 kg, catch up by males is seen. This weight of approximately 50 kg roughly corresponds to the age at which males also catch-up with the females in terms of their number of emerged teeth (Table 7.5). It is important to note that females show more variability in the number of teeth emerged by weight compared to males (Figure 7.3).

Figure 7.3. Number of emerged teeth by weight in males and females

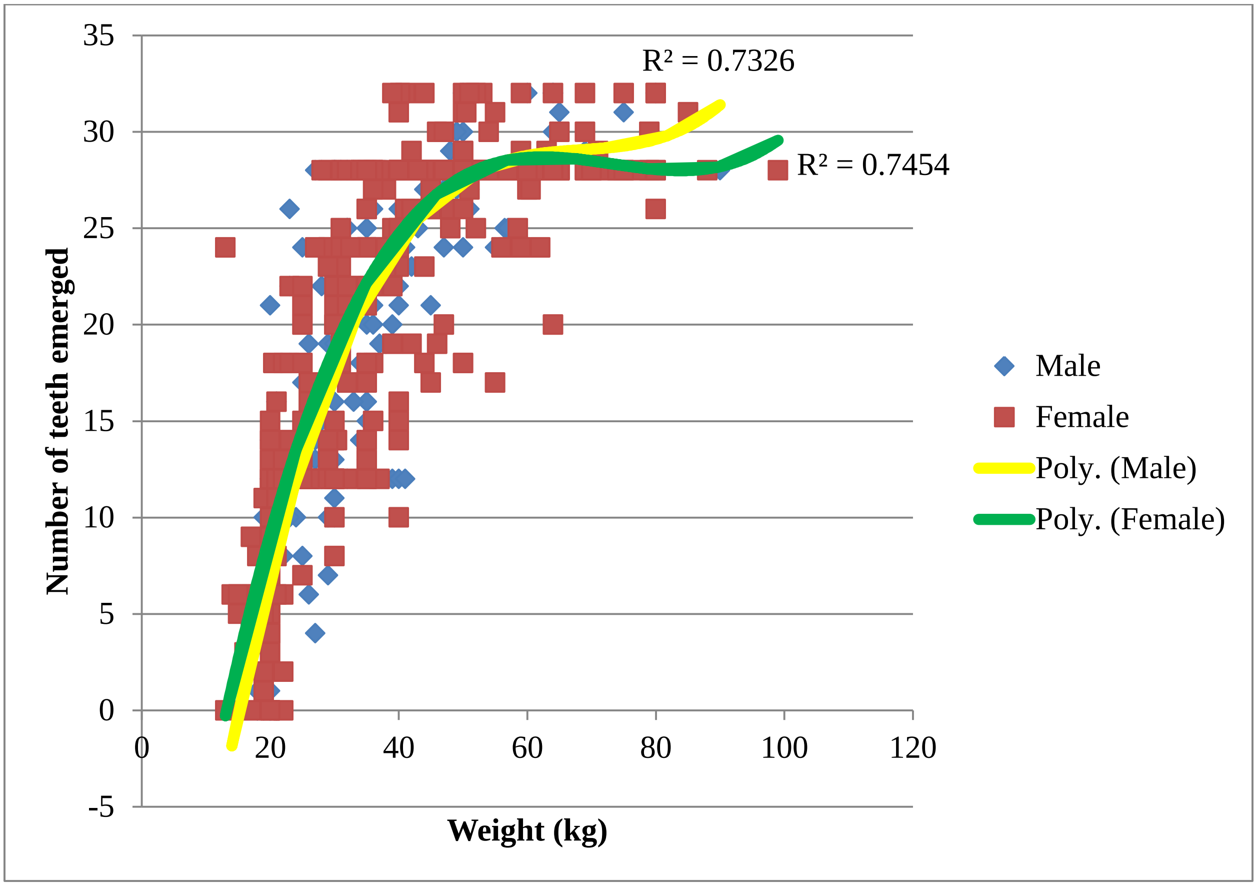


Table 7.8. Comparison of weight by sex, controlling for chronological age

Age (years)	Sex	n	Mean weight (kg)	SD	p	95% CI
5 - 5.99	M	10	18.10	2.08	0.26	[-3.66, 1.06]
	F	13	19.40	3.07		
6 - 6.99	M	27	21.15	5.55	0.24	[-1.01, 3.96]
	F	28	19.68	3.43		
7 -7.99	M	6	23.08	3.14	0.54	[-8.13, 4.49]
	F	9	24.90	6.61		
8 - 8.99	M	32	26.31	4.90	0.76	[-2.24, 3.06]
	F	27	25.91	5.26		
9 - 9.99	M	34	28.38	5.46	0.87	[-3.13, 2.67]
	F	30	28.61	6.14		
10 -10.99	M	15	28.33	4.29	**0.05**	[-8.27, 0.03]
	F	22	32.45	7.06		
11 - 11.99	M	17	34.48	3.43	0.10	[-7.30, 0.66]
	F	36	37.81	10.77		
12 -12.99	M	20	36.63	7.04	**0.00**	[-15.52, -5.24]
	F	44	47.01	10.45		
13 - 13.99	M	15	38.47	11.06	**0.01**	[-14.03, -2.43]
	F	39	46.70	8.88		
14 - 14.99	M	29	47.44	12.58	0.28	[-8.73, 2.54]
	F	33	50.54	9.56		
15 - 15.99	M	25	49.99	8.77	**0.04**	[-14.19, -0.46]
	F	27	57.31	14.85		
16 -15.99	M	11	55.36	7.78	0.71	[-9.11, 6.24]
	F	28	56.80	11.52		
17 -17.99	M	20	55.00	11.14	0.71	[-8.61, 5.91]
	F	25	56.35	12.64		
18 -18.99	M	5	56.00	9.77	0.61	[-16.53, 10.03]
	F	12	59.25	12.33		

Significant values in bold.

7.3.5 Mid-upper arm circumference and number of emerged teeth

There is a weak relationship between MUAC and number of emerged teeth when controlling for sex (Figure 7.4). The coefficients of variability are low for both males (R^2=0.33) and females (R^2=0.27). Females matched with males of similar MUAC values have a greater number of teeth

(Figure 7.4). In most cases, females have higher MUAC values than males with significant differences found at ages 5, 12 and 13 years ($p<0.05$) (Table 7.9).

Figure 7.4. Number of emerged teeth by mid-upper circumference in males and females

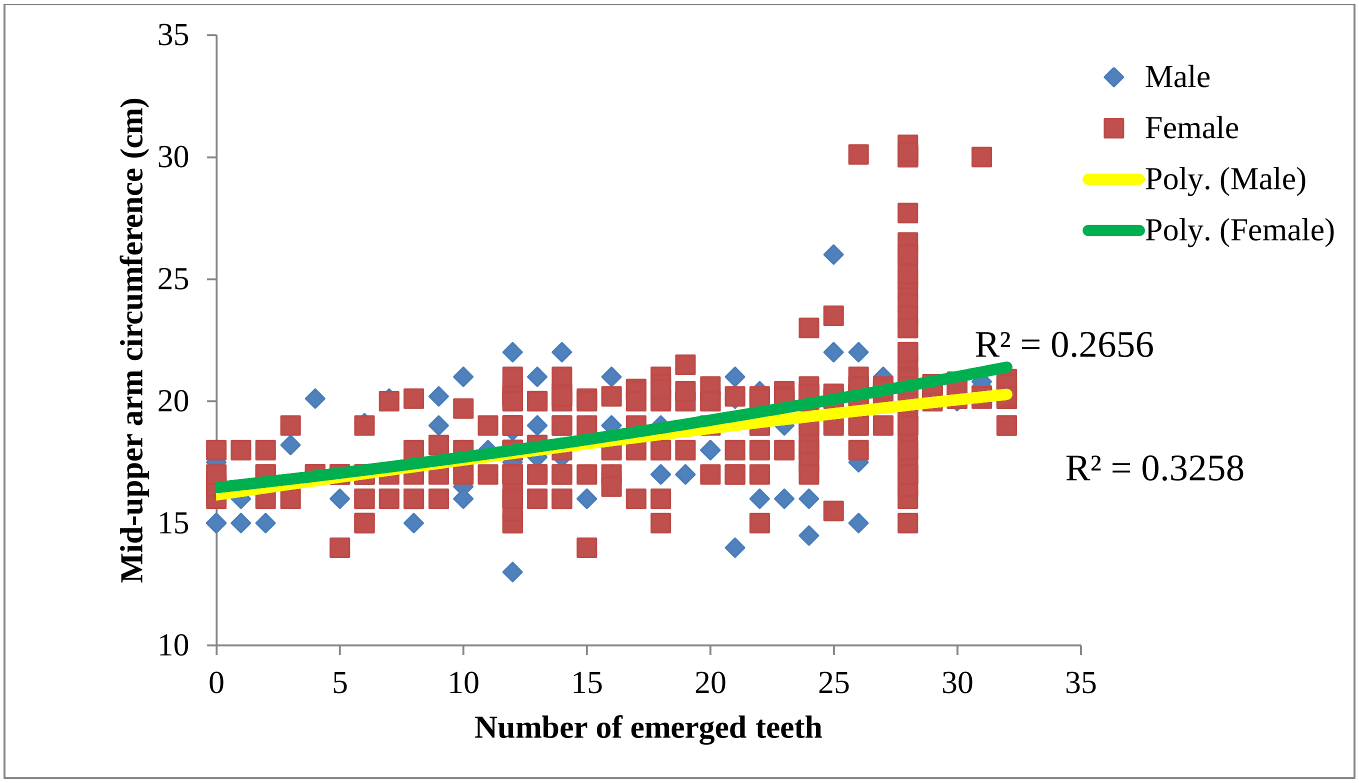


Table 7.9. Comparison of mid-upper arm circumference by sex, controlling for chronological age

Age (years)	Sex	n	Mean MUAC (cm)	SD	p	95% CI
5 - 5.99	M	10.00	15.90	0.74	**0.04**	[-2.06, 0.18]
	F	13.00	16.97	1.36		
6 - 6.99	M	27.00	16.78	1.67	0.66	[-1.00, 0.64]
	F	28.00	16.96	1.35		
7 -7.99	M	6.00	17.97	0.93	0.96	[-1.41, 1.48]
	F	9.00	17.93	1.44		
8 - 8.99	M	32.00	17.70	1.34	0.87	[-0.87, 0.74]
	F	27.00	17.77	1.75		
9 - 9.99	M	34.00	18.04	1.64	0.18	[-1.43, 0.28]
	F	30.00	18.62	1.79		
10 -10.99	M	15.00	18.71	2.47	0.41	[-1.75, 0.72]
	F	22.00	19.22	1.20		
11 - 11.99	M	17.00	19.36	0.84	0.48	[-1.14, 0.54]
	F	36.00	19.66	1.63		
12 -12.99	M	20.00	18.84	2.47	**0.02**	[-3.23, -0.23]
	F	44.00	20.57	2.91		
13 - 13.99	M	15.00	18.84	1.99	**0.04**	[-3.20, -0.09]
	F	39.00	20.48	2.73		
14 - 14.99	M	29.00	20.08	2.75	0.64	[-1.51, 0.93]
	F	33.00	20.36	2.04		
15 - 15.99	M	25.00	19.93	0.88	0.06	[-2.67, 0.06]
	F	27.00	21.23	3.29		
16 -15.99	M	11.00	20.27	0.50	0.42	[-2.27, 0.96]
	F	28.00	20.93	2.61		
17 -17.99	M	20.00	20.28	0.38	0.19	[-2.07, 0.43]
	F	25.00	21.10	2.75		
18 -18.99	M	5.00	20.44	0.36	0.80	[-0.29, 0.37]
	F	12.00	20.40	0.27		

Significant values in bold.

7.3.6 Head circumference and the number of emerged teeth

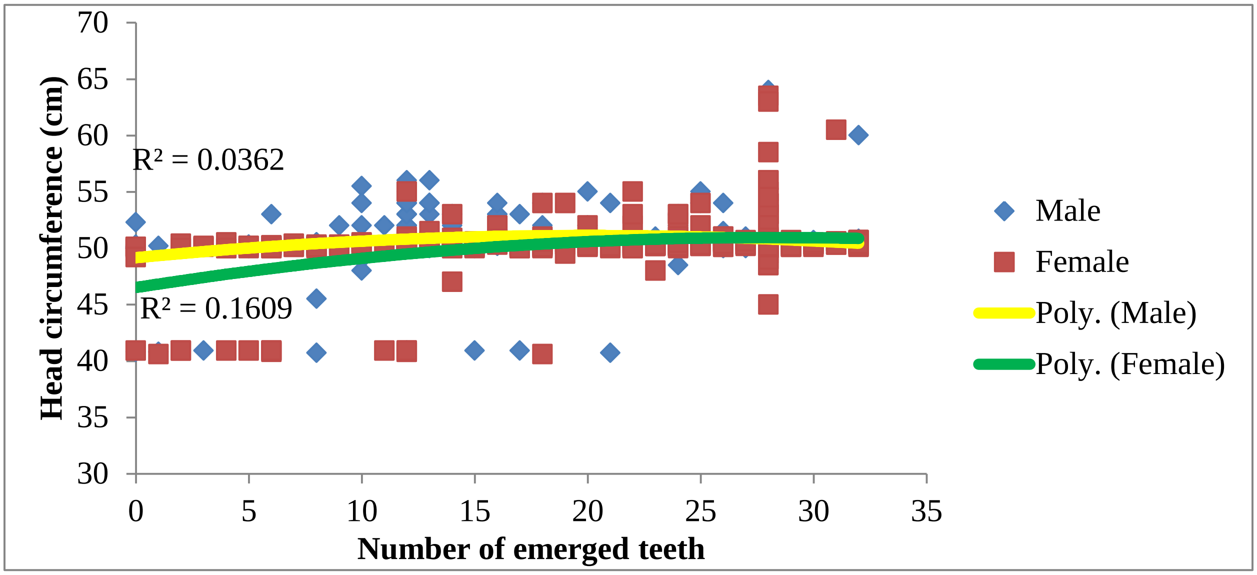
HC increases from ages 5 to 9 years in males and thereafter no further increase is seen. In females, HC growth occurs between ages 5 to 12 years. Males generally have larger, but mostly insignificantly larger, HCs compared to their female age counterparts (Table 7.10). No relationship exists between number of emerged teeth and head circumference when controlling for sex. The coefficient of variability of the number of emerged teeth and HC when controlling for sex is very low (R^2=0.04 and R^2=0.16 for males and females, respectively) (Figure 7.5).

Table 7.10. Comparison of head circumference by sex, controlling for chronological age

Age (years)	Sex	n	Mean HC (cm)	SD	p	95% CI
5 - 5.99	M	10	49.15	2.93	0.27	[-1.55, 5.27]
	F	13	47.29	4.49		
6 - 6.99	M	27	49.58	2.55	0.19	[-0.56, 2.82]
	F	28	48.45	3.60		
7 -7.99	M	6	50.68	1.14	0.28	[-1.41, 4.44]
	F	9	49.17	3.15		
8 - 8.99	M	32	51.08	3.45	0.06	[-0.04, 3.41]
	F	27	49.39	3.10		
9 - 9.99	M	34	51.68	2.54	**0.03**	[0.18, 2.91]
	F	30	50.13	2.93		
10 -10.99	M	15	50.00	4.05	0.87	[-1.93, 1.64]
	F	22	50.15	0.73		
11 - 11.99	M	17	51.07	1.32	0.94	[-1.22, 1.32]
	F	36	51.02	2.44		
12 -12.99	M	20	50.78	2.91	0.12	[-2.55, 0.28]
	F	44	51.91	2.49		
13 - 13.99	M	15	50.69	0.81	0.44	[-1.64, 0.73]
	F	39	51.14	2.21		
14 - 14.99	M	29	51.14	2.70	0.22	[-0.38, 1.69]
	F	33	50.53	0.82		
15 - 15.99	M	25	50.45	0.17	0.59	[-0.08, 0.14]
	F	27	50.42	0.21		
16 -15.99	M	11	50.45	0.17	0.33	[-0.07, 0.19]
	F	28	50.38	0.18		
17 -17.99	M	20	50.93	2.14	0.86	[-1.14, 0.37]
	F	25	50.81	2.03		
18 -18.99	M	5	50.48	0.13	0.14	[-0.05, 0.33]
	F	12	50.34	0.18		

Significant values in bold.

Figure 7.5. Number of emerged teeth by head circumference in males and females



7.3.7 Correlation analysis of the anthropometric variables and number of teeth emerged

Height and weight correlate significantly and strongly with the number of emerged teeth in both males (r=0.9, p=0.00 and r=0.8, p=0.00) and females (r=0.9, p=0.00 and r=0.8, p=0.00). MUAC and BMI show only moderate but significant correlations with the number of emerged teeth in males (r=0.6, p=0.00 and r=0.4, p=0.00) and females (r=0.5, p=0.00 and r=0.5, p=0.00). A moderately significant correlation is found between the number of emerged teeth and HC in females, (r=0.4, p=0.00), while it is weak but significant in males (r=0.1, p=0.00) (Table 7.7).

Correlational analysis was done for each age cohort to explore if the number of emerged teeth correlates with the anthropometric variables. No consistent pattern is seen across the age cohorts. However, height is significantly correlated with the number of the emerged teeth at ages 6, 8, 13 and 17 for males and ages 10 and 18 for females. Weight is only significantly correlated with tooth emergence at ages 6, 13, 17 and 18 for males. There is no such relationship for females at any age. HC correlates with number of teeth emerged for ages 7, 11, 12, 16 and 17 years for males. MUAC significantly correlates with number of teeth emerged at age 6 for males and 12 years for females. Moderate correlations are found between the number of emerged teeth and BMI in males and females (Table 7.11).

Table 7.11. Spearman's rho correlation between anthropometric variables and # teeth emerged, controlling for chronological age

Age (years)	Sex	n	Height	Weight	HC	MUAC	BMI
5 - 5.99	M	10	-	-	-	-	-
	F	13	0.10	0.36	0.10	0.86	0.92
6 - 6.99	M	27	0.75*	0.75*	0.15	0.43*	0.11
	F	28	0.20	0.32	0.17	0.07	0.25
7 -7.99	M	6	-0.20	-0.01	-0.88*	-0.56	0.17
	F	9	0.51	0.34	-0.12	0.07	.122
8 - 8.99	M	32	0.46*	0.16	0.25	-0.16	0.06
	F	27	0.01	-0.17	0.26	-0.22	0.24
9 - 9.99	M	34	0.30	0.23	-0.02	0.10	0.13
	F	30	0.16	0.33	0.07	0.27	0.30
10 -10.99	M	15	-0.13	-0.07	-0.30	-0.20	-0.02
	F	22	0.43*	0.35	0.32	0.25	0.18
11 - 11.99	M	17	0.46	-0.42	-0.51*	-0.36	-0.73
	F	36	0.22	0.09	0.12	-0.02	0.02
12 -12.99	M	20	0.12	0.19	0.45*	0.03	0.18
	F	44	0.25	0.25	0.26	0.35*	0.21
13 - 13.99	M	29	0.50*	0.37*	0.17	0.27	0.20
	F	33	0.23	-0.16	0.05	0.06	-0.28
14 - 14.99	M	25	-0.19	0.13	0.00	-0.01	0.28
	F	27	0.17	-0.15	0.29	-0.27	-0.21
15 - 15.99	M	25	-0.19	0.13	0.00	-0.01	0.28
	F	27	0.17	-0.15	0.29	-0.27	-0.21
16 -15.99	M	11	0.09	0.43	0.78**	0.52	0.36
	F	28	-0.30	-0.15	-0.14	-0.09	-0.02
17 -17.99	M	20	0.50*	0.70*	0.48*	0.37	0.54*
	F	25	0.17	0.24	0.23	-0.01	0.13
18 -18.99	M	5	0.17	0.94*	0.49	0.81	0.79
	F	12	0.68*	0.33	0.56	0.23	-0.25

*Significant difference at $p<0.05$
**Significant difference at $p<0.001$

7.3.8 Regression analysis of anthropometric variables (predictors) and number of emerged teeth (outcome)

The fact that a variable correlate significantly with another variable may not necessarily mean that there is a significant association or relationship. In a similar way, a significant relationship in a bivariate analysis neEds to be further tested with a multivariate analysis where confounders are controlled. Several variables in this study are correlated, therefore regression analysis was conducted to determine the relationships. In this model BMI, height and weight were significantly collinear. These variables were excluded from the model but no significant difference was noted in the output values. Therefore, the variables were included in the final model for generalized linear regression analysis. The model was built using forward selection. The regression model (negative binomial) shows that height, weight and BMI have significant associations with the number of emerged teeth in this study population ($p<0.05$) (Table 7.12). In these models, variables fitted were age, height, weight, HC, MUAC, BMI and the number of emerged teeth for males and females separately. The number of emerged teeth has a variance that is significantly greater than the mean and therefore does not correspond to a Poisson distribution. The goodness of fit test confirmed this. The generalized linear model (negative binomial) produced the best fit, judging by the normality of the deviance residuals.

Table 7.12. Generalized linear model (negative binomial) regression of predictors of emerged teeth

MALES

Variable	Coef	SE	Z	p	95% CI	
Age	0.09	0.04	2.08	**0.037**	0.01	0.17
BMI	0.20	0.09	2.31	**0.021**	0.03	0.38
Height	0.06	0.02	3.04	**0.002**	0.02	0.10
Weight	-0.09	0.04	-2.70	**0.007**	-0.16	-0.03
HC	0.01	0.03	0.45	0.651	-0.04	0.06
MUAC	0.01	0.05	0.14	0.888	-0.09	0.10
_cons	-7.73	2.86	-2.70	0.007	-13.33	-2.12

Goodness of fit Chi square (p = 0.102)

FEMALES

Variable	Coef	SE	Z	p	95% CI	
Age	0.04	0.01	6.92	**0.000**	0.03	0.06
BMI	0.15	0.07	2.29	**0.022**	0.02	0.28
Height	0.06	0.01	4.07	**0.000**	0.03	0.09
Weight	-0.06	0.03	-2.27	**0.023**	-0.12	-0.01
HC	0.02	0.02	0.97	0.332	-0.02	0.07
MUAC	0.01	0.03	0.30	0.761	-0.07	0.05
_cons	-6.67	2.20	-2.03	0.002	-10.98	-2.36

Goodness of fit Chi square (p = 0.498)
Significant values in bold.

7.4 Discussion

Dental development is thought to be a stable and reliable measure of growth and development because of its perceived relative immunity from environmental factors such as malnutrition, compared to the skeleton and other body systems. Well-designed studies specifically targeted at the timing of tooth emergence of children with severe malnutrition are lacking and difficult to undertake. This study considered dental emergence in a population of Black Southern African children with variable nutritional statuses, and found relationships between sex, height, weight, BMI, and the number of teeth emerged and the timing of emergence of individual teeth-- thus demonstrating that nutritional factors affect the timing of tooth emergence.

