AGE DETERMINATION, USING THE SPHENO-OCCIPITAL SYNCHONDROSIS; MEDIAL END OF THE CLAVICLE; AND S1 – S2 FUSION, ON A SOUTH AFRICAN POPULATION

Trisha – Jean Mahon

Supervisors

Dr G.M. Gordon (PhD) and Dr L.J. Friedling (PhD)

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DECLARATION

I, Trisha – Jean Mahon, student number 361694, declare that this dissertation is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my dissertation.

T. Mahon

On this the 09 day of January, 2018.

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of her dissertation.

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Authors	Name	Signature	Date
2nd Author	Dr Louise Jacqui Friedling		14/9/2017
3rd Author	Dr Guinevere Marianne Gordon		11/10/2017

To my mommy and grandmother who have stood by me no matter what.

In memory of my dad and grandfather.

Patrick Shaun Mahon

1959 – 2016

Charles George Hutchison

1929 – 2016

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

PUBLICATIONS

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ABSTRACT

Forensic Anthropologists are often tasked with establishing an osteo-demographic profile for a set of unknown skeletal remains. Estimating age is only one such demographic which needs to be established. Complete skeletal maturation occurs when three developmental markers completely fuse: spheno-occipital synchondrosis, medial end of the clavicle, and sacral elements S1 and S2. The aim of this study was to establish the age at which these developmental markers begin and complete fusion in a South African Black sample. Males and females from the Raymond A. Dart Collection of Human Skeletons were included. It was observed that the spheno-occipital synchondrosis begins fusion as early as 12 years in females and 15 years in males; while all individuals over the age of 20 years displayed complete fusion. No significant differences between the left and right clavicles, and staging score was observed ($p=0.9$). The mean age of partial union for the clavicle in males and females was 26.7 (±6.0) and 26.4 (±5.0), respectively. While complete union of this skeletal element was regularly noted in individuals above 30 years. The mean age of partial union for sacral elements S1 and S2 was established to be 29.1 (±7.0) years in males and 30.7 (±9.2) in females. Complete union of this skeletal element was observed more commonly in individuals over 35 years of age. The results obtained in this study demonstrate the importance of developing population-specific criteria – especially in a diverse, multi-cultural population such as South Africa.

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LIST OF ABBREVIATIONS

CBCT – Cone Beam Computed Tomography scans

CT – Computed Tomography scans

HCT – Helical Computed Tomography scans

LG - Laminagram

MDCT – Multidetector Computed Tomography scans

MRI – Magnetic Resonance Imaging

RG - Roentgenographs

USG - Ultrasonography

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Chapter 1

INTRODUCTION, AIMS AND OBJECTIVES

1.1 Introduction

Forensic Anthropology is the use of physical anthropological practices and knowledge in a medico-legal setting (Ubelaker, 1996). The discipline of forensic anthropology has undergone a paradigm shift over the last 20 years (Dirkmaat *et al*., 2008). The most significant turning point within the history of forensic anthropology was in 1972. That year saw the official introduction of the field of physical anthropology, and thus forensic anthropology its sub-discipline, within the American Academy of Forensic Science (Snow 1982; Işcan, 1988; Ubelaker, 1996). Forensic Anthropologists no longer only develop a demographic profile (age, sex, population affinity and stature) for an unknown set of skeletal remains, but they also assist in the recovery of decomposing and skeletal remains; the trauma analysis of these remains; establishing facial reconstruction or facial approximation for skeletal remains; have an understanding of taphanomic changes affecting remains; as well as the analysis of the scene (Sauer & Lackey, 2000; Dirkmaat *et al*., 2008; Boyd & Boyd, 2011).

From the aforementioned demographic profile, Sauer & Lackey (2000) stated that age-at-death estimation can be one of the most difficult demographics to determine. This is due to the significant differences in the age ranges obtained by Forensic Anthropologists, as well as the fact that once skeletal growth is completed, age related changes are highly variable between individuals (Sauer & Lackey, 2000; Scheuer, 2002). In juvenile remains, age-at-death can be estimated within a range of only a couple of months or years, whereas in adult remains age-at-death ranges can be several decades (Sauer & Lackey, 2000).

The term "estimation" instead of "determination" is used when formulating an age-at-death range, as an analysis of the biological age – and not the chronological or actual age – is being conducted (Milner & Boldsen, 2012). Despite current research that attempts to make biological age estimation as close to chronological age as possible, the difference between biological and chronological age remains one of the biggest limitations when estimating the age-at-death of an individual from a set of skeletal remains (Saunders, 1992; Milner & Boldsen, 2012). However, in spite of this, the estimation of age is an extremely useful and relevant demographic to determine. Biological or physiological age is defined as the age that the skeletal remains exhibit or the physical appearance of the skeleton; whereas chronological age is delineated as the actual age of the individual in calendar months and/or years or more specifically the time that has passed since birth to death (Johnston & Zimmer, 1989; Scheuer & Black, 2000; Garvin *et al*., 2012; Milner & Boldsen, 2012). The estimation of biological age is often compounded by numerous factors such as the environment, hereditary components, physical activity, and the health status of the individuals. All these factors have differing effects and consequences on the skeleton (Johnston & Zimmer, 1989; Scheuer & Black, 2000; Garvin *et al*., 2012; Milner & Boldsen, 2012). In comparison, chronological age is not affected by any external factors (Johnston & Zimmer, 1989; Scheuer & Black, 2000; Garvin *et al*., 2012). The processes used by Forensic Anthropologists to estimate age-at-death depends on the type and condition of skeletal remains received. Generally, the estimation of age-at-death is based on three phases observed on the skeleton: growth and maturation; stabilisation; and finally deterioration (Scheuer, 2002). Each phase represents a different age group. Children, sub-adults and young adults fall within the 'growth and maturation' phase; while middle to older adults form part of the other two groups.

Estimation of age-at-death by using the skeleton is divided into three stages of development: firstly timing of the appearance of primary ossification centres, the gross morphology of the bone and lastly the fusion of the secondary ossification centres (Scheuer & Black, 2000; Scheuer, 2002). Complete skeletal maturation (i.e. the change from juvenile or sub-adult to that of an adult), is determined when three skeletal elements have completely fused – the spheno-occipital synchondrosis; the medial end of the clavicle; and complete fusion of sacral elements one and two.

A relatively new scope of study within Forensic Anthropology is the estimation of age in living individuals. Guidelines such as those described by the Study Group on Forensic Age Diagnosis of the German Association of Forensic Medicine (*Arbeitsgemeinschaft für Forensische Altersdiagnostik der Deutschen Gesellschaft für Rechtsmedizin*) have been developed for establishing whether an individual is a minor or an adult (Schmeling *et al*., 2003; Schmeling *et al*., 2004a; Garamendi *et al*., 2005; Schulz *et al*., 2005; Mühler *et al*., 2006; Schmeling *et al*., 2006; Schulze *et al*., 2006; Schmidt *et al*., 2007; Schmeling *et al*., 2008; Schulz *et al*., 2008a; Schulz *et al*., 2008b; Quirmbach *et al*., 2009; Kellinghaus *et al*., 2010; Garamendi *et al*., 2011; Işcan & Steyn, 2013; Hillewig *et* al., 2013; Schulz *et al*., 2013). Along with other anthropometric techniques used to estimate living age, these guidelines specifically state that a radiological image of the clavicle needs to be examined in order to establish if the individual is over 18 or 21 years of age (Schmeling *et al*., 2003; Schmeling *et al*., 2004a; Schmeling *et al*., 2004b; Garamendi *et al*., 2005; Schulz *et al*., 2005; Mühler *et al*., 2006; Schmeling *et al*., 2006; Schulze *et al*., 2006; Schmidt *et al*., 2007; Schmeling *et al*., 2008; Schulz *et al*., 2008a; Schulz *et al*., 2008b; Quirmbach *et al*., 2009; Kellinghaus *et al*., 2010; Garamendi *et al*., 2011; Hillewig *et* al., 2013; Işcan & Steyn, 2013;

Schulz *et al*., 2013; Zhang *et al*., 2015). Estimating age from a living individual is of particular importance within the judicial system for a number of different reasons (Schulz *et al*., 2005; Schulze *et al*., 2006; Bassed *et al*., 2011; Garamendi *et al*., 2011). These include the influx of individuals from surrounding countries claiming refugee status without valid documentation; in criminal cases where individuals have committed a crime and for sentencing purposes their age needs to be established; individuals who have lost or did not receive any form of identification documentation; in cases of child pornography; in cases of individuals claiming pensions; and finally in competitive sports (Scheuer and Black, 2000; Schmeling *et al*., 2003; Schmeling *et al*., 2004a; Garamendi *et al*., 2005; Schulz *et al*., 2005; Introna and Campobasso, 2006; Lewis and Flavel, 2006; Schmeling *et al*., 2006; Schulze *et al*., 2006; Schmeling *et al*., 2007; Schmeling *et al*., 2008; Cunha *et al*., 2009; Santoro *et al*., 2009; Garamendi *et al*., 2011; Hjern *et al*., 2012; Işcan and Steyn, 2013)

Within South Africa, the field of physical anthropology has a vast history, with the majority of research being conducted in the fields of human evolution and palaeo-anthropology (Steyn *et al*., 1997). However, of late more attention to the field of forensic anthropology in South Africa has become apparent. One of the most contributing aspects to this increase in the importance of this discipline is due to the high numbers of unknown bodies found in South Africa (Steyn *et al*., 1997; L'Abbè & Steyn, 2012). Age-at-death estimation of these unknown individuals may aid in establishing a positive identification. Little or no information regarding the age at which fusion of the spheno-occipital synchondrosis; medial end of the clavicle; and sacral elements S1 and S2 in a South African population occurs. Thus research regarding these skeletal developmental markers is required.

1.2 Aim and Objectives

This project aimed to determine the age at which fusion of the spheno-occipital synchondrosis; the medial end of the clavicle; and sacral elements one and two, occurs within a South African population group.

The objectives of the study were:

1. To determine the age at which the spheno-occipital synchondrosis starts and completes fusion in a South African Black skeletal sample.
2. To evaluate observer error using a three-stage scoring method designed by Shirley & Jantz (2011) for the degree of fusion of the spheno-occipital synchondrosis.