7.4.1 Influence of nutritional factors on the timing of emergence and number of emerged teeth

Socioeconomic or environmental factors are known to directly influence nutrition, with a resulting impact on child development including tooth emergence (Adler 1963; Lee et al. 1965). However, Friedlaender and Bailit (1969) argued that the environmental influence on emergence times of permanent teeth was relatively unimportant. More recently, Elamin and Liversidge (2013) suggested that there was no significant impact of malnutrition on tooth formation in their study of severely undernourished children in South Sudan. Their study did not consider the effects of nutritional deficiency on tooth emergence.

The present study did find a significant relationship between BMI and number of teeth emerged, with obese and overweight children having more teeth emerged. We also found that overweight children are significantly advanced in the timing of tooth emergence compared to the underweight children. This is similar to previous studies (Sánchez-Pérez et al. 2010; Must et al. 2012). Elevated BMI has been related to accelerated linear growth and early sexual maturation (Aksglaede et al. 2009; Sánchez-Pérez et al. 2010) and height (He and Karlberg 2001). Obesity is considered to be the most common cause of accelerated growth (Slyper 1998). There is evidence suggesting that over-nutrition during childhood causes hyperinsulinemia and may also increase insulin-like growth factor-1 (IGF-1) secretion and growth hormone receptors (Sinha et al. 2002). It is likely that the metabolic changes caused by obesity that are known to have an impact on bone growth also affect tooth emergence. However, more studies are required to identify a specific mechanism involved in tooth emergence timing that is affected by high body fat content in children and adolescents.

Another factor to consider in the relationship between BMI and tooth emergence is that emergence is affected by primary tooth loss (Smith 1991). If obese or overweight children lose more primary teeth to decay from higher consumption of carbohydrates, this might contribute to the differences in emergence timing among BMI groups observed in this study.

In the present study, bivariate analyses show significant differences in the mean ages of emergence by BMI categories in many instances. One of the limitations of bivariate analysis is that it is merely descriptive and does not take into account the influence of confounders. It does not factor in how one variable could influence another and therefore it cannot give an explanation for the relationship between two variables. Therefore, explanatory analysis is needed to infer cause and effect (Spicer 2005). The multiple regression analysis (negative binomial model) used in the study documents that proxies of nutrition, such as height, weight and BMI, have significant effects on the number of teeth emerged. Children who are obese or overweight have a greater number of emerged teeth than the normal and underweight children.

A limitation of our study is the small sample of participants in the underweight category. Very few of the Southern African females were classified as underweight compared to the males. This could be viewed as lending support to the hypothesis that females are more buffered from environmental stressors. This hypothesis is based on evidence for sex differences in pre- and postnatal mortality and morbidity (McMillen 1979; Waldron 1983), differential responses to prenatal stress (Frisancho 1977), and climate (Haas et al. 1980). Greater investment in reproduction by females and the need to support pregnancy and lactation might have increased selection for better buffering from environmental stresses (Stini 1975, 1982; Stinson 1985).

The global burden of undernutrition and stunting is largely borne by developing nations including South Africa. A national school feeding program was started in all public primary and secondary schools in 1994 (Rendall-Mkosi et al. 2013). This might account for the low percentage of undernutrition recorded for the children in this study.

7.4.2 Relationship between height, weight and tooth emergence

There is a relationship between number of emerged teeth and height in Southern African Black children. This is similar to a study of Japanese children that determined height and weight had direct influences on tooth emergence (Niswander and Sujaku 1960). Oziegbe et al. (2009) found similar results for primary tooth emergence in Nigeria. Hence it is expected that any increase in height should lead to a corresponding acceleration in the timing of tooth emergence.

This study found a significant relationship between number of emerged teeth and weight of the children. A few other studies found a relationship between the number of emerged teeth and weight (Haddad and Pires Correa 2005; Hilgers, et al. 2006;); Sánchez-Pérez et al. 2010; Must et al. 2012). Children who have lower than average weight and height have been shown to have later emergence times than those who are within the normal range (Adler 1963; Billewicz and McGregor 1975; Lee et al. 1965; Triratana and Kiatiparjuk 1989).

Not all studies have found that height or weight relate to dental emergence. Kutesa et al. (2013) did not find any significant relationship with weight in Ugandan children. However, their conclusion was only based on correlational analysis, which is not robust enough to determine any relationship other than a linear relationship. Furthermore, Khan (2011), in a study conducted among Pakistani children, observed that heavy and short children had early tooth emergence while tall children showed delayed emergence regardless of their weight. No reason was given for those findings.

7.4.3 Mid-upper arm circumference and tooth emergence

To the best of our knowledge, the use of MUAC has not been evaluated as a prognostic indicator for tooth emergence. The present study did not find any association between the numbers of teeth emerged and MUAC. Craig et al. (2014) found poor accuracy for MUAC in classifying the nutritional status of Black South African males aged 5-9 years. This appears to be the case for our sample as well. MUAC is described as a very good measure for identifying those who are at risk for severe malnutrition. Although the MUACs of the children in our study were generally low, there were not many children who were severely malnourished according to the other nutritional proxies.

7.4.4 Relationship between head circumference and tooth emergence

HC has been shown to be a good measure of brain development but a poor predictor of nutritional status because brain growth is ‘favored’ over other growth processes. We found no significant relationship between head circumference and number of permanent teeth emerged, although the head circumference of the males increases from ages 5 to 9 in males and ages 5 to 12 in females. This later pattern of brain growth is much less dramatic than what is observed at earlier ages. There is evidence that brain development continues into adolescence (Kipke 1999). However, the influence of this form of development on the cranial capacity of Southern African children neEds to be further investigated to understand the protracted period of growth documented for the females in our study.

7.4.5 Influence of sex on tooth emergence

South African females are more advanced in tooth emergence than males. This occurs from the early years up to age 16, when there is catch-up with the females. The difference between males and females is most pronounced at age 9-10 years, which is around the time of the prepubertal growth spurt in females (Norris and Richter 2005). The prepubertal growth spurt occurs earlier in females than males. This might account for the spike in the difference in the number of teeth emerged between males and females around this age. There is an agreement from studies on tooth emergence that permanent teeth emerge earlier in females than males (Eskeli et al. 1999; Nyström et al. 2001; Ekstrand et al. 2003). Only one study (Kochar and Richardson 1998) of Irish children showed earlier emergence of second molars in boys. This was also viewed as a catch-up development because of the later onset of puberty in the males.

7.5 Conclusion

In the present study, height, weight and BMI were found to significantly influence the timing of emergence and the number of emerged permanent teeth in Southern African Black children. Obese and overweight children are more advanced in the timing of emergence and have more emerged teeth than underweight individuals in the same age cohort. No relationship was found between head circumference or mid-upper arm circumference and the number of emerged teeth". These findings should be verified by longitudinal data.

7.6 References

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Contents

Chapter 8

Dental development and life history variables of Black Southern African children

Abstract

Background: Strong correlations have been found between measures of skeletal, somatic and sexual development. It is not clear what relationship exists between sexual maturity and dental development in humans. Although earlier studies reported there is little or no relationship, newer work is challenging that view.

Aim: This study investigates the association between the timing of tooth emergence, age of attainment of specific tooth formation stages and life history events (age of sexual maturity, tempo of brain size increase) in a Black Southern African population.

Method: This is a cross-sectional study of 642 Black Southern African school children. Data for dental development, height, mid-upper arm circumference (MUAC) and head circumference (HC) were compared with mean age of tooth emergence and formation stages (following Demirjian et al. 1973) and mean age of attainment of sexual maturity stages and menarche (from Lundeen et al. (2015) and Norris and Richter (2005)) to identify if any developmental events co-occur. Correlation analysis was used to determine associations between HC, MUAC and dental maturity. Statistical significance was inferred at $p>0.05$.

Results: The overall pattern of correlations is the same for both sexes, although the strength of the correlation with HC is somewhat stronger in females. The number of teeth emerged in males correlates strongly with chronological age ($r=0.91$, $p=0.00$) and height ($r=0.89$, $p=0.00$), with weaker correlations with MUAC ($r=0.61$, $p=0.00$) and HC ($r=0.16$, $p=0.00$). In females, the number of teeth emerged correlates with chronological age ($r=0.88$, $p=0.00$) more than height ($r=0.83$, $p=0.00$), MUAC ($r=0.59$, $p=0.00$) or HC ($r=0.38$, $p=0.00$). Similar patterns of correlation are found for dental maturity.

The emergence of the maxillary and mandibular M2s co-occurs at approximately 12.6 years with the G2 stage of gonad development and the PH2 stage of pubic hair development in males. The M2s emerge around seven months earlier in females, coincident with the attainment of Tanner's B2 breast stage and the PH2 pubic hair stage. Notably, age of menarche does not coincide with any of the determined ages for emergence of teeth.

The timing of tooth formation also coincides with specific sexual maturity stages. Attainment of the final (H) stage of development for the C1 co-occurs with the G2 stage of gonad development and shortly after the pubic hair stage PH2 in the males. In females, the attainment of the H stage of C_1 formation occurs shortly before the attainment of the B2 stage of breast development. Furthermore, the H stage of P_1 formation coincides with the PH2 stage of pubic hair development, shortly after the attainment of the stage B2 of breast development. The attainment of the H stage in P_2 formation coincides with the age of menarche at approximately 13 years.

Conclusion: Dental development correlates more strongly with chronological age than it does with the measures of skeletal or somatic development in Black Southern African children. The onset of puberty is concurrent with the emergence of the mandibular and maxillary M2s and the final (H) stage of mandibular canine and first premolar formation in both males and females. Menarche appears to coincide with the attainment of the H stage for the mandibular P2s. The pattern of life history events in Black Southern Africans is not different from what is observed for other modern human populations, but the timing of the life history events is notably advanced, which should be factored into future forensic and anthropological research in Southern Africa.

8.1 Introduction

Life history is concerned with the strategy an organism uses to achieve growth, development, reproduction, and survival (Smith 1989; Smith and Tompkins 1995). A life history perspective employs an evolutionary comparative framework to consider how reproductive development, post-reproductive behavior and life span are shaped by natural selection (Stearns 2000). Variables or events that are typically considered in life history research include the pattern and tempo of juvenile development, age of sexual maturity and first reproduction, number of offspring, interbirth interval, level of parental investment, senescence and death.

The onset of puberty is accompanied by rapid biological changes characterised by sexual maturation, increases in height and weight, completion of skeletal growth with increase in skeletal mass, and changes in body composition commonly referred to as the adolescent growth spurt (Bogin 2010). The sequence of these events is consistent among adolescents during pubertal growth, although variation in the timing, duration, and tempo of pubertal changes is considerable within and between populations (Strang and Story 2005). These variations are due to genetic and environmental influences (Bogin 1998). Hence, sexual development may be used to gauge the magnitude and pattern of growth and developmental disruption in response to environmental stresses.

Indices of growth and development such as skeletal, dental and sexual maturity have been correlated with life history variables for many primates, and strong relationships between life history variables and tooth emergence characterise extant and fossil species (Harvey and Clutton-Brock 1985; Smith 1989; Smith 1991; Smith 1992; Smith and Tompkins 1995; Bogin and Smith 1996; Bogin 1997; Bogin 2010; Thompson and Nelson 2011; Kelley and Schwartz 2012; Lee

2012). A strong correlation was found between age at weaning, age at sexual maturity, interbirth interval, age at first breeding and first molar emergence across primate species (Smith et al. 1989). Nonetheless, modern humans have a unique life history. We have relatively short birth intervals, helpless newborns, a high rate of postnatal brain growth, an extended period of offspring dependency, intense levels of maternal and paternal care, a prolonged period of maturation, a typically marked adolescent growth spurt and delayed reproduction cycles (De Castro et al. 2003).

The life history perspective is also used to explore biological and behavioral diversity among human populations. A recent study of the Baka population from the Republic of the Cameroon attempted to correlate earlier timing of tooth emergence with life history events (Ramirez Rozzi 2016). The Baka (one of the groups formerly referred to as ‘African pygmies’) are characterized by short adult stature due to a slow rate of growth early in their development (Ramirez Rozzi et al. 2015; 2016). As the Baka are advanced in their tooth emergence relative to other populations, the possible precocity of other life history events was investigated. The life history of the Baka was found to be similar to that of other human populations, leading Ramirez Rozzi (2016) to suggest that the relationship between life history events and tooth emergence is disrupted in humans compared to other primates. This would allow adaptive variations in tooth emergence in response to different environmental controls while at the same time maintaining the unique human life cycle.

This study investigates the association between dental development (the timing of tooth emergence and the attainment of specific tooth formation stages) and the life history events (age of development of secondary sexual characteristics, menarche, tempo of brain size increase) in a Black Southern African population.

8.1.1 First molar (M1) emergence and life history events

Relationships between the timing of first molar emergence and life history events in primates are well documented (Smith 1989; Smith et al. 1994). Mandibular M1 emergence was found to strongly correlate (r>0.9) with the age at weaning, age at sexual maturity, and somatic measurements (adult brain mass, neonatal body mass and brain mass) (Smith 1989). Similarly, the age at M1 emergence is highly correlated with the ages of emergence of all other tooth types and the duration of tooth eruption among primates (Smith 1992; Smith et al. 1994). It was therefore argued that the strong relationship between tooth emergence and life history events makes it possible to use dental development for drawing conclusions about life history events in humans (Harvey and Clutton-Brock 1985; Smith 1991; Smith and Tompkins 1995; Bogin 2010; Thompson and Nelson 2011; Kelley and Schwartz 2012; Lee 2012). For example, the earlier age of M1 emergence in hominin (human ancestral) fossil species, compared to wild great apes, was interpreted as an indication that hominins have more rapid life histories dating to the beginning of their evolutionary history (Kelley and Schwartz 2012). However, the extension of life history patterns seen in certain species to other species is questioned because exceptions were identified when closely related great apes were compared (Dirks and Bowman 2007; Robson and Wood 2008; Guatelli-Steinberg 2009; Humphrey 2010).

Orangutan, gorilla, and chimpanzee M1s emerge prior to their weaning (Robson and Wood 2008). M1 emerges through the gingiva in humans at around six years of age (Hillson 2014) compared to shortly after three years of age in wild chimpanzees (Smith 2013). Humans are typically weaned long before the emergence of M1, while chimpanzees continue to breastfeed (Robson and Wood 2008; Smith 2013). Thus, in contrast to the situation for great apes, humans are weaned early relative to their permanent dental development (Humphrey 2010; Robson and

Wood 2008). Therefore, Humphrey (2010) concluded that there is no correspondence between weaning age and M1 eruption in humans. This is not unexpected, given that weaning in modern humans is highly variable and culturally determined.

8.1.2 Dental development and brain development

Brain development is an essential component of life history patterns. Brain metabolism and energy processing comprise the pacemaker of vertebrate growth and aging (Sacher and Staffeldt 1974). Unlike brain growth and development, which can be greatly influenced by environmental factors (as seen in the recent impact of the Zika virus), dental development has a very low variance and is viewed as very resistant to environmental perturbations (Lewis and Garn 1960). Therefore, brain weight and the pattern of tooth formation and emergence contribute two potentially different aspects to defining the life history of a primate species. Brain size is highly correlated with dental development in primates (Smith 1994; Allman and Hasenstaub 1999; Kelley and Schwartz 2010). Strong correlations were found between age at M1 emergence and age at completion of tooth emergence and brain size in hominids (brain weight for neonates (0.99 in both cases) and adults (0.98 and 0.97, respectively) (De Castro et al. 2003).

8.1.3 Dental development and sexual maturity

It is not clear what relationship exists between sexual maturity and dental development in humans. Earlier studies reported low to moderate correlations. Lewis and Garn (1960) found a moderate correlation (0.61) between the age of attainment of the occlusal level for the M2 and the onset of menarche. Nanda (1960) had a similar correlation (0.59) between the age of completion of permanent tooth emergence and menarche; furthermore, M3 emergence was also found to be strongly correlated with life history and somatic variables, especially age of sexual maturity. Conversely, Björk and Helm (1967) found a low correlation between age at menarche and tooth emergence. Similarly Filipsson and Hall (1975) found a low correlation between dental maturity and different measures of sexual development (age at menarche, breast and pubic hair development) and the age of peak height velocity. Hägg and Taranger (1982), in a longitudinal study of Swedish children, found that the dental emergence stages were not useful as indicators of the pubertal growth spurt. Demirjian et al.'s (1985) study of French Canadian girls who had attained 90% of their dental development found no significant relationships with the other maturity indicators such as sexual maturity and peak height velocity. Therefore, Demirjian et al. (1985) argued that the mechanisms controlling dental development are independent of somatic and/or sexual maturity. This perspective has contributed to the view that dental development does not vary in the same way as other aspect of development,

Substantial variability in the results concerning the relationship between dental development and life history events, especially sexual maturity, in humans is a justification for further research. The different methods used in ascertaining age of attainment of specific tooth developmental stages, as well as the type of tooth and the stages of dental development studied, may contribute to the variation that has been documented to date. For this study, age of tooth emergence and dental maturity scores were examined in relation to sexual maturity data (age of menarche, breast developmental stage, male genital developmental stage, and pubic hair developmental stage) and measures of somatic development (height, head circumference and mid-upper arm circumference).

8.2 Materials and methods

This is a cross-sectional study of 642 clinically healthy Black Southern African children aged 5-20 years whose parents and grandparents are indigenous Southern Africans. Children who met the demographic inclusion criteria were randomly selected from those being treated during dental outreach to schools in the Johannesburg municipality.

Ethical approval (NO. M141001) was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg. Permission to carry out the study was obtained from the local education authority and respective school heads. Consent was obtained from the parents while assent was obtained from the children participating in the study.

All selected students were examined on a dental chair in a mobile dental van installed with a panoramic radiograph machine. Intra oral examination was done with a dental mirror and probe under a light source. Teeth present were recorded using the *Fédération Dentaire Internationale (*FDI) notation. After examination, panoramic radiographs were taken and those children diagnosed as having agenesis of lateral incisors and third molars were excluded from the study.

An emerged tooth was defined as a tooth with any part of its crown penetrating the gingiva and visible in the oral cavity (Al-Jasser and Bello 2003). In general oral surgery some children have their emerged teeth extracted due to consequences of untreated dental caries, trauma or for orthodontic purposes. Extracted teeth were considered to have emerged.

Height was measured with an anthropometric stadiometer (Weylux model 424) and recorded to the nearest 0.1 cm. The mid-upper arm circumference (MUAC) of the left upper arm was measured with a tape measure at the mid-point between the tip of the shoulder and the tip of the elbow (olecranon process and the acromion) and recorded to the nearest 0.1 cm. The head circumference (HC) was measured to the nearest 0.1 cm by placing a tape measure across the forehead and around the greatest circumference of the head.

Panoramic x-rays of 642 children comprising of 270 males and 372 females were reviewed and assessed for tooth formation. Dental age assessment was performed according to the original version of Demirjian's method (Demirjian et al. 1973). Each radiograph was evaluated for the development of the seven left permanent mandibular teeth, and rated on an 8-stage scale from A to H, based on the stages of crown and root formation with stage 0 for non-appearance. Each stage of the tooth was allocated a sex-specific biologically weighted score and the sum of the scores for each subject was used to determine the dental maturity measured on a scale from 0 to 100. Each stage of development was dichotomized into presence or absence to allow for calculation of age of attainment using probit regression analysis (Hayes and Mantel 1958).

The study was pilot tested on 40 randomly selected students. Intra-examiner test and retest reliability of measurements were calculated using Lin's Concordance Coefficient (height r=0.99, head circumference r=0.92, and mid-upper arm circumference r=0.96). The investigator was the only rater for the developmental stages of the teeth. Intra-examiner reliability of dental age assessment for the Demirjian method was calculated using Cohen's Kappa (κ=0.97) (Landis and Koch 1977). All of these acceptable values attest to the precision of the data collection process.

The estimated mean age for each of the pubertal stages and age of menarche are from Lundeen et al. (2015) and Norris and Richter (2005). Both of these studies are part of the "Birth to Twenty" longitudinal data set for children from Soweto who are demographically very similar to our study population. Sexual maturity stages were based on Tanner stages of sexual development (Marshall and Tanner 1969; 1970). Breast development was subdivided into four stages: B2, B3, B4 and B5. The pubic hair development was characterised by four stages: PH2, PH3, PH4 and

PH5. The stage of gonad development, measured by the volume of the testicles, was also subdivided into four groups: GH2, GH3, GH4 and GH5.

The data were analyzed with Stata 12 for Windows. The analysis included frequencies and cross-tabulations. The mean age at the time of emergence and standard deviations were computed for each tooth using probit analysis and compared using Student's *t*-tests, after Liversidge (2003). Association between categorical variables was tested with chi square, while associations between continuous variables were tested with a Student's *t*-test. The mean ages of the pubertal developmental stages of males and females were compared to the mean age of tooth development to identify the events that co-occur. Correlation analysis was used to determine association between head circumference, mid-upper arm circumference and dental maturity. Statistical significance was inferred at $p>0.05$.

8.3 Results

8.3.1 Tooth emergence

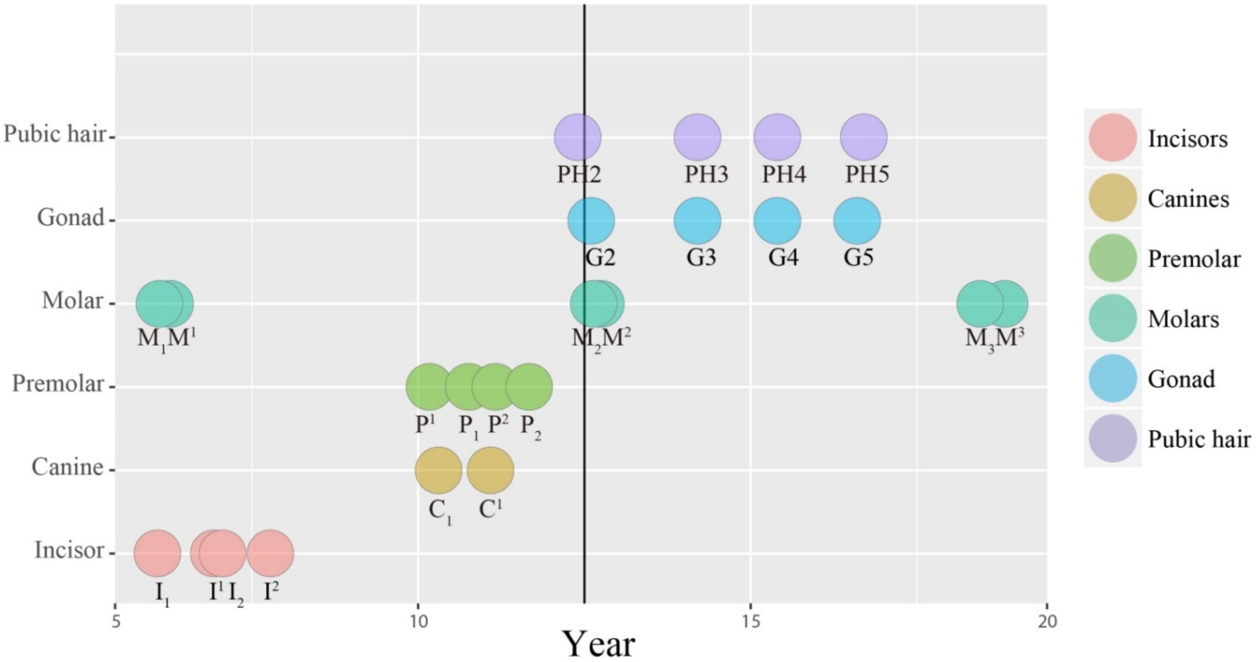
The age of emergence of maxillary (12.75±1.78 years) and mandibular (12.64±2.07 years) M2s appears to co-occur with the G2 stage of gonad development (12.6±1.6 years) and the PH2 stage of pubic hair development (12.4±1.5 years) in males (Table 8.1 and Figure 8.1). In females, maxillary and mandibular M2s emerge earlier (maxilla 12.07±1.30; mandible 11.69±1.51), and coincident with the age of attainment of the B2 breast stage (11.9±1.2 years) and the PH2 pubic hair stage (12.2±1.5 years) (Table 8.2 and Figure 8.2). Notably, age of menarche does not coincide with any of the determined ages for emergence of teeth.

Table 8.1. Mean age (years) of permanent tooth emergence and sexual maturity stages in Southern African males

Maxilla			Mandible			Sexual development (Lundeen et al. 2015)					
Tooth type	Combined		Tooth type	Combined							
	Mean	SD		Mean	SD	Genital stage	Mean age	SD	Pubic hair stage	Mean age	SD
I^1	6.93	0.87	I_1	6.08	0.34						
I^2	7.78	1.07	I_2	7.06	0.95						
C^1	11.09	1.69	C_1	10.17	1.67						
P^1	10.31	1.72	P_1	10.76	1.76	G2	**12.6***	1.6	PH2	**12.4***	1.5
P^2	11.16	1.74	P_2	11.67	2.42	G3	14.2	1.4	PH3	14.2	1.2
M^1	6.27	0.29	M_1	6.11	0.28	G4	15.4	1.3	PH4	15.4	1.2
M^2	**12.75***	1.78	M_2	**12.64***	2.07	G5	16.6	1.3	PH5	16.7	1.2
M^3	18.82	1.45	M_3	18.45	1.97						

*****Similar timing of appearance

Figure 8.1. Point plot of co-occurrence of timing of permanent tooth emergence and sexual maturity stages in Southern African males



Simultaneous events are located on the bold line.

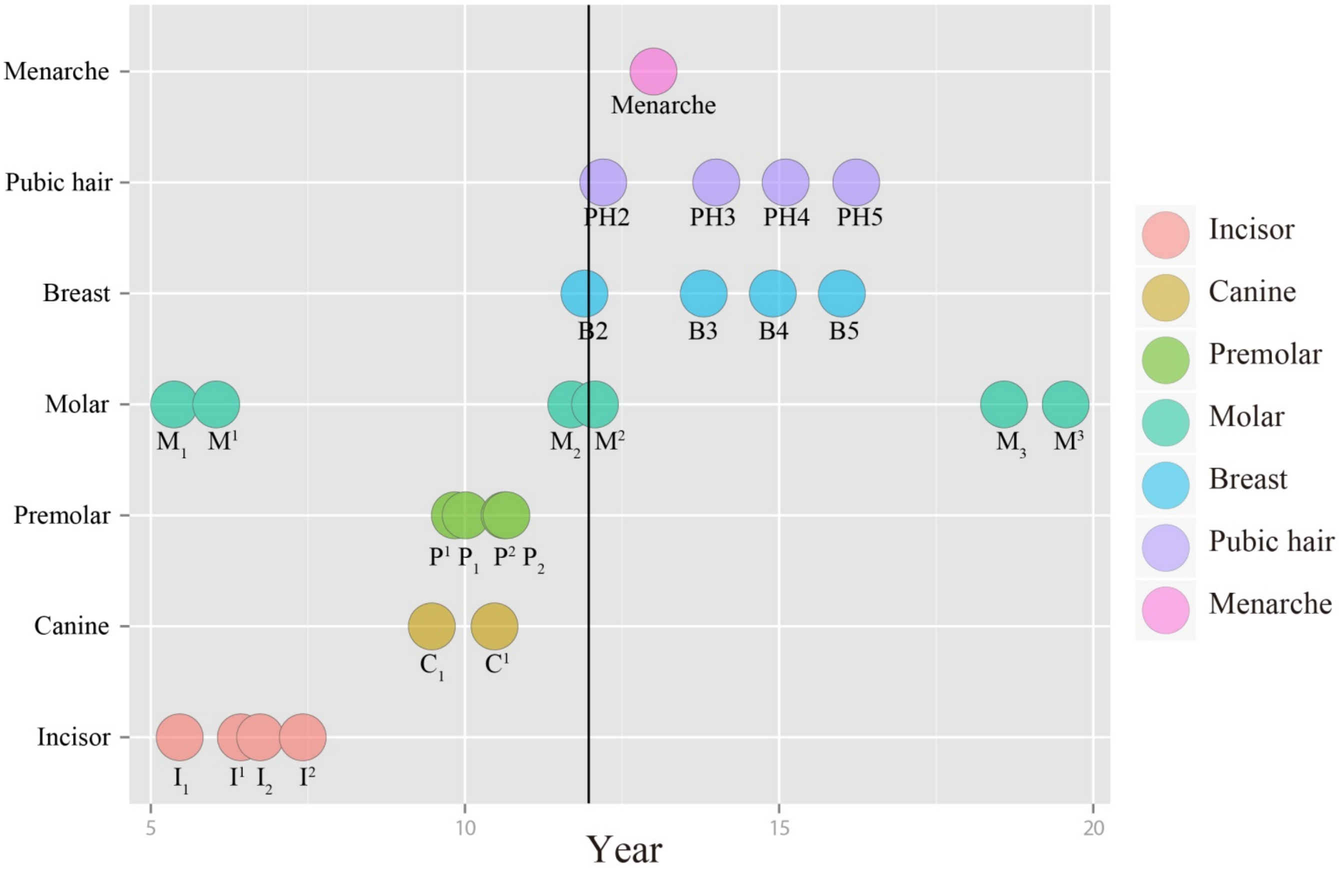
Table 8.2. Mean age (years) of permanent tooth emergence and sexual maturity stages in Southern African females

Maxilla			**Mandible**			**Sexual development (Lundeen et al. 2015)**							
Tooth type	**Combined**		**Tooth type**	**Combined**									
	Mean	**SD**		**Mean**	**SD**	**Breast stage**	**Mean age**	**SD**	**Pubic hair stage**	**Mean age**	**SD**	**Menarche^**	
												Mean	**SD**
I^1	6.43	0.66	I_1	5.46	0.94								
I^2	7.42	0.92	I_2	6.74	1.68								
C^1	10.47	1.46	C_1	9.47	1.37								
P^1	9.84	1.34	P_1	10.01	1.28	B2	**11.9***	1.2	PH2	**12.2***	1.5		
P^2	10.63	1.65	P_2	10.66	1.29	B3	13.8	1.4	PH3	14.0	1.3		
M^1	6.04	0.89	M_1	5.37	1.13	B4	14.9	1.4	PH4	15.1	1.4		
M^2	**12.07***	1.30	M_2	**11.69***	1.51	B5	16.0	1.5	PH5	16.3	1.4	13.0	1.3
M^3	19.56	2.89	M_3	18.58	2.49								

*****Similar timing of occurrence

^ Norris and Richter (2015)

Figure 8.2. Point plot of co-occurrence of timing of permanent tooth emergence and sexual maturity stages in Southern African females



Simultaneous events are located on the bold line.

8.3.2 Correlations between the number of emerged teeth and anthropometric variables

The Spearman's rho correlations (Table 8.3) show that the number of emerged teeth correlates strongly and significantly with the chronological age (r=0.91, p=0.00), while the correlation for height is slightly lower (r=0.89) as is the correlation for MUAC (r=0.61, p=0.00) in males. However, the number of emerged teeth is poorly correlated with HC in males even though the relationship is significant (r=0.16, p=0.007). In females, the number of emerged teeth correlates strongly and significantly with the chronological age (r=0.88, p=0.00) compared to height (r=0.83, p=0.00), There is a moderate correlation with MUAC (r=0.59, p=0.00), and even less with HC (r=0.38, p=0.00) (Table 8.3). The overall pattern of correlations is the same for both sexes, although the strength of the correlation with HC is somewhat stronger in females.

Table 8.3. Spearman's rho correlations between numbers of teeth emerged, age and anthropometric variables

Variable	**# teeth emerged Males (N=266)**		**# teeth emerged Females (N=373)**	
	r	p	r	p
Age	0.91	0.00	0.88	0.00
Height	0.89	0.00	0.83	0.00
MUAC	0.61	0.00	0.59	0.00
HC	0.16	0.01	0.38	0.00

8.3.3 Tooth formation

Females are advanced in the age of attainment of the final (H) stage of development in all the teeth compared to the males. The greatest difference in timing is seen in the canines (Tables 8.4 and 8.5).

The mean age of attainment of the H stage of canine (C1) development (12.75±0.07 years) co-occurs with the G2 stage of gonad development (12.6±1.6 years) and shortly after pubic hair stage PH2 (12.4±1.5 years) in the males (Table 8.4 and Figure 8.3). The age of attainment of the H stage of first premolar (P1) formation (12.97±0.10) occurs shortly after the G2 stage of gonad development (12.6±1.6 years) and the PH2 stage of pubic hair development in males (12.4±1.5 years) (Table 8.4 and Figure 8.3).

In females, the age of attainment of the H stage during C_1 formation (11.62±0.1 years) occurs shortly before the attainment of Tanner's stage B2 of breast development (11.9±1.2 years) (Table 8.5 and Figure 8.4). Furthermore, the attainment of the H stage of P_1 formation (12.15±0.10 years) coincides with the PH2 stage of pubic hair development (12.2±1.5 years) and shortly after the age attainment of the Tanner's stage B2 of breast development (11.9±1.2 years). The mean age of attainment of the H stage in P_2 formation (12.95±0.08 years) coincides with the age of menarche (13.0±1.3 years) (Table 8.5 and Figure 8.4).

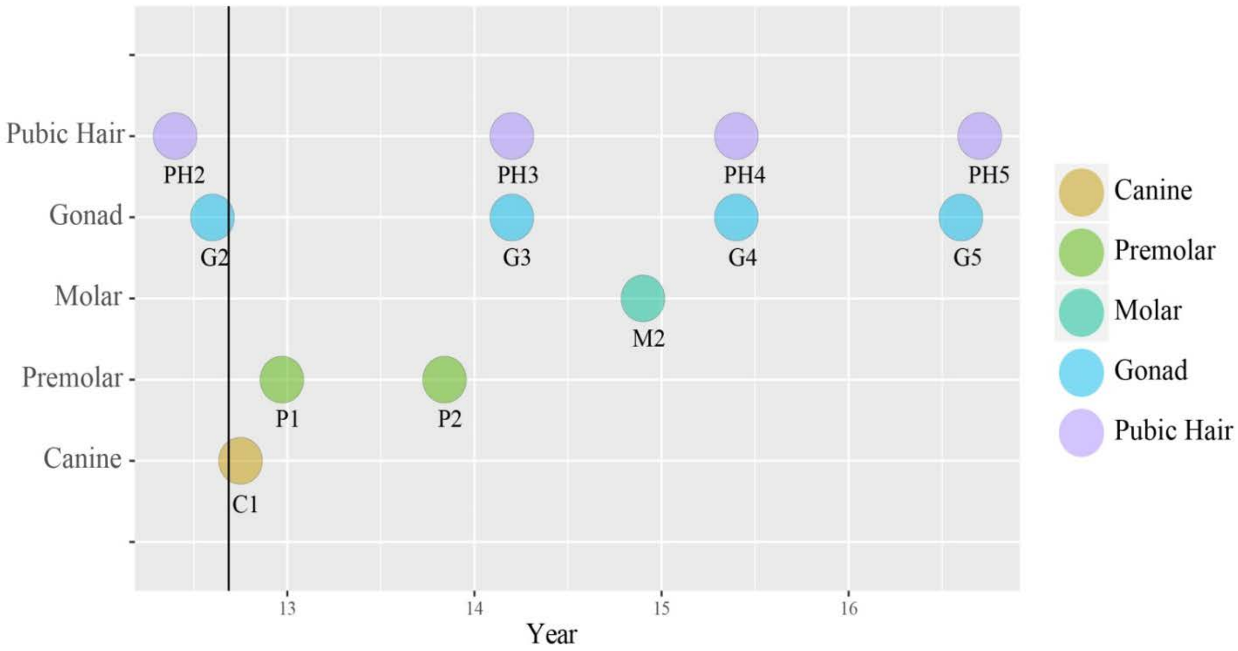
Table 8.4. Comparison of mean age of attainment of dental and sexual maturity stages in males

Stage	Dental maturity			Sexual maturity+					
	Mean age	**SD**	**n**	**Genital stages**	**Mean age**	**SD**	**Pubic hair stage**	**Mean age**	**SD**
Canine formation									
D	3.17	0.47	33						
E	7.01	0.10	24	G2	**12.6***	1.6	PH2	**12.4***	1.5
F	8.16	0.15	77	G3	14.2	1.4	PH3	14.2	1.2
G	11.34	0.12	27	G4	15.4	1.3	PH4	15.4	1.2
H	**12.75***	0.07	108	G5	16.6	1.3	PH5	16.7	1.2
First premolar formation									
D	3.17	0.47	39						
E	7.54	0.09	42	G2	**12.6***	1.6	PH2	**12.4***	1.5
F	9.11	0.09	50	G3	14.2	1.4	PH3	14.2	1.2
G	11.18	0.11	34	G4	15.4	1.3	PH4	15.4	1.2
H	**12.97***	0.10	104	G5	16.6	1.3	PH5	16.7	1.2
Second premolar formation									
D	4.74	0.27	44						
E	7.96	0.12	37	G2	12.6	1.6	PH2	12.4	1.5
F	9.28	0.11	56	G3	14.2	1.4	PH3	14.2	1.2
G	11.75	0.12	41	G4	15.4	1.3	PH4	15.4	1.2
H	13.84	0.09	86	G5	16.6	1.3	PH5	16.7	1.2
Second molar formation									
C	5.06	0.09	18						
D	6.09	0.16	42						
E	8.28	0.17	68	G2	12.6	1.6	PH2	12.4	1.5
F	11.01	0.13	38	G3	14.2	1.4	PH3	14.2	1.2
G	12.98	0.07	39	G4	15.4	1.3	PH4	15.4	1.2
H	14.90	0.05	64	G5	16.6	1.3	PH5	16.7	1.2

*Close values

+ Data from Birth to Twenty Study (Lundeen et al. 2015)

Figure 8.3. Point plot of co-occurrence of stages of permanent tooth formation and sexual maturity stages in Black Southern African males



Simultaneous events are located on the bold line.

Table 8.5. Comparison of mean age of attainment of dental and sexual maturity stages in females

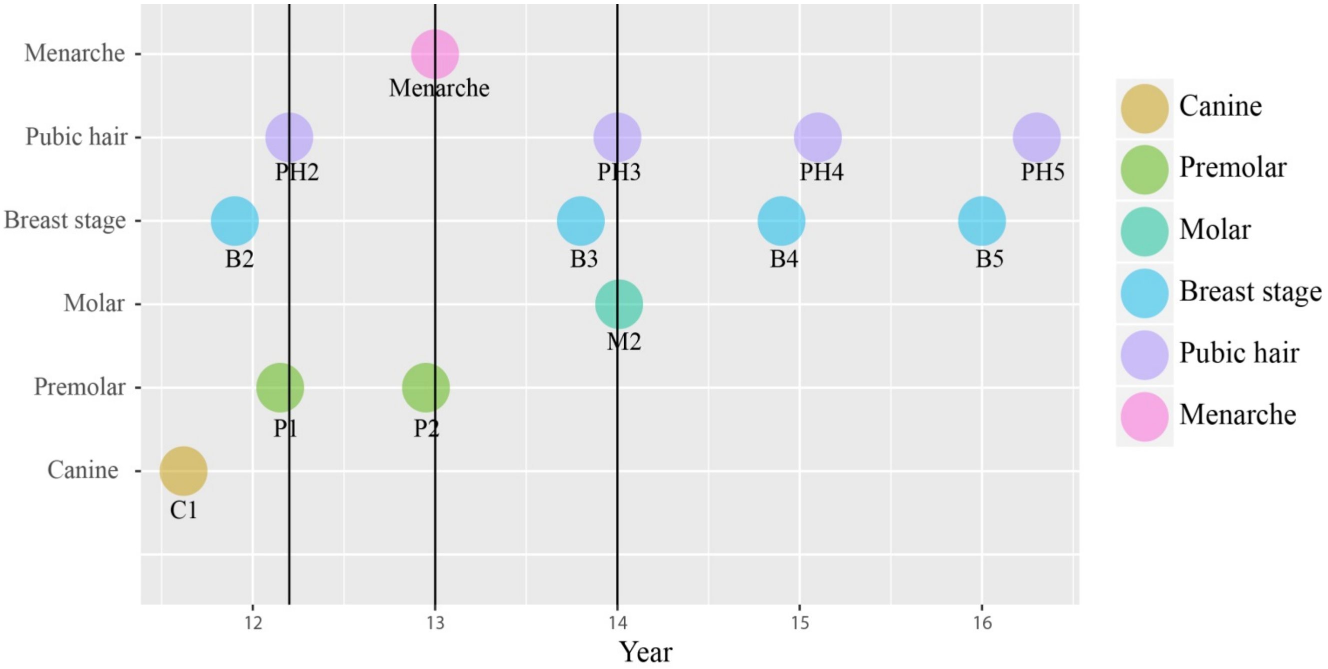
Stage	Dental maturity			Sexual maturity+					
	Mean age	**SD**	**n**	**Breast stage**	**Mean age**	**SD**	**Pubic hair stage**	**Mean age**	**SD**
Canine formation									
E	6.52	0.11	11	B2	**11.9***	1.2	PH2	12.2	1.5
F	7.17	0.12	59	B3	13.8	1.4	PH3	14.0	1.3
G	9.72	0.09	59	B4	14.9	1.4	PH4	15.1	1.4
H	**11.62***	0.10	372	B5	16.0	1.5	PH5	16.3	1.4
First premolar formation									
E	7.17	0.10	41	B2	**11.9***	1.2	PH2	**12.2***	1.5
F	7.81	0.12	64	B3	13.8	1.4	PH3	14.0	1.3
G	10.30	0.10	62	B4	14.9	1.4	PH4	15.1	1.4
H	**12.15***	0.10	194	B5	16.0	1.5	PH5	16.3	1.4
Second premolar formation									
E	7.86	0.14	12	B2	11.9	1.2	PH2	12.2	1.5
F	8.35	0.15	69	B3	13.8	1.4	PH3	14.0	1.3
G	10.88	0.10	75	B4	14.9	1.4	PH4	15.1	1.4
H	**12.95****	0.08	164	B5	16.0	1.5	PH5	16.3	1.4
Second molar formation									
D	5.29	0.19	51	B2	11.9	1.2	PH2	12.2	1.5
E	8.15	0.15	60	B3	13.8	1.4	PH3	14.0	1.3
F	10.48	0.06	69	B4	14.9	1.4	PH4	15.1	1.4
G	12.49	0.07	59	B5	16.0	1.5	PH5	16.3	1.4
H	14.01	0.08	124						

**Age of menarche =13.0 ±1.3 years (Norris and Richter 2005) is close to age of attainment of stage H of second premolar

*Close values

+ Data from Birth to Twenty (Lundeen et al. 2015)

Figure 8.4. Point plot of co-occurrence of timing of stages of permanent tooth formation and sexual maturity stages in Black Southern African females



Simultaneous events are located on the bold line.

8.3.4 Correlations between dental maturity and anthropometric variables

The Spearman's rho correlations in Table 8.6 show that the dental maturity score correlates strongly and significantly with chronological age (r=0.96, p=0.00) compared to height (r=0.92. p=0.00) and MUAC (r=0.64, p=0.00) in males. However, dental maturity is poorly correlated with HC in males even though the relationship is significant (r=0.18, p=0.007). In females, the dental maturity score correlates strongly and significantly with chronological age (r=0.94, p=0.00) compared to height (r=0.86, p=0.00) and MUAC (r=0.60, p=0.00), while it is weakly correlated with HC (r=0.34, p=0.00). Again, the correlation with head circumference is stronger in females. These results follow an identical pattern to what is observed for tooth emergence (Table 8.3).

Table 8.6. Correlation between dental maturity score, age and anthropometric variables

Variable	**Maturity score Male (N=270)**		**Maturity Score Female (N=372)**	
	R	p	r	P
Age	0.96	0.00	0.94	0.00
Height	0.92	0.00	0.86	0.00
HC	0.18	0.00	0.34	0.00
MUAC	0.64	0.00	0.60	0.00

8.3.5 Correlation between height and stages of individual tooth formation

There are significant correlations between height and the stages of formation of all the teeth. P1s show the strongest correlation with height (r=0.89, p=0.00) followed by M2s (r=0.88, p=0.00) and C1s (r=0.87, p=0.00) in males. In the females P1s strongly correlates with height (r=0.88, p=0.00), followed by C1s (r=0.86, p=0.00) and then M2s (r=0.79, p=0.00). All the other teeth moderately correlate with height in both males and females (Table 8.7).

Table 8.7. Correlation between height and individual tooth formation

Tooth	Height Male (270)		Height Female (372)	
	r	P	r	p
11	0.57	0.00	0.45	0.00
I2	0.68	0.00	0.49	0.00
C1	0.87	0.00	0.86	0.00
P1	0.89	0.00	0.88	0.00
P2	0.81	0.00	0.78	0.00
M1	0.65	0.00	0.69	0.00
M2	0.88	0.00	0.79	0.00

8.3.6 Correlation between chronological age, MUAC, HC and stages of individual tooth formation

In males, the stages of formation of the C1s and P1s have similarly strong and significant correlations with the chronological age (r=0.9, p=0.00) followed by the M2s (r= 0.89, p=0.00) and the P2s (r=0.83, p=0.00). All the other teeth (I1s, I2s and M1s) moderately correlate with chronological age. In females, the M2s strongly and significantly correlate with chronological age (0.89, p=0.00) followed by the P1s (r=0.87, p=0.00), P2s (r=0.85, p=0.00) and C1s (r=0.84, p=0.00) (Table 8.8).