3. To determine the age at which the medial end of the clavicle begins and finishes union in a South African Black skeletal sample.
4. To compare the rates of fusion between the left and right clavicles in a South African Black skeletal sample.
5. To determine the age at which sacral elements one and two fuse in a South African Black skeletal sample.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

Estimating age-at-death from a set of skeletal remains plays a critical role in the development of an osteo-biological profile (Saunders, 1992; Baccino & Schmitt, 2006; Garvin *et al*., 2012). Age estimation may assist in narrowing the search in a missing persons' database, as this demographic feature could aid in the identification of decomposed, unidentifiable, or skeletal human remains (Ritz-Timme *et al*., 2000; Scheuer & Black, 2000; Baccino & Schmitt, 2006; Lewis & Flavel, 2006; Konigsberg *et al*., 2008; Cunha *et al*., 2009; Garvin *et al*., 2012; Milner & Boldsen, 2012). Estimating age-at-death is one of the more challenging demographic features for forensic/physical anthropologists to determine (Cunha *et al*., 2009; Milner & Boldsen, 2012). It relies heavily on the type of skeletal remains received, the state of these remains, and the experience of the person conducting the analysis (Sauer & Lackey, 2000; Baccino & Schmitt, 2006; Cunha *et al*., 2009).

Forensic anthropologists, at a quick glance of the remains, can determine if they are dealing with a juvenile, sub-adult, or an adult individual. They can then proceed with the relevant techniques that are used when estimating the age-at-death of the skeletal remains with which they are presented. Different techniques are used depending on the broad age group initially assigned to the remains. This is due to differing age indicators, applications, and accuracies of these methods (Lewis & Flavel, 2006; Milner & Boldsen, 2012). This therefore highlights one of the most important disparities when it comes to age-at-death estimation, as no set protocol has been established as to which method(s) and/or technique(s) are needed during relevant anthropological cases (Baccino & Schmitt, 2006; Cunha *et al*., 2009; Garvin *et al*., 2012; Milner & Boldsen, 2012).

Age-at-death estimations in juveniles are based on the simple concept of growth and development of the skeleton during intrauterine life, the postnatal period, and puberty (Scheuer & Black, 2000; Scheuer, 2002; Lewis & Flavel, 2006; Franklin, 2010; Rissech *et al*., 2013). These alterations are usually more closely related to chronological age and are thus relatively more accurate in estimating age-at-death in these cases (Johnston & Zimmer, 1989; Saunders, 1992; Scheuer, 2002; Schmeling *et al*., 2007; Cunha *et al*., 2009; Franklin, 2010; Garvin *et al*., 2012; Rissech *et al*., 2013). Due to the consistent growth and easily distinguishable morphological changes of the skeleton during this time, a narrower age-at-death range can be obtained for these skeletal remains (Johnston & Zimmer, 1989; Saunders, 1992; Ritz-Timme *et al*., 2000; Garvin *et al*., 2012). Generally, the mineralisation and eruption of the dentition, ossification, the length of the long bones, and morphological characteristics displayed by certain skeletal elements are used when estimating the age of juvenile remains (Johnston & Zimmer, 1989; Saunders, 1992; Scheuer & Black, 2000; Scheuer, 2002; Lewis & Flavel, 2006; Cunha *et al*., 2009; Garvin *et al*., 2012; Rissech *et al*., 2013).

Sub-adult (from puberty to 20 years of age) and young adult (20 to 35 years of age) age-at-death estimates rely on the eruption of the third molar, the fusion between epiphysis and diaphysis of long bones, and the complete fusion of various synchondroses in the skull (Ritz-Timme *et al*., 2000; Scheuer, 2002; Lewis & Flavel, 2006; Cunha *et al*., 2009; Garvin *et al*., 2012; Milner & Boldsen, 2012; Rissech *et al*., 2013). Furthermore, a number of techniques that are used when estimating the age in adults can be employed in these types of cases as well. This includes the use of the sternal ends of the fourth rib, the auricular surface, and the pubic symphysis of the os coxa (Brooks & Suchey, 1990; Oettlè & Steyn, 2000; Buckberry & Chamberlain, 2002). Although these additional techniques are useful when estimating age for a set of skeletal remains, they tend to give a vast age-at-death range (Garvin *et al*., 2012; Milner & Boldsen, 2012). It is therefore imperative that these techniques are used in combination with the methods initially discussed for estimating age in sub-adults in order to lessen the age-at-death range obtained (Ritz-Timme *et al*., 2000; Schmeling *et al*., 2007; Konigsberg *et al*., 2008; Cunha *et al*., 2009; Franklin, 2010; Garvin *et al*., 2012; Milner & Boldsen, 2012; Rissech *et al*., 2013). Due to variations in skeletal maturation rates between males and females, it is important to establish the sex of the skeletal remains first (Scheuer & Black, 2000; Lewis & Flavel, 2006; Franklin, 2010). It is well noted in studies that females mature faster (by approximately two years) when compared to males; thus placing great emphasis on the accuracy of initially estimating sex during skeletal analysis (Scheuer & Black, 2000; Lewis & Flavel, 2006; Franklin, 2010).

Not only do sex specific criteria need to be used during the estimation of age, but population specific data should also be incorporated when developing an age-at-death range (Johnston & Zimmer, 1989; Saunders, 1992; Scheuer & Black, 2000; Baccino & Schmitt, 2006; Lewis & Flavel, 2006; Cunha *et al*., 2009; Franklin, 2010). Once complete fusion of the spheno-occipital synchondrosis, medial end of the clavicle and sacral elements S1 and S2 are observed, the skeletal remains can be broadly classified as those of a young, middle, or older aged adult individual (Scheuer, 2002; Lewis & Flavel, 2006). Hence, the inclusion of additional techniques and methods that measure the degenerative changes of the skeleton are now used to estimate age-at-death.

In South Africa, population-specific techniques and methods are lacking regarding the estimation of age in all age groups (L'Abbè & Steyn, 2012). At present, South African population-specific data exists when estimating age from the sternal end of the fourth rib, vertebral lipping, cranial suture closure, and the histological examination of the femur (Oettlè & Steyn, 2000; Van Der Merwe *et al.*, 2006; Dayal, 2009; Keough *et al.*, 2009). With only a few population-specific age-at-death techniques and methods that can be used in South Africa during relevant forensic anthropological cases, there is an exigent need for additional age-at-death studies to be developed on a modern South African population.

The transition from a sub-adult to an adult, as noted above, occurs when three skeletal elements completely fuse: the spheno-occipital synchondrosis, medial end of the clavicle, and sacral elements S1 and S2 (Scheuer, 2002; Lewis & Flavel, 2006). The estimated age at which each of these skeletal features starts ossification and completes fusion will be further discussed in more detail.

2.2 Spheno-occipital Synchondrosis

The spheno-occipital synchondrosis is located on the inferior aspect of the skull between the sphenoid and occipital bones (Scheuer & Black, 2000; White & Folkens, 2005; Drake *et al*., 2010). The basic function of this synchondrosis is to allow for the lengthening of the skull base during growth (Nemzek *et al*., 2000; White & Folkens, 2005; Allan & Kramer, 2010; Krishan & Kanchan, 2013). Another term for this anatomical structure is the basilar suture. However, some authors argue that the use of the term "suture" is incorrect by definition (Redfield, 1970). A synchondrosis is an example of an epiphyseal plate, which is a cartilaginous joint located in between two bones that are unable to move (Sahni *et al*., 1998; Allan & Kramer, 2010; Bassed *et al*., 2010). This cartilaginous joint eventually ossifies into bone during skeletal maturation (Sahni *et al*., 1998; Allan & Kramer, 2010; Bassed *et al*., 2010).

The occipital bone comprises of four bones at birth: a single squama occipitalis and pars basilaris bones, as well as two pars lateralis bones (Nemzek *et al*., 2000; Scheuer & Black, 2000). These four component parts all fuse at differing times to form the adult occipital bone (Nemzek *et al*., 2000; Scheuer & Black, 2000). The sphenoid bone, in comparison, consists of seven bones during intrauterine life, namely: two lesser wings, two greater wings, and two pterygoid plates, including a single central body (Scheuer & Black, 2000). The previously-mentioned seven skeletal elements fuse in variable ways to form the sphenoid bone in adults (Scheuer & Black, 2000). The basilar part of the occipital bone forms the posterior border of the spheno-occipital synchondrosis, while the anterior border is formed by the central body of the sphenoid (Scheuer & Black, 2000). Only the basilar part of the occipital bone and the body of the sphenoid bone will be further discussed.

The pars basilaris is a fairly dense bone that is quadrilateral in shape and is the site of numerous muscle attachments (Sahni *et al*., 1998; Scheuer & Black, 2000). Posteriorly, it forms the anterior border of the foramen magnum and laterally, it articulates with the left and right temporal bones (Scheuer & Black, 2000). As noted by Scheuer & Black (2000), the pars basilaris undergoes several morphological changes, both in size and shape, making it useful in estimating the age-of-death in juvenile remains. It is also a robust bone that is often found during skeletal cases and frequently forms part of the skeletal assemblage in forensic cases (Redfield, 1970). The body of the sphenoid is cuboidal in shape and consists of numerous components and articulates with several other bones, which include the ethmoid, frontal, zygomatic, parietal, and temporal bones as well as the vomer (Scheuer & Black, 2000).

Fusion of the spheno-occipital synchondrosis commences on the endocranial surface of the cranium and progresses towards the ectocranial surface (Powell & Brodie, 1963; Scheuer & Black, 2000; Bassed *et al*., 2010; Shirley & Jantz, 2011; Krishan & Kanchan, 2013; Can *et al*., 2014). This synchondrosis is an important feature in forensic anthropological cases due to the late complete fusion of this skeletal element. Subsequently, it can be used during the estimation of age-at-death in sub-adult remains as it has been noted to correlate well with age (McKern & Stewart, 1957; Redfield, 1970; Scheuer & Black, 2000; Kahana *et al*., 2003; Bassed *et al*., 2010; Shirley & Jantz, 2011; Krishan & Kanchan, 2013; Can *et al*., 2014; Franklin and Flavel, 2014).