MUAC is moderately and significantly correlated with the stages of tooth formation of all the permanent teeth in males. In females, low correlations were found with the I1s, I2s and M1s while moderate and significant correlations were found with the C1s, P1s, P2s and M2s. Weak but significant correlations were found between the I1s, I2s, M1s and HC in males. However, in the females, all the teeth showed significant but weak correlations with HC (Table 8.8).

Table 8.8. Correlation between age, HC, MUAC and individual tooth formation

Tooth		HC		MUAC		Age	
		M	**F**	**M**	**F**	**M**	**F**
I1	r	0.19**	0.21**	0.43**	0.28**	0.56**	0.41**
	p	0.00	0.00	0.00	0.00	0.00	0.00
I2	r	0.17**	0.25**	0.52**	0.26**	0.66**	0.45**
	p	0.01	0.00	0.00	0.00	0.00	0.00
C1	r	0.07	0.34**	0.55**	0.49**	0.90**	0.84**
	p	0.27	0.00	0.00	0.00	0.00	0.00
P1	r	0.09	0.33**	0.57**	0.51**	0.90**	0.87**
	p	0.15	0.00	0.00	0.00	0.00	0.00
P2	r	0.05	0.20**	0.47**	0.44**	0.83**	0.85**
	p	0.41	0.00	0.00	0.00	0.00	0.00
M1	r	0.22**	0.28**	0.47**	0.37**	0.63**	0.62**
	p	0.00	0.00	0.00	0.00	0.00	0.00
M2	r	0.03	0.19**	0.53**	0.47**	0.89**	0.89**
	p	0.63	0.00	0.00	0.00	0.00	0.00

**Significant at $p<0.01$

8.4 Discussion

The life history of modern humans is unique among primates, mainly for our long lifespan and the growth spurt at adolescence, coupled with early weaning, an extended period of offspring dependency, late onset of reproduction, relatively short interbirth intervals and menopause (Bogin 2001). While the relationship between dental development and life history variables has been widely studied in primates with high correlations found across species, very few studies have been done on *Homo sapiens.*

8.4.1 Relationships between dental age, chronological age and measures of somatic development

This study found that dental emergence and tooth formation more strongly correlate with chronological age in Black Southern African males and females than with somatic development. These findings are similar to earlier studies that found dental development to be less variable in relation to calendric age than skeletal maturity (Lewis and Garn 1960; Green 1961; Demirjian

1986; Demirjian et al. 1985). The reason for this is that dental developments are less affected by environmental influences such as diseases and chronic malnutrition compared to skeletal maturation.

The interrelationship of dental and skeletal development is often assumed to be strong, but the nature of their relationship is obscured because they are both highly dependent on the chronological age. Our results suggest that dental development and skeletal growth are highly correlated and not independent. We found that individuals who are dentally advanced relative to their peers tend to be skeletally advanced although a significant relationship was not found for all teeth (Chapter 5 of this thesis). Šešelj (2013) found a moderate correlation between skeletal development and dental development in a study of skeletal samples collected in the USA and Europe, while Demirjian et al. (1985) found a very low correlation in his study of French Canadian children. The reason for the differences may be due to different methodological approaches and developmental variables.

Previous studies found strong and significant correlations between stages of canine formation and skeletal development (Coutinho et al. 1993; Sierra 1987). Our study found strong and significant correlations between height and stages of the C1 and P1 formation in males while the M2, followed by the P1, strongly correlates with height in females. This suggests that dental and skeletal development may be under similar controlling influences. Contrary to our results, many studies show low correlation between stages of tooth formation and skeletal development (Garn et al. 1965; Steel 1965; Demirjian et al. 1985). Again, the reason for the differences may be due to methodological differences as varying methods of developmental assessment and use of only selected teeth characterise these earlier analyses.

Correlations between tooth formation and other parameters of physical development are generally low (Björk and Helm 1967; Filipsson and Hall 1976; Ekström 1982; Demirjian et al. 1985), however a slight covariation between tooth emergence and the adolescent growth spurt was noted by Chertkow (1980) in his study of White and Black South African clinical patients. Our study also found moderate to low correlations between dental development and MUAC and HC. The reason for the low variability has been attributed the lower level of environmental influence on tooth development compared to these parameters (Demirjian et al. 1985). The moderate correlation between tooth formation and emergence and MUAC compared to the strong correlation between tooth formation and emergence and chronological age shows that MUAC is highly variable and are more affected by environmental influence than is dental development.

A low correlation was found between dental development and HC in both sexes although the females show a stronger correlation compared to the males. The difference can be explained by the longer span of cranial growth in females. We found that HC increases in females from age 5 to 12 years while it only increased between 5 and 9 years in males (Chapters 5 and 7 of this thesis). This differing pattern of brain growth in males and females could be explained by the available fat reserves. Fat reserves are necessary for brain growth and development and children with higher fat reserve levels have better cognitive abilities compared to those with low fat levels (Innis 2007). In our study, the BMI of the females increases dramatically above the values for males from age 9 onward. This period coincided with the continued increase of the head circumference in females.

In a study of non-human primates, Godfrey et al. (2001) concluded that brain size is a better predictor of dental development than body size. However, this study found other measures of somatic maturity (height, MUAC) to be better predictors of dental development than brain development after age 5 years.

8.4.2 Timing of tooth emergence and sexual maturity

This study is the first to study the Tanner stages of sexual maturity in relation to dental development. Previous work only looked at the relationship between dental development and the onset of menarche and did not consider the different stages of sexual development (Chertkow 1980; Demirjian et al. 1985). The age of menarche is much easier to ascertain than the timing of the stages of sexual development, which requires a longitudinal study design. In addition, longitudinal data on tooth emergence would have been more informative; however there are difficulties in detecting the exact timing of tooth emergence under such a study design. Attempts to correlate the age of menarche with tooth development reported low correlations (Björk and Helm 1967; Filipsson and Hall 1976; Ekström 1982; Demirjian et al. 1985). A correlational approach to evaluating the association between life history variables can be misleading because tooth emergence and tooth formation may not have a linear relationship with events that are only measured by appearance, such as menarche. For this reason, the co-occurrence of these events would have been more reasonable to investigate. Thus, the present study investigated whether life history events such as sexual maturity occur at similar time periods as the attainment of dental maturity (measured by tooth emergence and calcification), rather than using a correlational approach.

Emergence of the maxillary and mandibular M2s occurs during the same time as the onset of sexual development (age of attainment of G2 of genital and PH2 stage of pubic hair development) in males. Similarly, emergence of mandibular M2s and the maxillary molars occurs around the same time as the age of attainment of the B2 stage of breast development and

PH2 stage of pubic hair development in females. The reason for the co-occurrence between M2 emergence and onset of sexual maturity is not known however, the similarity of the co-occurrence in both sexes is noteworthy and merits further investigation.

Age of attainment of menarche in Southern African Black females appears not to have any relationship with the timing of tooth emergence. This is in agreement with previous studies that demonstrated low correlations between the emergence of premolars, molars and menarche (Garn et al. 1965).

8.4.3 Age of attainment of tooth formation stages and sexual maturity

The attainment of the final (H) stage of mandibular canine formation appears to co-occur with the age of attainment of Tanners G2 stage of genital development and shortly after the PH2 stage of pubic hair development in males while it occurs shortly before the B2 stage of breast development in females. The relationship found in the present study between the H stage of mandibular canine formation and the onset of puberty may be a reflection of the circumpubertal increase in stature and acceleration in the growth of the craniofacial structures reported in other studies (Hunter 1966; Brown et al. 1971).

A previous clinical study in South Africa found that mandibular canine root completion prior to apical closure (comparable to stage H) occurs around the onset of puberty in Whites but later in Blacks (Chertkow 1980). It was then suggested that this relationship may be used clinically as a maturity indicator for White South Africans (Chertkow 1980). No similar association was found in this study.

The present study also found the H stage of P2 calcification occurs around the same age as the attainment of menarche in females. Contrary to Demirjian et al.’s (1985) assertion that tooth formation and sexual development are not under same controlling influence, these findings suggest that certain teeth and sexual maturity may be under the same controlling influence, presumably linked to specifically timed hormonal effects that impact upon growth trajectories.

8.4.4 Comparison of life history events and dental development in Black and White Southern Africans

Earlier tooth formation has been documented for Black Southern Africans compared to their White counterparts (Phillips, and van Wyk Kotze 2009,). It is worth noting that other aspects of development, such as age at menarche, are also slightly advanced in Black Southern Africans (Jones et al. 2008). Similarly, African Americans are advanced in age of menarche and skeletal development compared to Americans of European ancestry and Hispanics (Freedman et al. 2002; Wu et al. 2002; Karapanou and Papadimitriou 2010). The earlier occurrence of these life history events and developmental processes among Black Southern African children strongly suggests that somatic development and dental development are under the same genetic influences, contrary to the views of others such as Demirjian et al. (1985). The life history pattern of the Black Southern African population corresponds with findings that other sub-Saharan Africans and African Americans are advanced in tooth development and have earlier sexual maturation compared to European ancestry populations (Freedman et al. 2002; Karapanou and Papadimitriou 2010; Wu et al. 2002).

8.5 Conclusion

In this sample of Black Southern African children, dental development correlates strongly with chronological age more than it does with the measure of skeletal or somatic developments such as height and mid-upper arm circumference. Dental development shows low correlation with brain development. Age of emergence of mandibular and maxillary second molars appears to occur concurrently with the onset of puberty in both males and females. Similarly, age of attainment of the H stage of mandibular canine and first premolar formation coincides with the onset of puberty in males and females. Age at menarche appears to coincide with the age of attainment of the H stage of second mandibular premolar formation. The pattern of life history events in Black Southern Africans is not different from what is observed for other modern human populations, but the timing of their life history events is advanced.

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Contents

Chapter 9

Dental development references for Southern African Black children

Abstract

Background: Dental maturity charts and tables of conversion of maturity scores previously developed did not take into consideration the advanced tooth emergence and formation pattern observed in children of African ancestry.

Aim: To develop a population-specific dental maturity scores, and an atlas of tooth emergence and formation for age estimation of Black Southern Africans aged 5-20 years.

Method: This was a cross-sectional study of 642 Black Southern African children. Panoramic radiographs of the children were collected and analysed using the Demirjian et al. (1973) method. The WITS Atlas was developed using the tooth formation stage with the highest frequency for each tooth. This stage was considered the developmental standard for tooth for an age cohort. To develop population-specific maturity scores, panoramic radiographs from 540 participants aged 5-15.99 years were assessed. Southern African tables of conversion of maturity scores were generated separately for males and females using polynomial regression function (third degree). Maturity curves for boys and girls were plotted to determine the dental maturity curves for Southern African children. The dental age, calculated from the population-specific tables of conversion of maturity scores, was compared to the Willems and Demirjian methods of age estimation.

Results: At age 9.5 years, the canines, premolars and second molars are at least a year ahead in the WITS Atlas compared to London Atlas. The third molar formation and emergence occur three years earlier in the WITS Atlas compared to the London Atlas. The tables of population-specific maturity scores show there is no significant overestimation of the chronological age of the males ($p>0.05$) and in females ($p>0.05$). Compared to the Willems and Demirjian methods, the Southern African specific maturity tables showed the least overestimation and the least mean absolute error for males and females.

Conclusion: This study provides a new dental atlas (WITS Atlas), age prediction models and tables of conversion of maturity scores for Black Southern African children. The tables of conversion of Southern African specific maturity score to dental age show the highest accuracy in the Southern African population when compared with the Demirjian and Willems methods. Our findings suggest that these new tables of conversion can be used for age estimation for forensic, anthropological and clinical purposes in Southern Africa. Furthermore, similarities in dental development across sub-Saharan African populations suggest that the WITS Atlas and the new age prediction model and conversion tables can be used for those populations as well.

9.1 Introduction

Quantifying developmental milestones through the use of the dentition is proven to be the most reliable and accurate method for estimating age and development compared to other indices such as sexual maturity and skeletal maturation (Flood et al. 2013; Cavrić et al. 2016, Feijoo et al. 2012). Accurate age estimation methods are important for assessing chronological age as well as growth and development in children and adolescents for forensics and clinical purposes. Methods such as dental atlases and age specific maturity stages from radiographs of developing dentition are used for such purposes (Elamin and Liversidge 2014). However, existing methods are known to overestimate age for most non-European populations, so researchers are increasingly moving toward the development of population-specific reference values. Those values are not available for sub-Saharan African populations. Instead, researchers rely on growth and development references formulated for European or US children to interpret norms for Africans. The outcome of such practice and their application can lead to misrepresentations of the health status of a population and inaccurate decisions in clinical practice and forensic anthropology. Therefore, this study presents a dental atlas for tooth emergence and formation in Black Southern Africans, and a new population-specific table for conversion of maturity scores to dental age.

9.1.1 Dental maturity charts

There are numerous dental maturity charts with tooth-specific crown and root formation stages. None of these charts take into consideration the advanced tooth emergence and formation patterns observed in Southern African Black children.

The atlas developed by Schour and Massler (1941) is one of the earliest dental aging charts. It is a sequence of 21 drawings describing stages of dental development from in-utero to adulthood. The drawings show developing teeth and their eruption status in relation to an undefined line (possibly the gingival line) and a corresponding age (AlQahtani et al. 2014). The age categories are arranged in consecutive years to the age of 12 years, after which the next age group is 15 years. The last two drawings show fully emerged and formed teeth at 21 and 35 years. This atlas has been criticized because of its many inherent ambiguities. AlQahtani and co-workers (2014) emphasized that very few details of the sample from which the chart was derived are known. The chart appears to have been based on dental development of terminally ill American children, although it probably incorporated other anatomical and radiographic sources, including the work of Logan and Kronfeld (1933). Furthermore, there is no information on the subjects and how they were analyzed, the tooth stages and eruption level are undefined, and the age range of the subjects is limited. Smith (1991) pointed out that 19 of the possible total of 29 subjects were younger than 2 years of age. Other limitations of the chart are the observed poor correlation with measures of skeletal age, and the lumping of boys and girls together in the tooth development charts.

Ubelaker’s (1978) chart has improvements over the Schour and Massler atlas (Smith, 2005). He adjusted the error ranges and the original graphical descriptions of the rates of eruption and tooth formation, most especially the development of the canine from the age of 18 months to 2 years. He included published results on dental development of Native American Indians and other non-European populations. Ubelaker’s chart was modified by Blenkins and Taylor (2012) to include separate schemes for females and males by adjusting the age of each drawing.

There are many other dental charts: Nanda and Chawla (1966), Gustafson and Koch (1974), Brown (1985) and Kahl and Schwarze (1988). The latter two were based on Schour and Massler's (1941) atlas. However, all these aging systems have serious limitations that hamper their use as the tooth developmental stages are not well-defined.

The London atlas is arguably the most widely used chart for forensic and anthropological purposes. It was developed to overcome some of the limitations of previous systems (AlQahtani et al. 2014). The London atlas is based on skeletal samples from Portugal, The Netherlands, Canada and France, with additional data from panoramic radiographs of living children of Bangladeshi and British origin. AlQahtani et al. (2014) showed that the London atlas is better at estimating dental age compared to the Schour and Massler (1941) and Ubelaker (1978) charts. The atlas is tooth-specific and illustrates tooth development and eruption for 31 age categories. Tooth stages and eruption levels are both described and illustrated. Another advantage of the London atlas is that the teeth are spaced such that each tooth is clearly visible (AlQahtani et al. 2010). A limitation is the combining of data on children from different populations despite the established pattern of population variation in tooth formation (Koshy and Tandon 1998; Willems et al. 2001; Flood et al. 2013; Khorate et al. 2014; Zhai et al. 2016). For example, Black children from Africa are well advanced in dental emergence and formation compared to European and Asian populations (Oziegbe et al. 2014; Cavrić et al. 2016). Therefore, the London atlas could potentially yield biased age estimations for many populations.

9.1.2 Dental maturity methods

The most widely used dental maturity method is that described by Demirjian et al. (1973) and Demirjian and Goldstein (1976). It is based on a large reference sample of French Canadian children and adolescents. This method involves the assessment of eight stages of tooth formation for the seven left mandibular teeth. The dental maturity is calculated by adding together the specific biologic weight assigned to each tooth stage. The basis of the biological weights is the skeletal development research of Tanner et al. (1962). Tables of 50th percentile dental maturity values for males and females, developed from the reference sample, are used to convert the maturity scores to dental age (Demirjian et al. 1973).

Numerous studies reported advanced dental development in their study populations compared to the reference sample of French Canadians used in the Demirjian method (Willems et al. 2001; Nik-Hussein et al. 2011; Feijoo et al. 2012; Flood et al. 2013; Djukic et al. 2013; Amberkova et al. 2014; Ye et al. 2014; Cavrić et al. 2016). This has been attributed to real population differences rather than methodological problems. Therefore, the tables proposed by Demirjian and colleagues cannot be generalized to other populations, justifying the need for population-specific reference data (Koshy and Tandon 1998; Willems et al. 2001; Khorate et al. 2014; Zhai et al. 2016).

9.2 Methods

This study is a cross-sectional study of the panoramic radiographs of 642 healthy Southern African Black school children. Permission to carry out the study was obtained from the local education authority and respective school heads. Written consent was obtained from the parent/guardian and assent from the child was required before participation. Ethical clearance (N0. M141001) was obtained from the Human Research Ethics (Medical) Committee of the University of the Witwatersrand.

9.2.1 Sample size

The sample size formula is $N = 4z_{\alpha}^{2}S^{2} \div W^{2}$, where S=standard deviation, W=desired total width and Zα is the standard normal deviate for the 95% the confidence level. Cameriere et al. (2008) found a mean of 1.076 and a standard deviation of 0.824 derived from a standard error of 0.030. Using a width of 0.2, the minimum sample size total required for the 14 cohorts was 280. For the development of the population-specific atlas, a total of 642 children aged 5-20 years while 540 children aged 5-15.99 years were included for the development of Southern Africa specific maturity scores.

9.2.2 Data collection

Panoramic radiographs of children screened during visits of the Community Oral Health Outreach Program (Department of Community Dentistry, University of the Witwatersrand) to primary and secondary schools in the Johannesburg metropolis were collected and analyzed. Radiographs that showed gross pathology or low-quality resolution were excluded. Children with systemic diseases that can affect development of teeth, mandibular hypodontia, children with any form of tooth impaction and agenesis and those who had lost their teeth on both sides of the mandible were excluded.

9.2.3 Assessment of tooth emergence

All selected participants were examined by the author in a mobile dental van equipped with a panoramic radiograph machine. Intra oral examination was done with a sterile dental mirror and probe under a light source. Teeth present including third molars were recorded using Fédération Dentaire Internationale (FDI) notation. An emerged tooth was defined as a tooth with any part of its crown penetrating the gingiva and visible in the oral cavity (Al-Jasser and Bello 2003).

Extracted teeth were considered to have emerged and the occlusal relationship of mandibular third molars to the maxillary third molars was checked and recorded.

9.2.4 Reliability test

The magnitude of inter and intra-examiner error of interpretation and detection was conducted prior to the commencement of the study. For the development of the WITS Atlas, the author and two assisting dentists were calibrated by assessing the stages of dental development of 10 radiographs. The Cohen's Kappa for inter-examiner reliability was found to be 0.87. For the development of the Southern African specific conversion tables of maturity score to dental age, the investigator assessed the maturation stage of the seven left mandibular permanent teeth, without knowledge of the chronological age or sex, using the Original and Modified Demirjian methods. Twenty-five radiographs (with 175 individual tooth ratings) were randomly selected and assessed by the investigator at day one and day three. Intra-examiner reliability of dental age assessment for the Demirjian method was calculated using Cohen's Kappa (Landis and Koch, 1977) and was found to be 0.97.

9.2.5 Southern African specific dental maturity prediction model

For this aspect of the study, panoramic radiographs from 540 of the 642 participants aged 5-15.99 years were assessed. Children over 16.00 years were excluded because the Demirjian conversion tables do not extend beyond that age. The panoramic radiographs of each child were enhanced using Microsoft Office Picture Manager, properly labeled with a unique identity number and digitally archived. Dental age assessment was performed by the author according to the Original and revised (Modified) versions of the Demirjian method (Demirjian et al. 1973;

Demirjian and Goldstein 1976) and the Willems method (Willems et al. 2001). Each radiograph was assessed for the development of the permanent teeth on the left side of the mandible. Every tooth was rated using the 8-stage scale (A-H) based on the stages of tooth formation identified by Demirjian et al. (1973). Stage 0 was assigned for non-appearance of a tooth.

9.2.6 Population-specific maturity scores

Population-specific tables for conversion of maturity scores to dental age were generated separately for males and females. This was accomplished using a polynomial regression function by modelling the maturity score calculated from the Demirjian et al. (1973) Original method against the chronological age. Model fitting analysis indicated that the polynomial function (3rd degree) had the best fit (for males $R^2=0.914$, for females $R^2=0.897$). The maturity scores were then plotted to determine the dental maturity curves for Southern African children.

The dental ages calculated from the population-specific tables for conversion of maturity scores were compared to chronological ages of the540 participants (aged 5-15.99 years). The results (overestimation or underestimation) were compared to the overestimation or underestimation of dental ages obtained from the Willems, Original Demirjian and Modified Demirjian age estimation methods.

9.2.7 Development of the WITS Atlas of dental development

For the development of Wits Atlas, the stages of formation of all the teeth including third molars was done according to the Demirjian method. Following the above analyses, an atlas of tooth formation and tooth emergence for Black Southern African children was developed. This is modeled after the London atlas, with one key difference. AlQahtani et al. (2010) used the Moorrees method to determine the tooth development phases. The WITS Atlas uses the

Demirjian stages of dental development as most researchers are familiar with that system, and it further subdivides stages that would have been merged if AlQathani (2010) method was used thus, preventing overestimation of age in general. Also, the teeth in the atlas are drawn according to the Demirjian stages whereas the drawing in London atlas merged many of the developmental stages

A-H stages of tooth formation

The tooth formation stages proposed by Demirjian et al. (1973) can be described as follows:

A. Beginning of calcification at the most superior level of the crypt in the form of cones or inverted cones. These calcified points are not yet fused.
B. Fusion of the calcified points to form the occlusal surface.
C. -Complete formation of the occlusal enamel with initial projection and convergence towards the cervical region.

    -Formation of the dentine begins.

    -Curved outline of the pulp chamber is seen at the occlusal level.
D. -Crown formation is complete to the level of the cemento-enamel junction.

    -Upper border of the pulp is well delineated and concave towards the cervical region. Pulp horns appear umbrella-shaped if present; in molars the pulp chamber is trapezoidal.

    -Root formation starts and can be seen as a spicule.
E. Uniradicular teeth:

    -Pulp horns are larger than in the previous stage. The wall of the pulp cavity appears straight except where the profile is broken by the pulp horn.

    -Length of the root is less than the crown height.

    Molars:

-Radicular bifurcation begins, seen as a calcified point or semilunar in shape.

-Length of the root is still less than the crown height.

F. Uniradicular teeth:

-Pulp chamber wall resembles an isosceles triangle with a funnel-shaped apex.

-Crown height is less or equal to the root length.

Molars:

-Calcification of the radicular bifurcation extends further down the root. Ends of the roots are funnel-shaped in outline.

-Crown height may be less or equal to the root length.

G. Walls of the root canals are parallel, but the apex is yet to close.

H. -Root apex is closed.

-Around the root apex, the periodontal ligament has a uniform width.

To construct the atlas, the following steps were taken:

1. Assessment of the right maxillary and mandibular teeth was done using the Demirjian et al. (1973) stages of tooth formation. This is usually done on the left side, but for comparative purposes the right side was used as this is what appears in most atlases.
2. Tables of frequencies for the stages of tooth development for each tooth were generated separately for each age cohort and by sex. The most frequently occurring (modal) stage of tooth formation was considered the signature or standard developmental stage. Where a tooth had two developmental stages with equal frequencies, the more advanced stage was taken as the standard stage for that tooth if they were contiguous stages. Following the procedure of AlQahtani et al. (2010), if

the greatest difference in the age of attainment of specific tooth formation stages was not more than one developmental stage, the use of combined data is justified.

3. The relationship of the occlusal surfaces of the third molars with occlusal tables on the radiographs were checked and compared with the intraoral findings.
4. The diagram for the reference stage was drawn, following the sketches in the Demirjian diagram for stages of tooth development (Fig. 1, Demirjian et al. 1973).
5. The panoramic radiographs were sorted by age cohorts and enhanced using Microsoft Picture Manager. One author (TAE), along with a specialist paediatric dentist and a general dentist, jointly reviewed each radiograph and sorted them according to their patterned similarities in overall dental development. The commonest occurring (modal) pattern was chosen as the standard of dental development for that age cohort.
6. To validate the selected radiograph patterns, they were compared with the calculated frequency tables for each age cohort. Where they did not match, the radiograph sorting process was repeated. If the same situation of non-agreement occurred, the more advanced stage of tooth development of the two variants was considered as the standard pattern. Data for males and females were combined for the atlas. Although there are significant differences in the timing of formation and emergence between males and females, the greatest difference in the age of attainment of specific tooth formation stages was not more than one developmental stage; thus the use of combined sex data is justified.
7. The age cohort reference patterns were drawn using CorelDraw Graphic Suite X8. The age cohorts represent the midyear development beginning at 5.5 years and ending

at 17.5 years. Data on occlusion status is incorporated into the altas by showing the true spatial relationship between opposing teeth.

The WITS Atlas was visually compared with the London atlas by age cohorts and any differences are presented in the results.

9.2.8 Data analysis

For the development of the Black Southern African population-specific maturity score, the data were analyzed using IBM SPSS (version 22) software for Windows. The level of analysis was the entire group as well as each sex and age cohort. The Black Southern African dental age (SADA), generated from the Southern African specific tables of conversion, was compared to the chronological age (CA) for males and females separately. The difference between the SADA and the CA was tested using paired *t*-tests at a significance level of $p<0.05$. A Bland Altman procedure was done to determine the accuracy of the SADA derived from the Black Southern African specific maturity scores with the chronological ages.

The mean difference between the chronological age and the predicted age (SADA) using the Black Southern African table of conversions was compared to the mean differences calculated by the two Demirjian methods and the Willems method. The mean absolute error (MAE) between the SADA and the CA was calculated to express accuracy independent of bias. This was then compared to the MAE obtained for the Demirjian and Willems methods. Statistical significance was inferred at $p<0.05$.

9.3 Results

Table 9.1 provides the frequency of the developmental stages of each age cohort for males and females combined. The developmental stages with the highest frequency (modes) were chosen as the standard developmental stage for that tooth in that age cohort. The standard stage for each age cohort appears in bold.

The WITS Atlas for dental development for ages 5.5 to 17.5 years is presented in Figure 9.1. The drawings are modified for clarity, but they are close approximations of the Demirjian stages (Demirjian et al. 1973). In contrast with the London atlas (Figure 9.2), the sequence of formation and the tooth position relative to the occlusal plane and the maxillary and mandibular alveolar ridges during the process of emergence are clearly illustrated.

Table 9.1. Tooth developmental stages (combined for sex): Age cohorts 5, 6 and 7 years. Standard stages are in bold.

	Maxilla								Total	Mandible								Total
5-5.99	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**		**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	
I1					8	**18**	0	0	26					8	**14**	4	0	26
I2					**24**	2	0	0	26				4	**14**	8	0	0	26
C1				**26**	0	0	0	0	26				**22**	4	0	0	0	26
P1				**26**	0	0	0	0	26				**26**	0	0	0	0	26
P2			1	**25**	0	0	0	0	26				**26**	0	0	0	0	26
M1					13	**13**	0	0	26					6	**18**	2	0	26
M2			**14**	9	0	0	0	0	26			8	**18**	0	0	0	0	26
6-6.99																		
I1				1	3	**36**	8	6	54					4	**26**	22	2	54
I2				7	22	**22**	2	1	54					20	**28**	4	2	54
C1			2	**42**	8	2	0	0	54			1	**32**	14	7	0	0	54
P1			2	**52**	0	0	0	0	54				**48**	3	3	0	0	54
P2			12	**42**	0	0	0	0	54			7	**46**	0	1	0	0	54
M1					10	**36**	8	0	54					6	**40**	7	1	54
M2			22	**30**	2	0	0	0	54			8	**42**	4	0	0	0	54
7-7.99																		
I1						0	3	**12**	15						3	4	**8**	15
I2						3	**7**	5	15						3	**7**	5	15
C1				3	**9**	3	0	0	15				1	1	**13**	0	0	15
P1				5	**7**	3	0	0	15				4	**8**	3	0	0	15
P2				**12**	2	1	0	0	15				**10**	3	2	0	0	15
M1						4	**9**	2	15						2	**10**	3	15
M2				**10**	5	0	0	0	15				**11**	4	0	0	0	15
M3	**4**	0	0	0	0	0	0	0	15	**4**	0	0	0	0	0	0	0	15

Table 9.1 continued. Age cohorts 8, 9 and 10 years. Standard stages are in bold.

	Maxilla								Total	Mandible								Total
8-8.99	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**		**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	
I1							10	**50**	60							18	**42**	60
I2							25	**35**	60							28	**32**	60
C1				6	9	**41**	4	0	60					4	**47**	9	0	60
P1				8	**34**	18	0	0	60				2	4	**50**	4	0	60
P2				10	**35**	15	0	0	60				6	7	**47**	0	0	60
M1							27	**33**	60							18	**42**	60
M2			3	17	**32**	8	0	0	60				24	**36**	0	0	0	60
M3	**40**	6	0	1	0	0	0	0	60	**47**	3	0	0	0	0	0	0	60
9-9.99																		
I1							10	**56**	66							3	**63**	66
I2						8	13	**45**	66							6	**60**	66
C1					18	**37**	11	0	66					3	**32**	28	3	66
P1				5	25	**27**	9	0	66					12	**41**	12	1	66
P2				5	27	**29**	5	0	66				7	10	**40**	9	0	66
M1						7	11	**48**	66							5	**61**	66
M2				19	**33**	14	0	0	66				15	**36**	15	0	0	66
M3	**54**	7	5	0	0	0	0	0	66	**57**	6	3	0	0	0	0	0	66
10-10.99																		
I1							3	**35**	38								**38**	38
I2							4	**34**	38							2	**36**	38
C1						**31**	7	0	38						14	**14**	10	38
P1					8	**20**	10	0	38						**25**	11	2	38
P2				3	9	**21**	5	0	38			1	1	1	**25**	10	0	38
M1							1	**37**	38								**38**	38
M2				8	**28**	2	0	0	38				1	**28**	9	0	0	38
M3	**24**	6	5	3	0	0	0	0	38	11	**16**	9	2	0	0	0	0	38

Table 9.1 continued. Age cohorts 11, 12 and 13 years. Standard stages are in bold.

	Maxilla								**Total**	**Mandible**								**Total**
11-11.99	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**		**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	
I1								**53**	53								**53**	53
I2								**53**	53								**53**	53
C1						20	**30**	3	53						7	**33**	13	53
P1						26	**26**	1	53						9	**34**	10	53
P2						**29**	22	2	53						19	**24**	10	53
M1								**53**	53								**53**	53
M2					23	**23**	7	0	53					8	**36**	9	0	53
M3	6	16	**16**	15	0	0	0	0	53	3	13	**19**	14	3	0	1	0	53
12-12.99																		
I1								**65**	65								**65**	65
I2								**65**	65								**65**	65
C1						21	**29**	15	65						0	20	**45**	65
P1						8	**33**	24	65						2	23	**40**	65
P2						18	**42**	5	65						5	**37**	23	65
M1								**65**	65								**65**	65
M2					13	**34**	14	4	65						**29**	23	13	65
M3			4	**50**	6	5	0	0	65		2	3	**33**	15	7	5	0	65
13-13.99																		
I1								**65**	65								**65**	65
I2								**65**	65								**65**	65
C1							7	**58**	65							4	**61**	65
P1							10	**55**	65							7	**58**	65
P2						7	21	**37**	65							15	**50**	65
M1								**65**	65								**65**	65
M2						21	**35**	9	65						12	**32**	21	65
M3			7	27	**29**	2	0	0	65			3	22	**35**	3	2	0	65

Table 9.1 continued. Age cohorts 14, 15 and 16 years. Standard stages are in bold.

	Maxilla					**Total**	**Mandible**					**Total**
14-14.99	**D**	**E**	**F**	**G**	**H**		**D**	**E**	**F**	**G**	**H**	
I1					**28**	28					**32**	32
I2					**28**	28					**32**	32
C1			1	1	**26**	28				1	**31**	32
P1			1	3	**24**	28				1	**31**	32
P2			2	11	**15**	28				4	**28**	32
M1					**28**	28					**32**	32
M2			2	**19**	7	28			2	**18**	12	32
M3	8	**17**	3	0	0	28	10	**15**	7	0	0	32
15-15.99												
I1					**25**	25					**27**	27
I2					**25**	25					**27**	27
C1					**25**	25					**27**	27
P1				1	**24**	25					**27**	27
P2				1	**24**	25					**27**	27
M1					**25**	25					**27**	27
M2				6	**19**	25				1	**26**	27
M3	4	**13**	7	1	0	25	2	**13**	10	1	1	27
16-16.99												
I1					**12**	12					**28**	28
I2					**12**	12					**28**	28
C1					**12**	12					**28**	28
P1					**12**	12					**28**	28
P2					**12**	12					**28**	28
M1					**12**	12					**28**	28
M2					**12**	12					**28**	28
M3		3	2	**5**	2	12		6	6	**14**	2	28

Table 9.1 continued. Age cohorts 17 and 18-20 years. Standard stages are in bold.

	Maxilla				Total	Mandible				Total
17-17.99	**E**	**F**	**G**	**H**		**E**	**F**	**G**	**H**	
I1				**45**	45				**45**	45
I2				**45**	45				**45**	45
C1				**45**	45				**45**	45
P1				**45**	45				**45**	45
P2				**45**	45				**45**	45
M1				**45**	45				**45**	45
M2				**45**	45				**45**	45
M3		7	9	**29**	45		9	11	**25**	45
18-20.00										
I1				**5**	17				**12**	17
I2				**5**	17				**12**	17
C1				**5**	17				**12**	17
P1				**5**	17				**12**	17
P2				**5**	17				**12**	17
M1				**5**	17				**12**	17
M2				**5**	17				**12**	17
M3		4	2	**11**	17		4	3	**10**	17

9.3.1 Comparison of the WITS Atlas (Figure 1) to the London Atlas (Figure 2)

The key differences between the two atlases are described in Table 9.2. In general, the Southern African children are considerably advanced in their dental development compared to the children represented in the London atlas. The WITS Atlas illustrates considerable differences in both the timing of emergence and the stages of tooth formation when compared to the London atlas. There is advanced development of the mandibular central incisors at ages 5.5 and 6.5. The maxillary lateral incisors are in occlusion at age 7.5 with the root stages almost completed, whereas in the London atlas these teeth are yet to emerge. Another important difference is that the canines and premolars emerge at least one year earlier. The timing of third molar development is the most striking difference between the two atlases. Third molars emerge at age

15.5 years and are in occlusion at 17.5 years. In contrast, third molars emerge four years later in the London atlas and are not in occlusion until 21.5 years, which is six years later than the Wits Atlas.

Table 9.2. Comparison of the WITS and London atlases

Age cohort	**WITS Atlas**	**London atlas**
5.5	Resorption of primary I1s is more advanced Crown completion of MI and root stages more than crown length	Resorption of primary I1 yet to commence Crown completion of M1s. Root stage commencement
6.5	Advanced root development of mandibular I1 More resorption of the mandible primary I2 and close to emergence	Not as advanced as WITS Atlas
7.5	All incisors are emerged and in occlusion The roots of M1 are more advanced Beginning of root formation of M2	Maxillary I2 yet to emerge. The roots of MI are less advanced Crown completion of M2
8.5	Developing mandibular C1 roots are more advanced Significant root resorption of premolars Furcation calcification appears in M2	Primary canine roots not resorbed Crown calcification completed in M2
9.5	Mandibular P1 emerging M2 root development advanced M3 crown formation completed Maxillary canines moving more medially	Mandibular P1 yet to emerge M2 root development not as advanced
10.5	Maxillary and mandibular P1 emerged and in occlusion M3 furcation calcification	P1s yet to emerged M3 crown formation not completed
11.5	Maxillary P2 emerged, mandibular P2 emerging M3 root development is advanced	P2s are yet to emerge This M3 stage occurs at 14.5
12.5	M3 crown advanced	
13.5	M2 root development almost completed M2s are in occlusion M3 root development more than length of the crown	
14.4	M3 half formed roots	M3 half formed roots at 16.5
15.5	M3 emerged	M3 emerged at 18.5
16.5	M3 in occlusion	M3 in occlusion at 21.5

Figure 9.1. WITS Atlas of tooth development

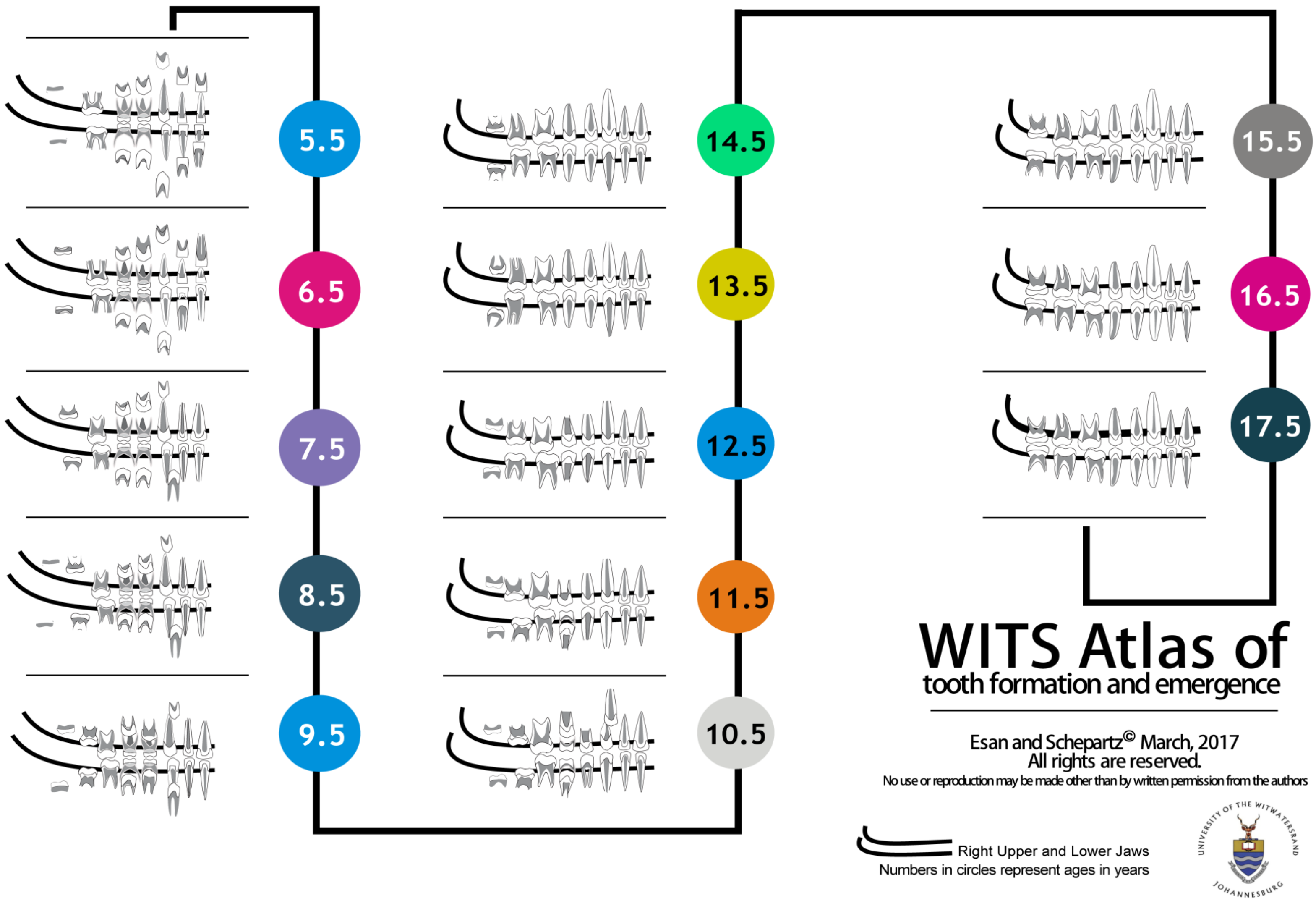
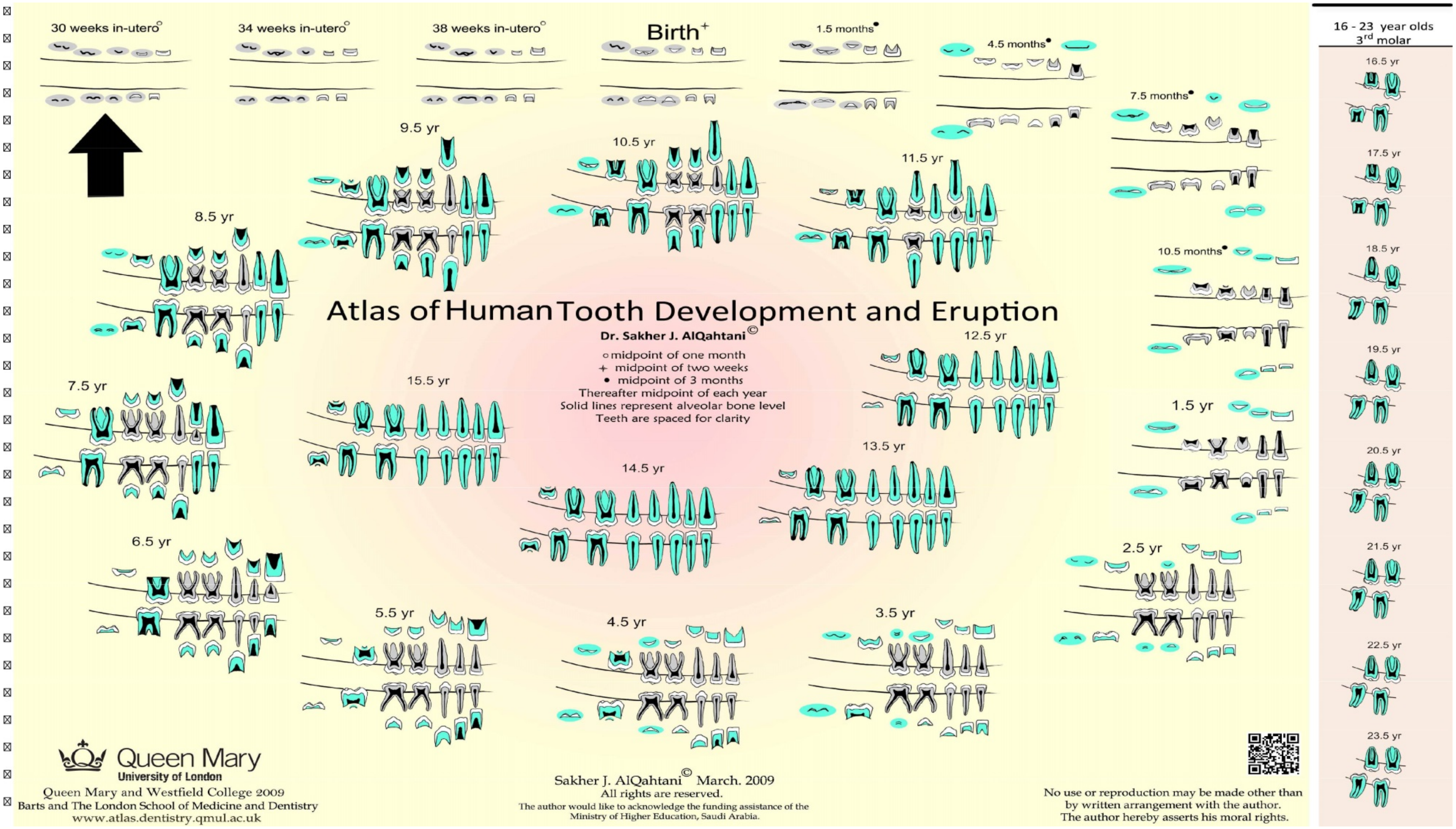


Figure 9.2 London Atlas



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9.3.2 Southern African specific age prediction models

Polynomial function equations (third order) were generated for males and females. For males, the formula is $y = 0.1434^3 - 5.4219x^2 + 68.775x - 197.34$ ($R^2=0.9137$) and for females it is $y = 0.1358x^3 - 5.2085x^2 + 66.593x - 185.74$ ($R^2=0.8971$).

Where y=maturity score and x=chronological age. The maturity score graphs for Black Southern Africans are presented in Figures 9.3 and 9.4 separately for males and females.