A considerable amount of research has been conducted on the age at which the spheno-occipital synchondrosis completes fusion, which could possibly account for the huge discrepancies in age-at-death ranges presented in the literature (Scheuer & Black, 2000). Studies on the age at which this particular synchondrosis fuses have also been carried out in a variety of ways. Techniques have been developed using dry bone, wet bone, radiographic images, computed tomography (CT) scans, and magnetic resonance imaging (MRI). Moreover, numerous staging methods and observation procedures (endocranial or ectocranial) have also been used. All of these differing techniques and methods may also account for the differences in the age ranges obtained (Scheuer & Black, 2000). A summary of the population affinity and number of individuals included, technique used, staging method, and the age-at-death ranges obtained in each documented study are presented in Table 2.1.

Currently, within South Africa, there is sparse literature available documenting the age at which the spheno-occipital synchondrosis commences and completes fusion. This therefore highlights the need to develop population-specific data for this country.

2.3 The Clavicle

The clavicle forms part of the movable shoulder/pectoral girdle in humans. The main function of the clavicle is to provide structural support for the arm and an anchor for muscle attachment (Scheuer & Black, 2000; White & Folkens, 2005; Auerbach & Raxter, 2008; Drake *et al*., 2010). It also offers protection to a number of important blood vessels and nerves passing between the neck and the upper limb (Scheuer & Black, 2000).

The clavicle attaches medially to the manubrium of the sternum and laterally to the acromial process of the scapula (Scheuer & Black, 2000; White & Folkens, 2005; Drake *et al*., 2010). Once fully developed, the clavicle is easily distinguishable from other long bones due to its S-shaped appearance (Scheuer & Black, 2000; White & Folkens, 2005; Drake *et al*., 2010). It has been noted that due to the robust nature of the outer compact/cortical layer of bone found on the clavicle, it can withstand a number of taphonomic changes, leading to this skeletal element often being recovered in forensic anthropological cases (Black & Scheuer, 1996; Scheuer & Black, 2000). Hence, the clavicle is of critical importance in forensic anthropology investigations as a number of biological traits can be assessed from this skeletal element, including estimating sex and age-at-death (Krogman & Işcan, 1986; White & Folkens, 2005; Işcan & Steyn, 2013).

Due to it being the first skeletal element to ossify during fetal life and the last skeletal element to completely fuse in adult individuals, the clavicle is of particular interest when it comes to age estimation (Stevenson, 1924; Todd & D'Errico, 1928; McKern & Stewart, 1957; Webb & Suchey, 1985; Black & Scheuer, 1996; Kreitner *et al*., 1998; Scheuer & Black, 2000; Meijerman *et al*., 2007; Auerbach & Raxter, 2008; Quirmbach *et al*., 2009; Langley-Shirley & Jantz, 2010; Singh & Chavali, 2011; Garvin *et al*., 2012; Işcan & Steyn, 2013). The clavicle grows at a fairly constant rate and measures approximately 40 – 41 mm at birth (Black & Scheuer, 1996; Scheuer & Black, 2000). Growth can only occur at either end of the clavicle (like other long bones), giving rise to two secondary centres of ossification –

medial (sternal) and lateral (acromial) epiphyses (Ogata & Uhthoff, 1990; Black & Scheuer, 1996; Scheuer & Black, 2000; Auerbach & Raxter, 2008).

There is little consensus amongst authors about the development of a lateral epiphysis (Todd & D'Errico, 1928; Black & Scheuer, 1996; Scheuer & Black, 2000). However, two basic theories exist regarding the presence of the lateral epiphysis. The first theory states that there is no lateral epiphysis formed during the development of the clavicle; whereas the second theory postulates that the lateral epiphysis develops around 19 – 20 years of age and completely fuses soon after it has formed (Todd & D'Errico, 1928; McKern & Stewart, 1957; Black & Scheuer, 1996; Scheuer & Black, 2000). An alternative theory, which supports the existence of a clavicular lateral epiphysis, is that this epiphysis never forms as a separate bony structure. Rather, the ridge and furrow system seen on the lateral aspect of the clavicle fades due to bone being deposited directly onto the epiphyseal surface (Todd & D'Errico, 1928; Black & Scheuer, 1996; Scheuer & Black, 2000; Langley, 2015).

The development of the medial epiphysis of the clavicle has been extensively researched, yet debates regarding the age at which this epiphysis begins and completes fusion are still ongoing. Most of the growth of the clavicle occurs at the medial end at a relatively slow rate. This is typically the final long bone epiphysis to completely fuse (Stevenson, 1924; Todd & D'Errico, 1928; McKern & Stewart, 1957; Webb & Suchey, 1985; Black & Scheuer, 1996; Kreitner *et al*., 1998; Scheuer & Black, 2000; Meijerman *et al*., 2007; Auerbach & Raxter, 2008; Quirmbach *et al*., 2009; Langley-Shirley & Jantz, 2010; Singh & Chavali, 2011; Garvin *et al*., 2012; Işcan & Steyn, 2013).

According to Black & Scheuer (1996) and Scheuer & Black (2000) the hyaline cartilage on the medial aspect of the clavicle begins to ossify around puberty. This small, ossified piece of bone begins to flatten in a posterior to superior manner, forming a separate bony structure that is flake-like in appearance on the medial epiphysis. Fusion of the medial epiphysis to the diaphysis of the clavicle begins at approximately 16 – 21 years of age and complete fusion of this epiphysis can occur as late as the third decade of life. This variation in fusion times of the medial aspect of the clavicle can be attributed to differences in individual maturation times as well as environmental and genetic factors (Black & Scheuer, 1996; Scheuer & Black, 2000).