Figure 9.3. Regression of male chronological age versus maturity scores

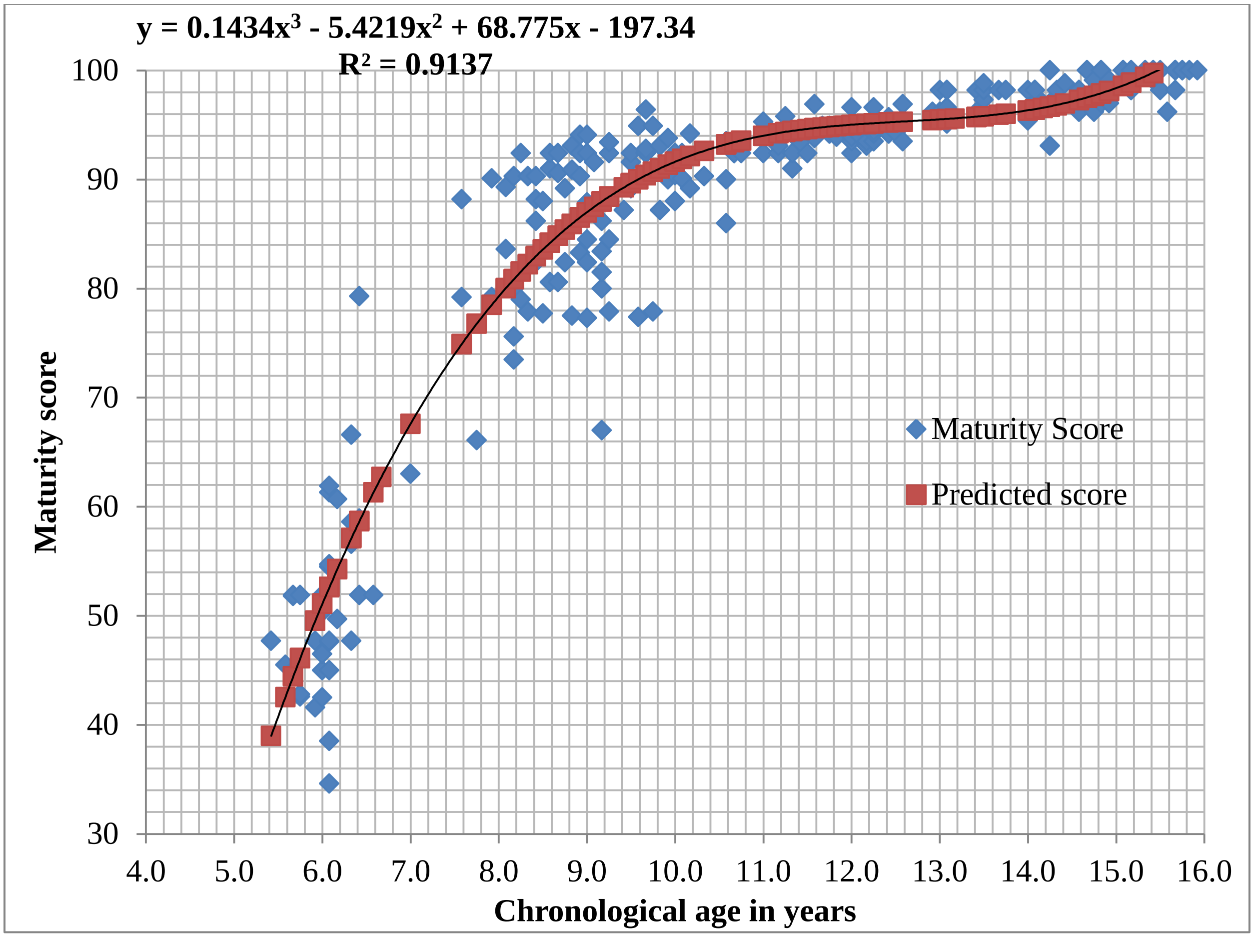
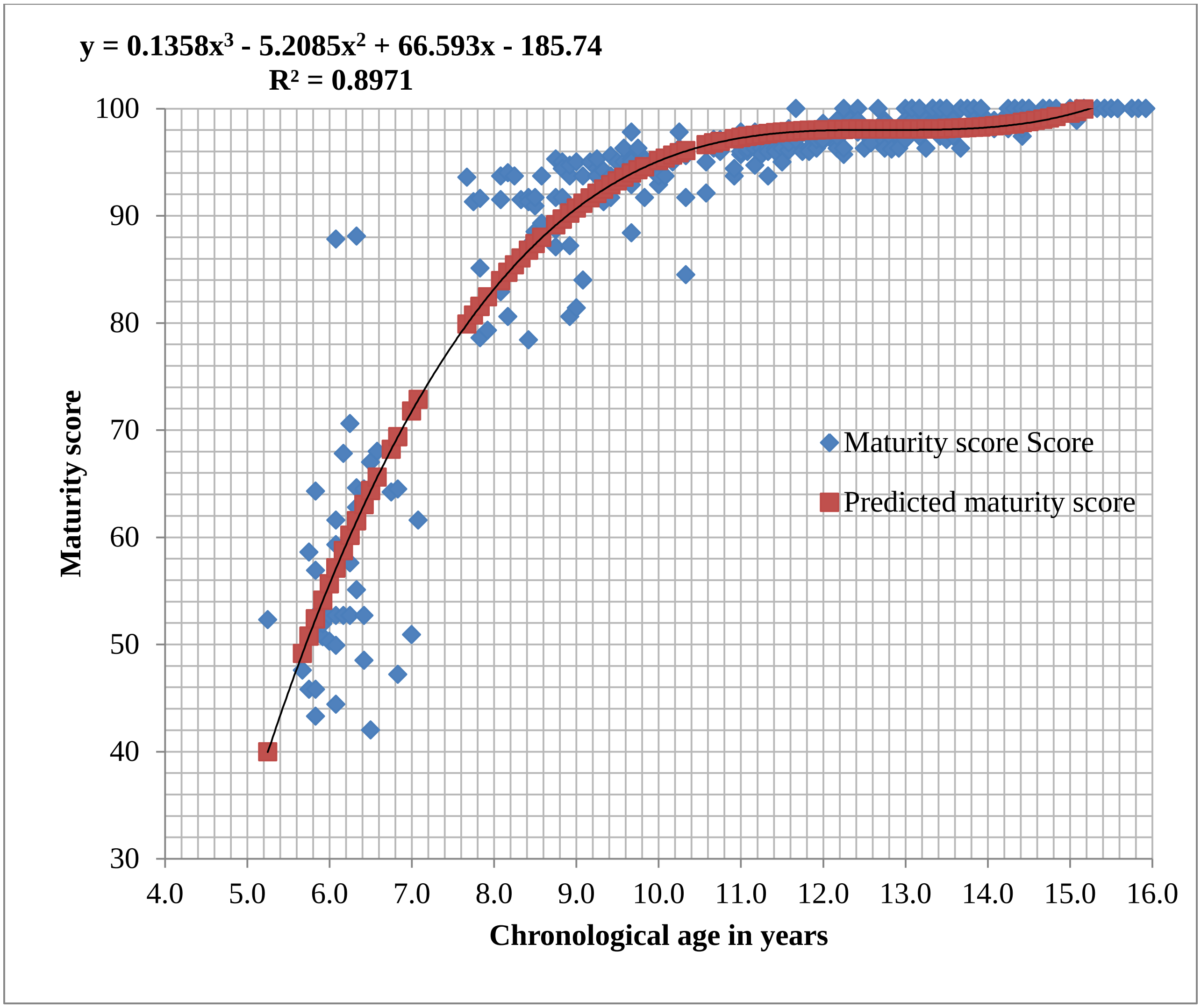


Figure 9.4. Regression of female chronological age versus maturity scores



9.3.3 The new Southern African specific maturity score

Southern Africa specific conversion tables of maturity to dental age were generated separately for males and females (Tables 9.3 and 9.4).

Table 9.3. Conversion of dental age scores in Southern African males

Age	Predicted Maturity score	Age	Predicted Maturity score	Age	Predicted Maturity score
5.4	38.98	**9.1**	87.47	**12.6**	95.25
5.5	40.75	**9.2**	87.98	**12.7**	95.29
5.6	42.52	**9.3**	88.41	**12.8**	95.35
5.7	44.45	**9.4**	89.27	**12.9**	95.40
5.8	46.12	**9.5**	89.64	**13.0**	95.44
5.9	49.54	**9.6**	90.00	**13.1**	95.48
6.0	51.10	**9.7**	90.39	**13.2**	95.53
6.1	52.61	**9.8**	91.02	**13.3**	95.58
6.2	54.28	**9.9**	91.34	**13.4**	95.65
6.3	57.13	**10.0**	91.62	**13.5**	95.74
6.4	58.67	**10.1**	91.88	**13.6**	95.80
6.5	59.99	**10.2**	92.16	**13.7**	95.87
6.6	61.30	**10.3**	92.61	**13.8**	95.94
6.7	62.73	**10.4**	92.98	**13.9**	96.02
6.8	64.90	**10.5**	93.10	**14.0**	96.15
6.9	66.15	**10.6**	93.22	**14.1**	96.31
7.0	67.60	**10.7**	93.41	**14.2**	96.41
7.1	68.98	**10.8**	93.57	**14.3**	96.54
7.2	70.40	**10.9**	93.80	**14.4**	96.67
7.3	71.78	**11.0**	94.00	**14.6**	96.96
7.4	72.98	**11.1**	94.10	**14.7**	97.14
7.5	74.00	**11.2**	94.24	**14.8**	97.28
7.6	74.91	**11.3**	94.44	**14.9**	97.48
7.7	76.15	**11.4**	94.54	**15.0**	97.67
7.8	76.76	**11.5**	94.62	**15.1**	98.11
7.9	78.50	**11.6**	94.69	**15.2**	98.32
8.0	79.50	**11.7**	94.77	**15.3**	98.57
8.1	80.03	**11.8**	94.83	**15.4**	98.86
8.2	80.85	**11.9**	94.95	**15.5**	99.41
8.3	82.22	**12.0**	95.00	**15.6**	99.75
8.4	82.95	**12.1**	95.05	**15.7**	100.00
8.5	83.58	**12.2**	95.10	**15.8**	100.00
8.6	84.18	**12.3**	95.14	**15.9**	100.00
8.7	84.84	**12.4**	95.22		
8.8	85.39	**12.5**	95.25		
8.9	86.51				
9.0	87.00				

Table 9.4. Conversion of dental age scores in Southern African females

Age	Predicted Maturity score	Age	Predicted Maturity score	Age	Predicted Maturity score
5.3	39.96	**9.1**	91.16	**12.7**	98.08
5.4	43.00	**9.2**	91.66	**12.8**	98.08
5.5	45.45	**9.3**	92.47	**12.9**	98.08
5.6	47.51	**9.4**	92.90	**13.0**	98.09
5.7	49.15	**9.5**	93.26	**13.1**	98.09
5.8	50.78	**9.6**	93.60	**13.2**	98.09
5.9	54.13	**9.7**	93.97	**13.3**	98.11
6.0	55.64	**9.8**	94.57	**13.4**	98.12
6.1	57.13	**9.9**	94.78	**13.5**	98.14
6.2	58.75	**10.0**	95.14	**13.6**	98.15
6.3	61.54	**10.1**	95.39	**13.7**	98.18
6.4	63.05	**10.2**	95.65	**13.8**	98.21
6.5	64.35	**10.3**	95.86	**13.9**	98.29
6.6	65.62	**10.4**	96.20	**14.0**	98.33
6.7	67.32	**10.5**	96.43	**14.1**	98.38
6.8	68.22	**10.6**	96.62	**14.2**	98.45
6.9	70.61	**10.7**	96.79	**14.3**	98.59
7.0	71.77	**10.8**	96.93	**14.4**	98.68
7.1	72.85	**10.9**	97.20	**14.5**	98.77
7.2	74.52	**11.0**	97.30	**14.6**	98.88
7.3	75.81	**11.1**	97.40	**14.7**	99.00
7.4	76.92	**11.2**	97.51	**14.8**	99.25
7.5	78.90	**11.3**	97.59	**14.9**	99.41
7.6	79.10	**11.4**	97.73	**15.0**	99.57
7.7	79.89	**11.5**	97.79	**15.1**	99.73
7.8	81.55	**11.6**	97.84	**15.2**	99.93
7.9	82.43	**11.7**	97.89	**15.3**	100.00
8.0	83.00	**11.8**	97.96	**15.4**	100.00
8.1	83.92	**11.9**	97.99	**15.5**	100.00
8.2	84.72	**12.0**	98.01	**15.6**	100.00
8.3	85.40	**12.1**	98.30	**15.7**	100.00
8.4	86.77	**12.2**	98.05	**15.8**	100.00
8.5	87.38	**12.3**	98.06	**15.9**	100.00
8.6	87.97	**12.4**	98.07	**15.9**	100.00
8.7	88.80	**12.5**	98.08		
8.8	89.67	**12.6**	98.08		
8.9	90.23				
9.0	90.71				

The age cohorts and the number of the participants (233 males and 307 females) are shown in Tables 9.5 and 9.6. The mean ages are 10.69±3.08 years and 11.15±2.89 years for males and females respectively. There is no significant difference between the mean ages of males and females ($p=0.078$). The estimated dental age and chronological age of the children are compared in Tables 9.5 and 9.6. Significant overestimation is only found for the age cohort 10 years in males ($p<0.05$), while it characterises the age cohort 8 years in females. No significant underestimation is found in males at any age, although it is found for females in the 10, 11 and 15-year age cohorts (Tables 9.7 and 9.8).

The mean difference (overestimation) between the dental ages (SADA) estimated by the Southern African specific maturity score method and the chronological ages (CA) for males is 0.06 years; for females, it is 0.08 years (Table 9.7). The one sample t-test does not show any significant difference between the estimated SADA and CA in both males and females indicating that there are no fixed biases ($p<0.05$) (Table 9.7). The presence of proportional bias was investigated by linear regression analysis of the mean difference between the estimated SADA and the CA against the average of the estimated SADA and the CA. A significant slope of the regression line (Males $p=0.02$; Females $p=0.02$) documents the presence of proportional bias in the measurements. This result indicates that the test values (SADA) do not agree equally with the CA throughout the range of age cohorts (Table 9.8). The Bland Altman plots illustrate the presence of proportional bias in the distribution of the plotted points. The male plot (Figure 9.5) has many scatter points above the upper limit of agreement (green line) and below the lower limit (yellow line). In the female plot (Figure 9.6), the greatly disproportionate distribution of the scatter points below the lower limit of agreement (yellow line) clearly illustrates a different patterning of the proportional bias.

Figure 9.5. Bland Altman plot between the mean difference of SADA and CA and the average of SADA and CA in males

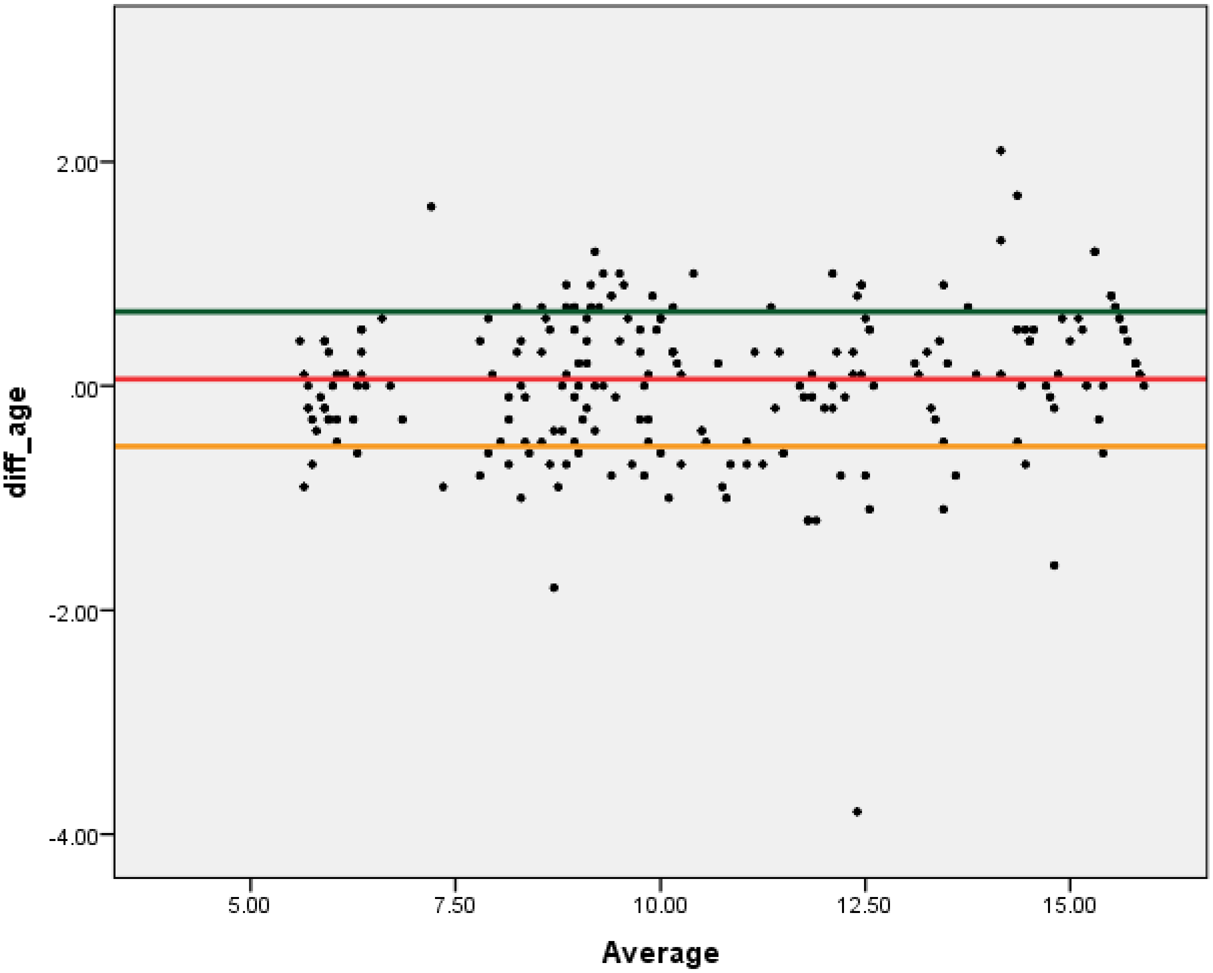


Figure 9.6. Bland Altman plot between the mean difference of SADA and CA and the average of SADA and CA in females

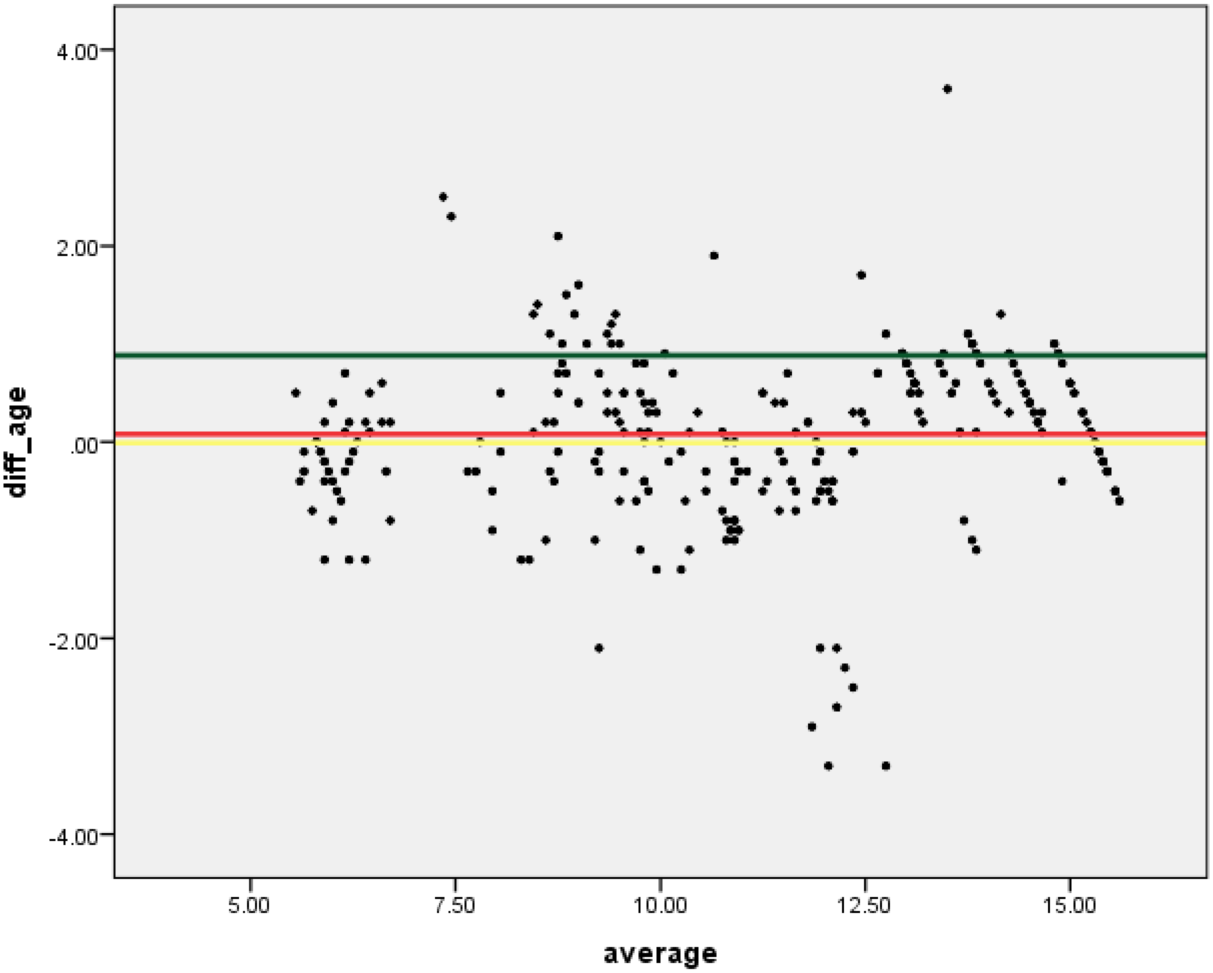


Table 9.5. Mean differences between Southern African specific dental age and chronological ages by age cohort in males

Age cohort (years)	N	Southern African dental age (SADA)		Chronological age (CA)		SADA-CA		T	p	95% CI		Mean Absolute Error (MAE)
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**			**Lower**	**Upper**	
5 – 5.99	10	5.79	0.21	5.71	0.15	0.08	0.28	0.91	0.39	-0.12	0.28	0.24
6 – 6.99	26	6.16	0.55	6.19	0.19	-0.03	0.49	-0.40	0.70	-0.24	0.16	0.34
7 – 7.99	6	7.73	0.76	7.63	0.34	0.10	0.60	0.40	0.71	-0.54	0.74	0.47
8 – 8.99	33	8.70	0.68	8.54	0.26	0.16	0.59	1.55	0.13	-0.05	0.37	0.51
9 – 9.99	36	9.48	0.74	9.38	0.31	0.10	0.64	0.83	0.41	-0.13	0.31	0.52
10 – 10.99	16	10.03	0.43	10.29	0.30	-0.27	0.44	-2.52	**0.02**	-0.52	-0.04	0.38
11 – 11.99	17	11.26	0.63	11.47	0.29	-0.21	0.57	-1.58	0.13	-0.51	0.08	0.47
12 – 12.99	21	12.26	0.54	12.27	0.23	-0.01	0.07	-0.20	0.85	-0.33	0.28	0.53
13 – 13.99	15	13.71	0.85	13.31	0.25	0.40	0.84	1.81	0.09	-0.07	0.86	0.63
14 – 14.99	28	14.53	1.03	14.45	0.31	0.07	0.92	0.33	0.75	-0.30	0.41	0.56
15 – 15.99	25	15.69	0.45	15.47	0.26	0.22	0.52	2.01	0.06	-0.01	0.42	0.34

Significant values in bold.

Table 9.6. Mean differences between mean South African specific dental and chronological ages by age cohort in females

Age cohort (years)	N	Southern Africa dental age (SADA)		Chronological age (CA)		SADA-CA		t	p	95% CI		Mean Absolute Error (MAE
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**			**Lower**	**Upper**	
5 – 5.99	13	5.81	0.32	5.77	0.17	0.04	0.33	0.42	0.68	-0.16	0.24	0.24
6 – 6.99	28	6.28	0.78	6.31	0.24	-0.03	0.81	-0.16	0.87	-0.34	0.29	0.53
7 – 7.99	9	7.94	1.34	7.64	0.35	0.30	1.10	0.82	0.44	-0.55	1.15	0.88
8 – 8.99	27	9.03	0.73	8.54	0.28	0.49	0.73	3.48	**0.00**	0.20	0.78	0.75
9 – 9.99	30	9.67	0.71	9.46	0.26	0.21	0.64	1.78	0.09	-0.03	0.44	0.52
10 – 10.99	22	9.95	0.66	10.42	0.33	-0.47	0.59	-3.71	**0.00**	-0.73	-0.21	0.52
11 – 11.99	36	11.24	0.95	11.41	0.28	-0.17	0.88	-3.86	0.24	-0.47	0.12	0.64
12 – 12.99	44	12.73	0.73	12.46	0.26	0.27	0.56	-0.48	**0.03**	0.09	0.43	0.56
13 – 13.99	39	13.51	1.33	13.41	0.28	0.10	1.30	2.47	0.66	-0.33	0.51	1.01
14 – 14.99	32	14.67	0.87	14.39	0.20	0.28	0.83	1.88	0.07	-0.02	0.57	0.66
15 – 15.99	27	15.28	0.12	15.41	0.29	-0.13	0.29	-2.42	**0.02**	-0.25	-0.02	0.27

Significant values in bold.

Table 9.7. One sample t-test of the mean difference between Southern African dental age and chronological age

Sex		**Test Value = 0**					
	N	**Mean Difference**	**SD**	**t**	**p**	**95% CI of the difference**	
						Lower	**Upper**
Male	233	0.06	0.54	1.30	0.20	-0.03	0.14
Female	307	0.08	0.73	1.78	0.08	-0.00	0.18

Table 9.8. Regression analysis between the overall mean difference and the average of SADA and CA in males and females to determine the presence of proportional bias

	Coefficients		t	Sig.	95% CI	
Male	B	Std. Error			Lower	Upper
Constant	-0.30	0.15	-1.97	0.05	-0.59	0.00
Average	0.03	0.01	2.43	**0.02**	0.01	0.06
Female						
Constant	-0.33	0.19	-1.75	0.08	-0.69	0.04
Average	0.04	0.02	2.27	**0.02**	0.01	0.07

Significant values in bold.

9.3.4 Comparison of age estimation using South African specific maturity scores with other methods

The mean difference between the estimated Southern African dental ages (SADA) and the chronological ages is less than 0.5 in all age cohorts for both males and females (Tables 9.9 and 9.10). The total mean difference between the estimated SADA and the CA is the lowest when compared to the total mean differences from the other methods (Original Demirjian, Modified Demirjian and Willems) in both males and females (Tables 9.9 and 9.10).

The mean absolute error for the estimated SADA values is 0.45 for males and 0.59 for females. None of the age cohorts in males and females have mean absolute errors of over one year. The mean absolute errors detected for the estimated SADA values are the lowest compared to the other methods (Table 9.11).

Table 9.9. Mean differences of the methods for age estimation in males

Age cohort (years)	n	Original Demirjian		Modified Demirjian		Willems		Southern African specific method	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
5 – 5.99	10	1.32	0.31	0.92	0.31	0.34	0.41	**0.08**	**0.28**
6 – 6.99	26	1.13	0.43	0.69	0.73	0.76	1.83	**-0.03**	**0.49**
7 – 7.99	6	1.23	0.92	1.46	1.13	0.82	0.98	**0.10**	**0.60**
8 – 8.99	33	1.21	1.05	1.38	1.03	0.81	0.77	**0.16**	**0.59**
9 – 9.99	36	0.89	1.33	0.73	1.22	0.32	0.97	**0.10**	**0.64**
10 – 10.99	16	0.53	0.76	1.01	0.89	-0.15	0.57	**-0.27**	**0.44**
11 – 11.99	17	0.57	0.88	1.18	0.76	-0.15	0.83	**-0.21**	**0.57**
12 – 12.99	21	0.11	0.84	0.87	0.73	-0.31	0.72	**-0.01**	**0.07**
13 – 13.99	15	1.42	1.14	1.36	0.95	0.24	0.81	**0.40**	**0.84**
14 – 14.99	28	0.56	1.12	0.54	0.91	-0.45	1.09	**0.07**	**0.92**
15 – 15.99	25	0.39	0.56	0.18	0.46	-0.15	0.87	**0.22**	**0.52**
Total mean diff	233	0.85	0.85	0.94	0.83	0.19	0.90	**0.06**	**0.54**

Table 9.10. Mean differences of the methods for age estimation in females

Age cohort (years)	**n**	**Original Demirjian**		**Modified Demirjian**		**Willems**		**Southern African specific method**	
		Mean diff	**SD**	**Mean diff**	**SD**	**Mean diff**	**SD**	**Mean diff**	**SD**
5 – 5.99	13	1.26	0.32	0.40	0.48	0.17	0.53	**0.04**	**0.33**
6 – 6.99	28	1.05	0.67	0.47	0.89	0.32	0.92	**-0.03**	**0.81**
7 – 7.99	9	1.05	1.11	-0.81	1.05	0.34	0.76	**0.30**	**1.10**
8 – 8.99	27	1.31	0.98	1.50	1.15	0.65	0.65	**0.49**	**0.73**
9 – 9.99	30	1.37	0.95	1.79	1.29	0.34	0.78	**0.21**	**0.64**
10 – 10.99	22	0.93	1.09	1.84	1.69	-0.05	0.76	**-0.47**	**0.59**
11 – 11.99	36	0.92	0.94	2.48	1.26	-0.02	0.85	**-0.17**	**0.88**
12 – 12.99	44	1.03	1.13	2.25	0.94	0.19	1.29	**0.27**	**0.56**
13 – 13.99	39	1.35	1.30	1.93	0.65	0.79	1.58	**0.10**	**1.30**
14 – 14.99	32	0.59	0.79	1.11	0.28	-0.03	1.10	**0.28**	**0.83**
15 – 15.99	27	0.54	0.35	0.38	0.29	0.31	0.42	**-0.13**	**0.29**
Total mean diff	307	1.04	0.88	1.21	0.91	0.27	0.88	**0.08**	**0.73**

Table 9.11. Mean absolute error by age cohorts

Age cohorts	SA specific maturity score method		Willems method		Original Demirjian method		Modified Demirjian method	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
5 – 5.99	0.24	0.24	0.38	0.44	1.19	1.25	0.78	0.33
6 – 6.99	0.34	0.53	0.90	0.64	1.20	0.86	0.89	0.63
7 – 7.99	0.47	0.88	0.99	0.34	1.51	0.96	1.75	0.76
8 – 8.99	0.51	0.75	0.91	0.64	1.54	1.46	1.47	1.43
9 – 9.99	0.52	0.52	0.83	0.61	1.49	1.50	1.28	1.94
10 – 10.99	0.38	0.52	0.57	0.65	0.68	1.24	1.06	2.46
11 – 11.99	0.47	0.64	0.56	0.67	0.63	0.86	1.08	2.47
12 – 12.99	0.53	0.56	0.47	0.98	0.73	1.01	1.02	2.00
13 – 13.99	0.63	1.01	0.65	1.28	1.73	1.55	1.56	1.81
14 – 14.99	0.56	0.66	1.06	1.00	1.01	0.97	0.70	1.08
15 – 15.99	0.34	0.27	0.41	0.22	0.36	0.34	0.21	0.22
Mean	**0.45**	**0.60**	**0.70**	**0.68**	**1.10**	**1.09**	**1.07**	**1.38**

9.4 Discussion

Variation in dental development among human populations is well documented. All previous research, as well as the present study, establishes that Black Southern African children are significantly advanced in dental emergence and tooth formation compared to European ancestry populations. Even so, dental reference developed from US and European populations are still used to estimate age in Africa due to a lack of population-specific reference data. This study introduces a new dental atlas (WITS Atlas) and new dental age predictive equations for age estimation for forensic, anthropological and clinical purposes in Southern Africa.

9.4.1 WITS Atlas

Dental atlases are quick and easy tools for assessment of dental development in children and adolescents. They require less specialized dental knowledge and obviate the use of sophisticated or destructive methods of age estimation in forensics and clinical applications (AlQahtani et al. 2010). The first dental atlas (Schour and Massler 1941) was broadly criticized for the obscured nature of the sample population, method of analysis and undefined tooth stages (Smith 1991; AlQahtani et al. 2014). This necessitated revisions and led to the development of new atlases such as Uberlaker (1978), Nander and Chawla (1966) and the most recently developed London atlas (AlQahtani et al. 2010). These atlases are based on populations from the US, Europe and Asia. None of the existing atlases take into consideration the significantly advanced tooth emergence and maturity found in African populations.

The number and magnitude of differences in the timing and stages of permanent tooth emergence and formation between the WITS and the London atlases demonstrates that the London atlas is not suitable for age estimation of Black Southern Africans. This is particularly true for forensic applications where the level of accuracy neEds to be within 6 months or at most one year (McKenna et al. 2002; Flood et al. 2011). For example, a Southern African Black child with emerged mandibular first premolars at age 9.5 would be aged as 11.5 using the London atlas. If the third molar is considered, the age discrepancy can be over 4 years. Hence there is a need to factor in the advanced dental development for anthropological, clinical and forensic purposes when estimating age for the Black Southern African population. This study further validates the population-based variability in dental development that has been reported by other authors (Davis and Hagg 1993; Willems et al. 2001; Chen et al. 2010; Cruz-Landeira et al 2010; Ogodescu et al. 2011; Baghdadi and Pani 2012; Erdem et al. 2013). Maturity score tables and atlases of tooth development based on data combined from diverse populations may not be useful where accuracy and specificity of age determination are required.

There were very few situations (3 out of 112 occurrences) where a tooth had two developmental stages with equal frequencies. It should be stressed that these 3 cases occurred between successive developmental stages. AlQahtani et al. (2010), when merging male and female data in their study, posited that there is no significant difference between the developmental stages of a tooth if they are contiguous. Hence, the more advanced stage chosen in these very few occasions would not result in overestimation of age in the WITS Atlas.

The similarity of tooth emergence times of Southern Africans and other sub-Saharan populations (c.f. Chapter 6 of this thesis as well as studies from Nigeria (Oziegbe et al. 2014) and Kenya (Hassanali and Odhiambo 1989)) suggests that the WITS Atlas may be used for clinical and forensic applications for sub-Saharan African children.

A limitation of this study is the absence of the younger age categories in the sample and the small sample size in age 18 and above. This is partly due to the study design, which is based on data collected during visits to schools and not the usual retrospective use of x-rays archived in hospitals, and the need to comply with the health regulations and ethics of South Africa that do not permit x-rays of very young children except when there is an absolute medical or dental condition that dictates their use. Furthermore, exclusion of participants with third molar impaction and agenesis from the study, and the limited number of participants above age 18 years in secondary schools, also contributed to the small sample size for age group 18-20 years. The available paediatric cadaver collections in South Africa are comprised of children of unknown ages and so their inclusion in this study was not possible. Future efforts to verify the ages of children in those collections will facilitate the expansion of the WITS Atlas to the younger age cohorts.

9.4.2 Age prediction equations for Southern African children

The Demirjian and Willems methods have become the most commonly applied procedures for dental age estimation using radiological data (Willems et al. 2001; Maber et al. 2006; Mani et al. 2008; Nik-Hussein et al. 2011; Urzel et al. 2013, Uys et al. 2014; Zhai et al. 2016). These methods are based on clearly defined and identifiable tooth developmental stages. Moreover, these methods have very good reproducibility for both intra- and inter-observer assessments (Willems 2001; Baghdadi 2014). However, the current study showed that these methods significantly overestimate the chronological age of Black Southern Africans. This warranted the development of population-specific maturity scores for dental age estimation in our population.

Our results demonstrate that the Southern African specific prediction models and tables of conversion provide a more accurate method for age estimation in males and females. This is evidenced by a mean difference value for the dental and the chronological ages that is nearest to zero compared to the Demirjian and Willems methods. Even so, we did find proportional bias with the application of our method, which indicates that the estimation of dental age by the Southern African specific maturity scores does not agree consistently with the chronological age across the age cohorts. This result is expected given the influence of environmental factors on tooth development. Children with higher nutritional status in our sample have advanced dental development compared with children of similar age with lower nutritional statuses (Chapters 5 and 7 of this thesis). Despite this shortcoming, the coefficients of the regression models for both males and females are very close to zero, indicating a high degree of accuracy and thus may be used for clinical, anthropological and forensic applications. Furthermore, the low mean absolute error value reported in this study for the dental age is further proof that the population-specific values developed here can be used for reliable age estimation of Black Southern African children. However, further studies are needed to validate this method in Black Southern African children.

The mean absolute error found for the dental age of Black Southern Africans is very low compared to the errors of the Demirjian and Willem methods. This means that the Southern African specific age estimation method is more accurate than any of the Demirjian or Willems methods. Therefore, the new age prediction model in this study may be used with a high degree of accuracy for both males and females. Our findings are consistent with those of previous studies, in that estimating the chronological age using a population-specific approach produces accurate results in their populations (Willems et al. 2001; Duangto et al. 2016).

9.5 Conclusion

This study provides new dental references in the form of the WITS Atlas, age prediction models and tables for conversion of maturity scores to dental age for Black Southern African males and females. The use of the tables in our reference population provides the highest accuracy for a Southern African population when compared with the Demirjian and the Willems methods of age estimation. The magnitude of over- or underestimation error for the specific method is negligible and within the acceptable limits for forensic purposes (McKenna et al. 2002, Flood et al. 2011). Even so, we did find proportional bias with the application of our method, which indicates that the estimation of dental age by the Southern African specific maturity scores does not agree consistently with the chronological age across the age cohorts. Further studies are needed to validate the use of these new tables of conversion for Southern Africa. Furthermore, similarities in dental development across sub-Saharan African populations suggest that the WITS Atlas and the new age prediction model and conversion tables can be used for those populations as well.

9.6 References

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Contents

Chapter 10

General discussion

References for dental development currently used in most clinical, forensic and academic settings in Southern Africa are based on references derived from other populations despite substantial evidence that population variation exists. The problems with using non-specific reference are complex, and their application may lead to misrepresentation of growth status in clinical, forensic and anthropological settings.

Reference for skeletal, somatic and sexual growth and development have been developed for Southern African children (Norris & Richter 2005; Vidulich et al 2006; Griffiths et al. 2008; Sheppard et al. 2009) through a large longitudinal investigation into child and adolescent health and development in the Johannesburg-Soweto metropolis, otherwise known as “birth to twenty” study (Richter et al. 2007). This thesis adds to that body of knowledge with a thorough investigation of dental development in a similar population from Johannesburg.

This is the first comprehensive study (comprised of eight separate investigations) that documents the timing of emergence for all permanent teeth and the age of attainment of specific maturity stages of tooth formation for Black Southern African children and further investigates the observed variation in terms of sexual dimorphism and responses to environmental stress. The results clearly indicate the need for population specific references for dental development. Based on our findings, two references, an atlas of tooth formation and emergence, and tables of population-specific maturity scores were developed for the Black Southern African population. The similarity of our results with other studies on dental development from other sub-Saharan African countries suggests that our reference can be used for the entire sub-Saharan African region.

There are three major conclusions that can be drawn from our series of studies on dental development. First, Black Southern African children, like other sub-Saharan African children and children of African ancestry world-wide, achieve dental maturity earlier than children of European and Asian ancestry. This is true even though they are more frequently subjected to greater environmental stresses. At the same time our study demonstrated significant effects of nutritional status on dental development contrary to prevailing opinions that dental development is largely resistant to such stresses. We also found that life history events co-occur with dental emergence and some specific stages of tooth formation in Black Southern African children suggesting that dental development and sexual development are under similar controlling influences that may involve hormonal regulation of general growth processes.

This study provides evidence on the accuracies of the common methods used in estimating dental age globally through a meta-analysis and also tests the validity of the methods in Black Southern African Children.

10.1 The Demirjian versus the Willems methods for dental age estimation in different populations: A meta-analysis of published studies

A systematic review and meta-analysis of published studies on the Original Demirjian (Demirjian et al. 1973) and Willems (Willems et al. 2001) age estimation methods was undertaken to determine their accuracies. This is the first systematic review to provide scientific evidence on the accuracy of the two most widely employed methods of age estimation.

This study posed the research question **“***Does the Demirjian method for dental age estimation provide a more accurate estimate of chronological age when compared to the Willems method of dental age estimation in different populations?”* We found that the Willems method is more accurate than the Demirjian method in estimating chronological age of children and adolescents globally. The Demirjian method significantly overestimated chronological age ($p<0.05$) in males and females when the studies were pooled by age cohorts and sex. The majority of studies using the Willems method did not report significant overestimation of ages in males or females, but when all the studies were pooled, significant overestimation was found. The weighted mean difference for the Demirjian method was 0.62 for males and 0.72 for females compared to 0.26 and 0.29 for males and females with the Willems method. Previous systematic reviews have reported overestimation for Demirjian methods in most populations (Jayaraman et al. 2013; Yan et al 2013). The overestimation seen in both methods may be due to population level genetic diversity and environmental influence. In summary, the Willems method significantly overestimates chronological age, yet it has significantly better accuracy than the Demirjian method.

10.2 Tooth Formation: Assessment of maturity scores and dental age estimation of Southern African children using Demirjian's method

Our study documented sexual dimorphism in dental maturity with females showing significantly advanced maturity scores compared to males. These are similar to those of other studies (Fanning 1961; Demirjian et al. 1973; Demirjian and Levesque 1980; Liversidge et al. 1999; Cavrić et al. 2016). This pattern of female advancement is also observed for many developmental indicators such as sexual maturity and skeletal development (Stang and Story 2005). Males are more affected by environmental stresses than females, particularly during the prenatal period (Tobias 1972; Wolanski and Kasprzak 1976; Stinson 1985), and this could be expressed as sexual dimorphism in dental development.

Black Southern African children are more advanced in dental maturity compared to Asian and European ancestry children. This finding is similar to the result of a previous study (Tompkins 1996) that found black Southern African children to have an earlier age of attainment of maturation of the enamel and dentine in incisors and molars compared to a Native American and French-Canadian child. This suggests a genetic basis for population variation in tooth formation.

10.3 Tooth Formation: Accuracy of the Willems method and Demirjian's seven tooth methods

This study investigated the accuracies and validity of three common methods estimating age (Original Demirjian, Modified Demirjian and Willems methods) in a Black Southern African population. We found significant overestimation of chronological age of Black Southern African children by these methods highlighting the need for population-specific reference

The Original Demirjian method significantly overestimated the chronological age in all the age cohorts studied. Our result is similar to other studies from European, Asian and African populations using Demirjian's method (Willems et al. 2001; Hegde and Sood 2002; Baghdadi and Pani 2012; Ifesanya and Adeyemi 2012; Jayaraman et al. 2013; Uys et al. 2014; Carneiro et al. 2015; Cavrić et al. 2016). Overall the Demirjian's method overestimated the age of males by 0.85 years and the ages of females by 1.0 years with a high mean absolute error of 1.1 years for both males and females. The reason for the overestimation may be due to advance dental maturity in the Southern African children compared to the Demirjian's reference sample of French Canadian children. In addition, genetic differences and environmental influences contribute to the inaccuracy of the method for age estimation in Black Southern Africans.

Similarly, the Modified Demirjian method overestimated chronological age by 0.9 years and 1.2 years in males and females with a high mean absolute error of 1.4 for females, the highest error of any method. Again, the overestimation may be due to genetic and environmental influences on tooth formation. Other studies also found significant overestimation with this method (Willems et al. 2001; Lee, et al. 2011; Flood et al. 2013; Akkaya et al. 2015).

The Willems method has significantly better accuracy at estimating chronological age of black Southern African children compared to the Demirjian methods. This is in agreement with other studies (Mani et al. 2008; El-Bakary et al. 2010; Nik-Hussein et al. 2011; Djukic et al. 2013; Urzel and Bruzek 2013; Ambarkova et al. 2014; Ye et al. 2014; Akkaya et al. 2015; Hegde et al. 2016). Although the Willems method significantly overestimates the chronological age, the low mean absolute error value of 0.70 and 0.68 years in males and females makes it a suitable tool for estimating chronological age in Black Southern Africa children. The reason for the better accuracy with the Willems method in many populations compared to Demirjian's method is not known. However, the removal of the cumbersome step of using a table of conversion to obtain the dental age might account for the better accuracy.

10.4 Effect of nutrition on tooth formation

Is the timing permanent tooth formation immune from environmental influences such as nutritional stress? Some authors believe that dental development is regulated strictly by genetic factors while others contend that factors such as nutrition play significant roles in the age of attainment of specific dental maturity stages.

Influence of nutrition and tooth formation

We used several anthropometric variables to provide information on the general nutritional status (height, weight, BMI, mid-upper arm circumference (MUAC) and head circumference (HC)). Our study used a combination of univariate and multivariate analyses to obtain our results. We found a significant influence of nutritional status on tooth formation in males and females, with a significant delay in age of attainment of H stages in children with low BMI values. This is in agreement with previous studies that found a significant influence of nutrition on tooth formation among children from Iran and the United States (Hilgers et al. 2006; Mack et al. 2013; Zangouei-Booshehri et al. 2011).

Our findings are at variance with previous reports that found no significant difference between underweight and overweight children (Bagherian and Sadeghi 2011; Eid et al. 2002; Elamin and Liversidge 2013). A reason for the lack of significant difference may be methodological differences in the use of BMI values.