Table 2.1: A summary of previous literature, using differing techniques and staging methods, detailing the mean age or age-at-death range at which the spheno-occipital synchondrosis commences and completes fusion.

Name of author(s) and year of publication	**Population affinity and number of individuals**	**Technique used**	**Staging method used**	**Age (years)**	
				Fusing synchondrosis	**Fused synchondrosis**
McKern & Stewart (1957)	American population (n=213)	Dry bone (ectocranial)	5	Not evaluated in this study	Male: >17
Powell & Brodie (1963)	American population (n=398)	LG and cephalometric LG	6	Female: >9 Male: >12	Female: 11–14 Male: 13–16
Konie (1964)	American population (n=314)	Midsagittal LG	5	Female: >10.6 Male: >12.6	Female: >13.6 Male: >16
Redfield (1970)	Yugoslavian medieval population (n=117)	Dry bone (ectocranial)	Unknown	In both sexes: <19	In both sexes: 20–29
Krogman & Işcan (1986)	American population (n=500)	Lateral x-rays	Unknown	Unknown	In both sexes: 20–25, mean age 23
Okamoto *et al*. (1996)	Japanese population (n=253)	CT	Unknown	Unknown	In both sexes: >13
Sahni *et al*. (1998)	Indian population (n=157)	A combination of x-rays and CT	3	Female: >12 Male: >13	Female: >13 Male: >15
Scheuer & Black (2000)	European population (n=unknown)	Dry bone (ectocranial)	Unknown	Unknown	Female: 11–16 Male: 13–18
Kahana *et al*. (2003)	American and Israeli population (n=91)	Wet bone (endocranial)	2	Female: 12.3 Male: 16.9	Female: 15.7 Male: 17.0
Akhlaghi *et al*. (2008)	Iranian population (n=106)	Wet bone (endocranial)	3	Male: 16.9	Male: 21.4

Akhlaghi *et al*. (2010)	Iranian population (n=376)	Wet bone (endocranial)	3	Female: 12.4 Male: 16.1	Female: 19.4 Male: 21.2
Bassed *et al*. (2010)	Australian population (n=666)	CT	5	In both sexes: >15	In both sexes: >17
Shirley & Jantz (2011)	American population (n=162)	Dry bone (ectocranial)	3	Female: 11.4 Male: 16.5	Female: 13.7 Male: 17.4
Franklin & Flavel (2014)	Australian population (n=312)	MDCT	4	Female: 8.6–15.5, mean 12.6 Male: 11.5–16.3, mean 14.3	Female: 11.7–25.6, mean 18.6 Male: 13.4–25.0, mean 19.8
Ekizoglu *et al*. (2016)	Turkish population (n=1078)	MRI	5	Female: 11.0–20.0, mean 13.1 Male: 10.0–21.0, mean 13.8	Female: 13.0–21.0, mean 17.5 Male: 13.0–21.0, mean 17.5
Kocasarac *et al*. (2015)	Turkish population (n=349)	CBCT	4	Female: 8.0–16.0, mean 12.3 Male: 8–14, mean 11	Female: 14.0– 25.0, mean 20.6 Male: 11.0– 25.0, mean 18.5
Lottering *et al*. (2015)	Australian population (n=864)	MDCT	6	Female: 9.6–12.7, mean 11.2 Male: 12.2–15.4, mean 13.8	Female: 14.7 Male: 19.8
Sinanoglu *et al*. (2016)	Turkish population (n=238)	CBCT	4	Female:8–15, mean 12 Male: 8–15, mean 13	Female: 13–25, mean 18 Male: 13–25, mean 20

Due to the early development (± 11 years) and late fusion of the medial end of the clavicle (± 30 years), it has been noted that this skeletal developmental marker is of significant importance for age estimation in living individuals as well as in skeletal remains (Stevenson, 1924; Todd & D'Errico, 1928; McKern & Stewart, 1957; Webb & Suchey, 1985; Black & Scheuer, 1996; Kreitner *et al*., 1998; Scheuer & Black, 2000; Meijerman *et al*., 2007; Auerbach & Raxter, 2008; Quirmbach *et al*., 2009; Langley-Shirley & Jantz, 2010; Singh & Chavali, 2011; Garvin *et al*., 2012; Işcan & Steyn, 2013).

Several studies have been done worldwide on the age at which the medial end of the clavicle starts ossification and completes union. However, the methods, techniques, and types of samples used in these studies differ. A summary of the findings in each of these studies has been tabulated and can be found in Table 2.2. There seems to be agreement amongst authors concerning the age at which the medial end of the clavicle fuses, compared to that of the spheno-occipital synchondrosis. Depending on the staging method and observation material used (such as dry bone, radiographic imaging (X-rays), computed tomography (CT), ultrasound studies, or magnetic resonance imaging (MRI)), differing age-at-death ranges are observed.

A review of literature shows that there are extensive volumes of research, spanning close to a century, regarding the age at which union of the medial end of the clavicle begins and completes. However, no published studies have yet been done in South Africa, giving rise to the need for the development of population-specific data.