Height, weight and tooth formation

Regression and correlational analysis showed that height significantly influenced maturity scores in both males and females. The result of this study is similar to the findings of Green (1961) and Demirjian et al. (1985). This study found no relationship between weight and tooth formation in the males, whereas a significant relationship was found in females. This may be due to the different pattern of weight gain in the two sexes (Geer and Shen 2009). The weight gain in females occurs earlier and continues till puberty while that of males occurs much later. These differences in the pattern of weight gain characterized the Black Southern African children.

Although our study found a strong correlation between weight and maturity scores in males and females, it is possible that the correlation may be due to weight gain as age increases.

MUAC and tooth formation

There was no significant relationship between the mid-upper circumference and tooth formation in this study. This is unexpected as MUAC is widely regarded as a good predictor of nutritional status. One reason for our result may be due to variation in the distribution of adipose tissue from one population to another (Gasperino 1996). Other measures of body composition, such as waist-to-hip ratios, may be more informative about nutritional status in Southern Africa. Furthermore, MUAC is affected by exercise, type of work or household chores and this may make the use of it solely for nutritional assessment unpredictable. Future studies are needed to explore this research area.

HC and tooth formation

A significant relationship was found between HC and tooth formation in females but not in males. This might be due to the longer period of increase in head circumference observed in the females. The HC measurement increased from age 5 to age 12 years in females and thereafter stabilized, whereas it only increased from age 5 to 9 years in males. This differing pattern of brain growth in males and females could be explained by the available fat reserves, which we found to be significantly greater in the females of our study population.

10.5 Permanent tooth emergence: Timing and sequence in black Southern African children

The present study documents the timing of emergence of all permanent teeth in a black southern African population. Females are significantly advanced in the timing of tooth emergence compared to males. This finding is in agreement with the pattern of female advancement in other populations (Savara and Stein 1978; Kochhar and Richardson 1998; Eskeli et al. 1999; Oziegbe et al. 2014; Leroy et al. 2003; Moslemi 2004; Khan 2011). Females also show advancement over males in most maturity indices (Almonaitiene et al. 2010) as they are more buffered from environmental insults (Stini 1982; Stinson 1985).

Sexual dimorphism was also noted in the sequence of tooth emergence. Females have the M_1 emerging before the I_1, as opposed to the I_1M_1 sequence in males. Black Southern African males have a similar sequence of emergence to children from sub-Saharan African, Europe and the US but differed from Asian populations. Southern African females and some females from sub-Saharan Africa and Pakistan show similar $M_I I_1$ polymorphism that differs from other populations. This may be attributed mainly to genetic influences on the timing and sequence of emergence of the permanent teeth.

Black Southern African children are advanced in the timing of emergence of permanent teeth compared to American, Asian and European children. Our finding is in conformity with other studies that reported the earlier timing of tooth emergence in African ancestry children compared to those of European descent (Garn et al. 1973; Lavelle 1976; Stewart et al. 1982; Blankenstein et al. 1990; Harris and McKee 1990; Koch and Poulsen 2001; Oziegbe et al. 2014). This may indicate that genetics play a strong role in the determination of permanent tooth development.

Similar emergence times of the permanent teeth were found in Southern African children and children from other sub-Saharan African populations from Nigeria (Oziegbe et al. 2014), the Gambia (Billewicz and McGregor 1975), Ghana (Houpt et al. 1967), Kenya (Hassanali and Odhiambo 1989) and Uganda (Krumholt 1971). Genetic affinity may be responsible for these similarities among Black sub-Saharan African populations as the majority are Bantu language speakers.

No temporal changes were found in the timing of emergence of incisors and first molars in this study and the Blankenstein et al. (1990) study, although some changes in the timing of emergence were expected due to the rapid demographic and socio-political changes and increasing gene flow in Southern Africans that occurred shortly after the Blankenstein et al. (1990) study was published. The short interval of time between the two studies may be responsible for our findings.

10.6 Influence of nutrition on permanent tooth emergence

Influence of BMI on tooth emergence

BMI is a well-regarded proxy of nutrition and it is frequently used to assess the nutritional status of children (WHO 1997). The present study found a significant relationship between BMI and number of teeth emerged. This is similar to the reports of previous authors from the USA and Mexico (Sánchez-Pérez et al. 2010; Must et al. 2012). High BMI enhances linear growth and early sexual maturation (Slyper 1998; Sánchez-Pérez et al. 2010). More studies are required to identify the specific mechanism involved in tooth emergence timing that is affected by high body fat content in children and adolescents.

Association between height, weight and tooth emergence

Our findings show a relationship between the number and the timing of permanent tooth emergence and height. This is similar to the findings of Niswander and Sujaku (1960) in a study of Japanese children. However, Kutesa et al. (2013) in a study on Ugandan children found mean tooth emergence times to directly correlate with height and not the weight of the children. On the contrary, Khan (2011) in a study conducted among Pakistani children observed that heavy and short children had early tooth emergence while tall children showed delayed emergence regardless of their weight.

No significant relationship was found between weight and the number of emerged teeth in this study. Weight is not an accurate measure of growth. It is affected by other confounding variables such as lifestyle and eating habits. Contrary to the findings of this study, a few studies (Haddad and Pires Correa 2005; Hilgers et al. 2006; Sánchez-Pérez et al. 2010; Must et al. 2012) found a relationship between the number of emerged teeth and weight in Mexican and USA populations. Those children with greater weight had higher mean number of emerged teeth. Furthermore, children who have lower than average weight and height have been shown to have later emergence times than those who are within the normal range (Adler 1963; Billewicz and McGregor 1975; Lee et al. 1965; Triratana and Kiatiparjuk 1989). The reason for the conflicting results is not clear but may be due to the difference in the sample population and method of analysis.

MUAC and number of emerged teeth

The present study did not find any relationship between the numbers of teeth emerged and MUAC. This could be due to population variation in the distribution of adipose tissue hence making the use of MUAC infective in distinguishing malnutrition. In addition, the present study did not include children who were severely malnourished or highly variable in their nutritional statuses. Craig et al. (2014) found poor accuracy for MUAC in classifying the nutritional status of black Southern African males aged 5-9 years. This appears to be the case for our sample as well. Further studies are needed to explore this relationship.

HC and number of emerged teeth

The study by Godfrey et al. (2001) on a large range of primates found brain development to be a better predictor of dental development than general somatic development. Similarly, a positive correlation between primary teeth and HC had been established in younger children (Vejdani et al. 2015). However, no significant relationship was found between the number of teeth emerged and head circumference in the present study. A relationship was expected because the head circumference increased gradually from age 5 to 9 years in males and 5 to 12 years in females. There is no similar study on permanent teeth to compare with the result of this study.

10.7 Dental development (tooth formation and emergence) and life history variables in Southern African children

Conflicting results have been published regarding the relationship between sexual development and tooth formation with most authors reporting low correlations. Methods of analysis could have been responsible for this because previous authors looked at correlations without examining similarities in the timing of occurrence of stages of tooth formation and Tanner stages of sexual maturity.

Tooth emergence and formation strongly correlate with chronological age more than height and other somatic measures. This finding is similar to earlier reports which show that dental development is less variable and also has low variability in relation to calendric age (Lewis and Garn 1960; Green 1961; Demirjian et al. 1985; Demirjian 1986), and a stronger association between chronological age and dental age than between skeletal age and dental age.

This is the first study comparing the Tanner stages of sexual development with dental development. Previous studies only considered how dental development relates to menarche. We found that the M_2 emerges approximately simultaneously with the onset of sexual development (age of attainment of G2 of genital development and the PH2 stage of pubic hair development) in males. In females, the M_2 emergence coincides with the attainment of B2 stage of breast development and PH2 stage of pubic hair development. Similarly, the attainment of the final (H) stage of mandibular canine development appears to occur at the same time period as the age of attainment of Tanner's G2 stage of genital development, and PH2 stage of pubic hair development in males, while it is only concurrent with the B2 stage of breast development in females. The relationship found in the present study between the mandibular canine and the onset of puberty may be a reflection of the circumpubertal increase in stature and acceleration in the growth of the craniofacial structures reported in many studies (Hunter 1966; Brown et al. 1971).

Age of attainment of menarche in Southern African females does not have any relationship with tooth emergence, yet the final (H) stage of the P_2 calcification is concurrent with the onset of menarche. Previous studies demonstrated low correlations between the emergence of premolars, molars, and menarche (Garn et al. 1965; Demirjian et al. 1985), with Demirjian et al. (1985) concluding that sexual and dental development may be under different controlling influences. The associations found in this study, in contrast, indicate similar controlling influences for some teeth and sexual development.

10.8 Tooth formation and emergence references for Southern African Black children

The significant overestimation of age by the Demirjian methods and the slight overestimation by the Willems method make it imperative to develop Black Southern African specific maturity scores. Polynomial function equations (3rd degree) were used to derive tables of maturity scores separately for males and females. The mean differences between dental age and chronological age for both males and females were not statistically significant. The mean differences and the mean absolute errors calculated for the Southern African specific methods were significantly lower compared to those derived from the Willems and Demirjian methods enabling the accurate estimation of age to less than one week of the chronological age. This demonstrates that tables of maturity scores based on data combined from diverse populations may not be useful where accuracy and specificity of age determination are required.

Researchers and clinicians typically compare growth in the population of interest to references formulated for other populations. The problems associated with using non-population specific references are numerous. Their application can lead to misrepresentation of the health status and inaccuracies of the age estimation. For these reasons, a new atlas of tooth emergence and formation called “the WITS Atlas” was derived from the panoramic radiographs of the Black Southern African children using the patterns of tooth formation and emergence that occurred most commonly in each age cohort. The number and magnitude of differences in the timing and stages of permanent tooth emergence and formation between the WITS and the London atlases demonstrates that the London atlas is not suitable for age estimation of Black Southern Africans. This is particularly true for forensic applications where the level of accuracy needs to be within 6 months or at most one year (McKenna et al. 2002; Flood et al. 2011). For example, a Southern African Black child with emerged mandibular first premolars at age 9.5 would be aged as 11.5 using the London atlas. If the third molar is considered, the age discrepancy can be over 4 years.

Our findings suggest that these new tables of conversion of maturity scores can be used for age estimation for forensic, anthropological and clinical purposes in Black Southern Africans. Furthermore, similarities in advanced dental development across sub-Saharan African populations suggest that the WITS Atlas and the new age prediction model and conversion tables can be used for those populations as well.

10.9 Conclusions

1. There is sex dimorphism in age of emergence with the females emerging their permanent teeth earlier. The Black Southern Africans show similarities in the ages and sequence of emergence of the permanent teeth with children from sub-Saharan African countries. However, they are advanced compared to children from the USA, Europe, Australia and Asia.
2. Globally, the Willems method is more accurate at predicting chronological age compared to the widely used Original Demirjian method.
3. Tooth formation in the Black Southern African children is more advanced compared to children of European and Asian ancestry.
4. The Willems method is more accurate at estimating chronological age of the Black Southern African children compared to the Original and Modified Demirjian methods.
5. Contrary to some studies, malnutrition has significant influence on the number of teeth emerged and the timing of emergence of permanent teeth. Obese/overweight/tall children tend to have more emerged teeth and earlier age of emergence than underweight/short children of the same age.
6. Similarly, obese/ overweight individuals attained H stage of dental development earlier than the underweight children.

7. Tooth emergence and formation are under similar controlling influence during growth and development. Emergence of second molars and the H stage of canine and first premolar formation co-occur with onset of puberty in males and females. Menarche appears to coincide with the attainment of the H stage of mandibular second premolar.
8. WITS Atlas and new population specific maturity tables for Black Southern Africans were developed. The WITS atlas differs significantly from the London atlas in earlier age of tooth formation and emergence. The population specific age estimation method showed good accuracy in the estimation of dental age. We conclude that this method could be used in other sub-Saharan African countries because of similarities in tooth formation and emergence

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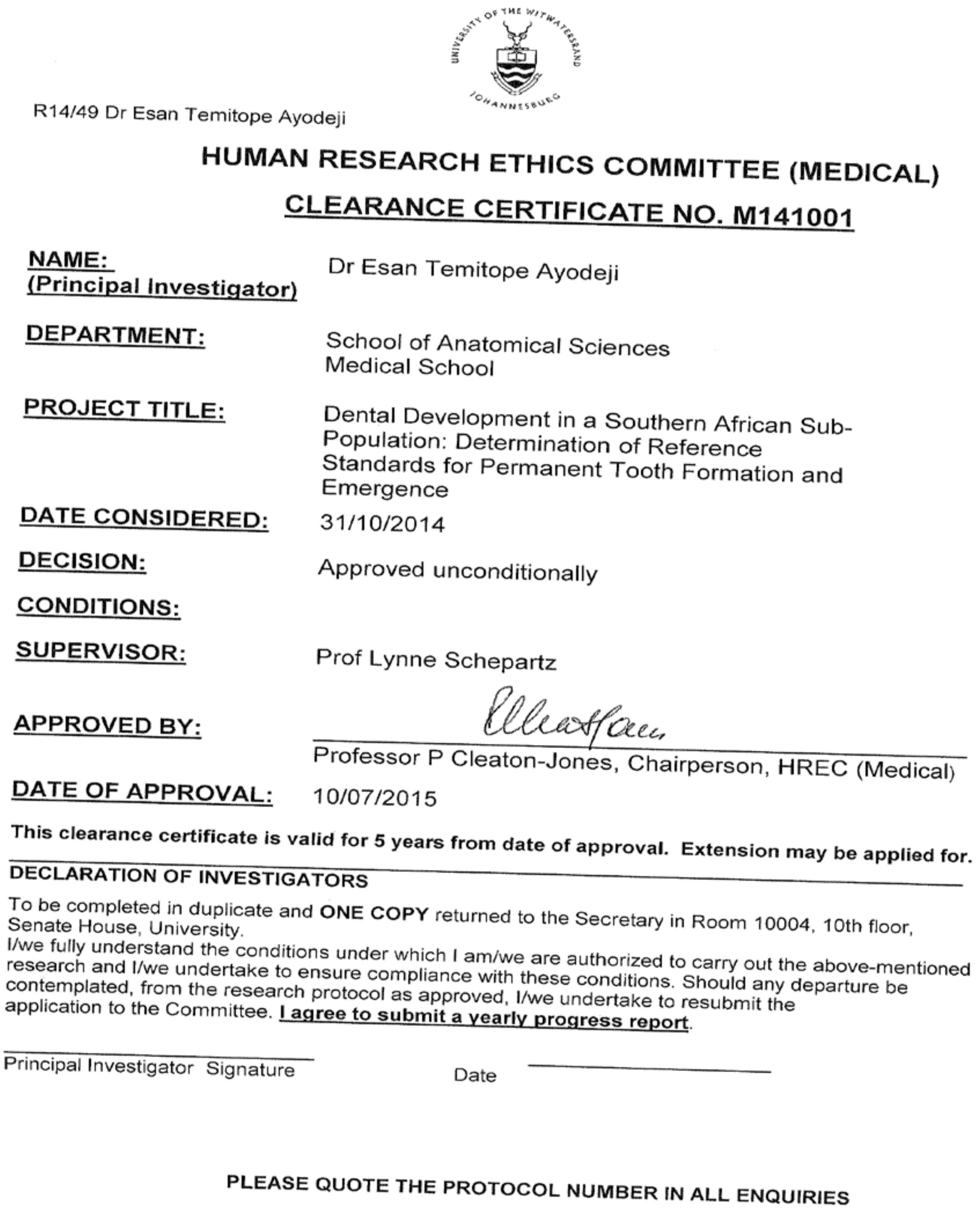
Appendix 1



UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Esan Temitope Ayodeji

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M141001

NAME:
(Principal Investigator) Dr Esan Temitope Ayodeji

DEPARTMENT: School of Anatomical Sciences
Medical School

PROJECT TITLE: Dental Development in a Southern African Sub-Population: Determination of Reference Standards for Permanent Tooth Formation and Emergence

DATE CONSIDERED: 31/10/2014

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof Lynne Schepartz

APPROVED BY: Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 10/07/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature ____________ Date ____________

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 2

DATA COLLECTION FORM 1

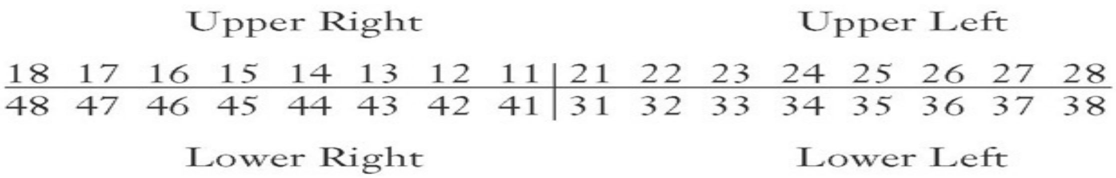
1. NAME OF SCHOOL
2. YEAR/CLASS
3. SUBJECT ID NO
4. AGE Date of Birth:. dd/mm/yy………
5. SEX
6. NATIONALITY
7. ETHNICITY
8. WHO IS RESPONSIBLE FOR YOUR UPKEEP?
9. IF FATHER, STATE FATHER’S OCCUPATION
10. IF MOTHER, STATE MOTHER’S OCCUPATION.
11. IF SOMEONE ELSE, STATE THE RELATIONSHIP

12. STATE THE OCCUPATION OF THE PERSON

13. BEEN TO THE DENTIST BEFORE? YES NO

14. HOW MANY TIMES DO YOU CLEAN YOUR TEETH?

15. TEETH PRESENT (Put X on teeth not erupted)



Upper Right Upper Left

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38

Lower Right Lower Left

16. TOOTH/TEETH EXTRACTED
17. TEETH FILLED
18. DECAYED TEETH
19. ORAL HYGIENE STATUS. (1. GOOD) (2. FAIR,) (3. POOR)

DATA COLLECTION FORM 2

Scoring of tooth development

1. SUBJECT ID 2. NAME OF SCHOOL
3. SCHOOL YEAR 4. DATE 5. SEX

Demirjian's Method

Tooth Type	**Demirjian Stage**	**Biological Weight**	**Maturity Score**
UPPER LEFT			
I^1			
I^2			
C^1			
P^1			
P^2			
M^1			
M^2			
LOWER LEFT			
I_1			
I_2			
C_1			
P_1			
P_2			
M_1			
M_2			
TOTAL SCORE			

Modified Demirjian's method

Tooth Type	**Demirjian Stage**	**Biological Weight**	**Maturity Score**
UPPER LEFT			
I^1			
I^2			
C^1			
P^1			
P^2			
M^1			
M^2			
LOWER LEFT			
I_1			
I_2			
C_1			
P_1			
P_2			
M_1			
M_2			
TOTAL SCORE			

Appendix 3: ***Self-Weighted Scores for Dental Stages of 7 Teeth (Mandibular Left* Side)**

Boys

Tooth	Stage 0	A	B	C	D	E	F	G	H
M_2	0	2.1	3.5	5.9	10.1	12.5	13.2	13.6	15.4
M_1				0	8	9.6	12.3	17	19.3
PM2	0	1.7	3.1	5.4	9.7	12	12.8	13.2	14.4
PM_1			0	3.4	7	11	12.3	12.7	13.5
C				0	3.5	7.9	10	11	11.9
I_2				0	3.2	5.2	7.8	11.7	13.7
I_1					0	1	4.1	8.2	11.6

NB: Stage 0 Is no calcification

Girls

Tooth	Score 0	A	B	C	D	E	F	G	H
M_2	0	2.7	3.9	6.9	11.1	13.5	14.2	14.5	15.6
M_1				0	4.5	6.2	9	14	16.2
PM2	0	1.8	3.4	6.5	10	12.7	13.5	13.8	14.6
PM_1			0	3.7	7.5	11.8	13.1	13.4	14.1
C				0	3.8	7.3	10.3	11.6	12.4
I_2				0	3.2	5.6	8	12.2	14.2
I_1					0	2.4	5.1	9.3	12.9

NB: Stage 0 Is no calcification

Appendix 4: *Turn-it-in Report*

Dt

by Temitope1 Esan1

Submission date: 18-Oct-2017 05:41AM (UTC+0200)

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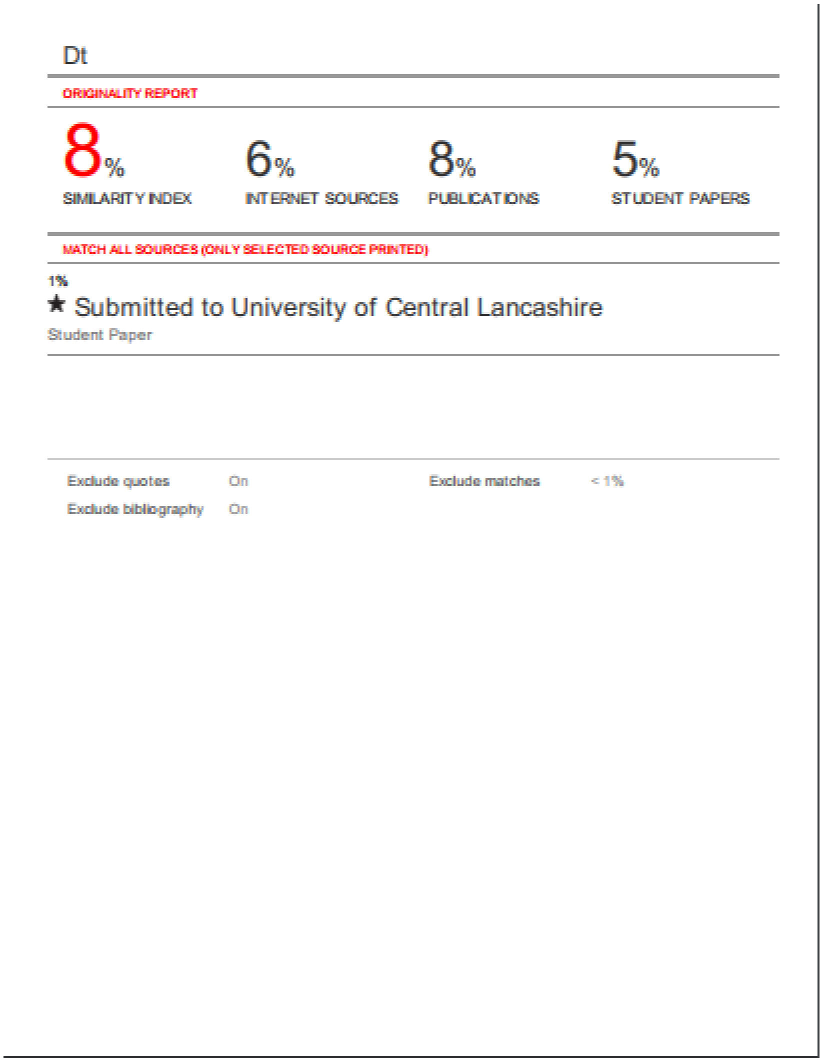

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