2.4 The Sacrum

The sacrum forms part of the lower spinal column and the immovable pelvic girdle in humans. Its main function is to assist with support and protection of the pelvic and abdominal regions, as well as to provide an area for muscle attachment (White & Folkens, 2005). Another function of the sacrum is to protect the lower region of the spinal cord (cauda equina) and to allow the nerves to pass into the lower limb region through the sacral foramina (Scheuer & Black, 2000; Drake *et al*., 2010).

Table 2.2: A summary of previous literature, using differing techniques and staging methods, detailing the mean age or age-at-death range at which the medial end of the clavicle begins to ossify and complete fusion.

Name of author(s) and year of publication	**Population affinity and number of individuals**	**Technique used**	**Staging method used**	**Age (years)**	
				Ossifying medial end of the clavicle	**Fused medial end of the clavicle**
Stevenson (1924)	American population (n=110)	Dry bone	4	In both sexes: 22	In both sexes: 27–28
Todd & D'Errico (1928)	American population (n=63)	Dry bone	4	In both sexes: 21	In both sexes: 25
Flecker (1932)	Australian population (n=655)	RG	2	Female: >11 Male: >12	In both sexes: >22
McKern & Stewart (1957)	American population (n=237)	Dry bone	5	Males: 18–25	Males: 25–31
Szilvássy (1980)	Unknown population (n=140)	Dry bone	3	In both sexes: 21–25	In both sexes: 26–30
Webb & Suchey (1985)	American population (n=859)	Dry bone (autopsy sample)	4	In both sexes: 16–22	In both sexes: >34
Black & Scheuer (1996)	European population (n=143)	Dry bone	5	In both sexes: 19–23	In both sexes: >25
Kreitner *et al*. (1998)	German population (n=380)	CT	3	In both sexes: >11	In both sexes: >22
Schmeling *et al*. (2004b)	German population (n=699)	X-rays	5	Female: 16.0–26.8 Male: 16.7–24.0	Females: 20.0 – 30.9 Males: 21.3 – 30.9
Schulz *et al*. (2005)	European population (n=556)	CT	5	Female: 15.0–21.6, mean 18.2 Male: 15.2–23.9, mean 18.9	Female: 21.5–29.9, mean 25.1 Male: 21.2–30.4, mean 25.2

Schulze *et al*. (2006)	European population (n=100)	CT	4	In both sexes: >18	In both sexes: >19.8
Schmidt *et al*. (2007)	European population (n=54)	MRI	4	In both sexes: 15.0–35.9	In both sexes: 23.8–40.4
Cardoso (2008)	Portuguese population (n=121)	Dry bone	3	In both sexes: 17–25	Female: 22 Male: 26
Schulz *et al*. (2008b)	European population (n=84)	USG	4	Female: 17.1–20.8, mean 18.7 Male: 18.7–21.4, mean 19.7	Female: 22.5–30.4, mean 25.9 Male: 22.9–28.3, mean 25.8
Quirmbach *et al*. (2009)	German population (n=77)	USG	4	Male: >19	Male: >20.5
Kellinghaus *et al*. (2010)	German population (n=502)	CT	5	Females: 13.1–19.3, mean 16.3 Males: 14.4–20.7, mean 17.8	Female: 21.3–35.2, mean 28.2 Male: 21.6–35.9, mean 29.6
Langley-Shirley & Jantz (2010)	American population (n=1289)	Dry bone	5	Female: 13.5–21.0 Male: 14.4–23.0	Female: >22.5 Male: >24.1
			3	Female: 14.1–27.1 Male: 14.9–28.5	Female: >22.1 Male: >24.0
Bassed *et al*. (2011)	Australian population (n=674)	CT	5	Female: 14.7–20.0, mean 17.4 Male: 14.5–22.9, mean 18.7	Female: 21.1–26.3, mean 23.7 Male: 20.6–26.4, mean 23.5
Faria (2011)	European population (n=246)	Dry bone	3	Female: 20.9 Male: 22.1	Female: 23.9 Male: 26.0
			4	Female: 21.7 Male: 23.0	Female: 23.9 Male: 26.5
Garamendi *et al*. (2011)	Spanish population (n=123)	X-rays	6	In both sexes: 20.1	In both sexes: 26.9

Hillewig *et al*. (2011)	European population (n=121)	MRI	5	Female: >19.8	Female: >24.5
Singh & Chavali (2011)	Indian population (n=314)	Wet bone (autopsy sample)	5	In both sexes: >18	In both sexes: >22, however it was more commonly observed at 31 years in females and 32 years in males
Hillewig *et al*. (2013)	European population (n=220)	MRI	5	Female: 16.0–26.0, mean 19.8 Male: 16.2–26.2, mean 20.6	Female: 18.1–26.9, mean 24.2 Male: 22.1–26.9, mean 24.9
Milenkovic *et al*. (2013)	Balkan population (n=67)	Wet bone (autopsy sample)	3	In both sexes: >21	In both sexes: >31
Schulz *et al*. (2013)	European population (n=616)	USG	4	Female: 14.1–22.2, mean 17.3 Male: 14.4–22.9, mean 17.6	Female: 18.9–25.9, mean 23.3 Male: 19.3–25.9, mean 23.5
Milenkovic *et al*. (2014)	Balkan population (n=181)	CT	5	In both sexes: 15.0–18.0, mean 16.9	In both sexes: 19.0–30.0, mean 23.8
Tangmose *et al*. (2014)	Nordic population (n=102)	MRI	4	In both sexes: Puberty	In both sexes: >19
Pattamapaspong *et al*. (2015)	Thai population (n=409)	CT	5	Female: 16.5–26.5, mean 20.9 Male: 15.2–27.8, mean 20.9	Female: 19.5–28.1, mean 24.0 Male: 18.1–29.1, mean 25.3
Zhang *et al*. (2015)	West China Han population (n=752)	CT	4	Female: 16.3–25.8, mean 21.6 Male: 16.7–26.0, mean 21.5	Female: 18.9–26.0, mean 23.7 Male: 20.0–25.8, mean 23.7

The sacrum articulates laterally with the left and right os coxa at the auricular surface, superiorly to the fifth lumbar vertebra, and distally to the coccyx. It is shaped like an upside-down triangle, usually made up of five vertebral segments. However, there can be as many as six or as few as four vertebral segments depending on individual variation (McKern & Stewart, 1957; Broome *et al*., 1998; Scheuer & Black, 2000; White & Folkens, 2005; Belcastro *et al*., 2008; Drake *et al*., 2010). Anteriorly, the sacrum is concaved; while posteriorly, it is convex in shape (Scheuer & Black, 2000; Drake *et al*., 2010).

The sacrum is of considerable importance with regards to age-at-death estimation, due to it being one of the few skeletal elements to complete ossification in early adulthood, thus aiding in narrowing age-at-death ranges (Belcastro *et al*., 2008; Rĭos *et al*., 2008). Fusion of the sacrum is extremely complex and will be discussed briefly.

According to Scheuer & Black (2000), the neural arches of each sacral element commence fusion with their corresponding lateral elements by the age of two to five years. This occurs before the neural arches fuse with their respective centrum, which usually occurs between two and six years of age. The laminae fuse to form the posterior spinous process by the age of seven years. At this stage, each sacral element remains separated until adolescence and 14 centres of secondary ossification begin to form. Just before puberty, the lateral elements start to fuse from the lower regions upwards. Thus S1 and S2 lateral elements are the last to complete fusion. This direction of fusion (caudocranial direction) is observed in all secondary centres of ossification in the sacrum and results in fusion between the anterior bodies of sacral elements one and two being the last to occur (Scheuer & Black, 2000). Due to the uniformed sequence of development and the late completion of ossification of the sacrum (though variable), it can be used during relevant forensic anthropological cases when it comes to age-at-death assessments (Belcastro *et al*., 2008; Rĭos *et al*., 2008). However, there is a paucity of literature documenting the age at which fusion between sacral elements S1 and S2 begins and ends. The majority of these studies that document the age at which sacral elements one and two complete fusion have been conducted on dry bone elements from skeletal collections (Table 2.3).

There is minimal documented research detailing the age at which the first and second sacral elements start and complete fusion on a South African population. This lack of research emphasises the need to develop population-specific criteria for this geographical region.

Table 2.3: A summary of previous literature, using differing techniques and staging methods, detailing the mean age or age-at-death range at which sacral elements one and two commence and complete fusion.

Name of author(s) and year of publication	**Population affinity and number of individuals**	**Technique used**	**Staging method used**	**Age (years)**	
				Fusing sacral elements	**Complete fusion sacral elements**
McKern & Stewart (1957)	American population (n=373)	Dry bone	5	Males: >17	Males: >25
Broome *et al*. (1998)	American population (n=110)	Conventional x-rays, MRI, HCT	Unknown	Unknown	In both sexes: 18–21
Belcastro *et al*. (2008)	European population (n=904)	Dry bone	4	In both sexes: >20	In both sexes: >30
Rĭos *et al*. (2008)	Portuguese population (n=242)	Dry bone	5	Females: 21.1 Males: 25.6	Females: 35 Males: 37
Passalacqua (2009)	American population (n=633)	Dry bone	2	Unknown	In both sexes: 20–30

2.5 South Africa and Forensic Anthropology

South Africa is known as the "rainbow nation" due to its various population groups and diverse cultural heritage. According to the mid-year data released by Statistics South Africa, there is an estimated 52 million individuals living in the country, with just under 13 million of these individuals residing in the Gauteng province alone (Statistics South Africa, 2014). Unfortunately, South Africa has been labelled a violent country, as 46.0% of documented injury deaths in 2000 were due to homicides, with the majority of victims being male individuals aged between 15 – 29 years (Norman *et al*., 2007). The National Injury Mortality Surveillance System (NIMSS) observed that in 2010, a total of 11 084 individuals died from other than natural deaths in Gauteng (NIMSS, 2012). NIMSS obtains its data from the Forensic Pathology Services (FPS) Medico-legal Mortuaries nationwide and documents the age, sex, population affinity, the cause and manner of death, as well as a number of other factors pertaining to the deceased individuals received at these facilities. In over one third of these unnatural deaths documented during 2010, the manner of death was violence seen mostly in male individuals aged between 15 – 40 years (NIMSS, 2012). The foremost cause of death resulting from this violence was observed to be that of firearm injury closely followed by sharp force injury (NIMSS, 2012).

During the mid-1990s in South Africa, Steyn *et al*. (1997) noticed a slow increase in the awareness of the discipline forensic anthropology within this country. These authors attributed this increase to the rise in the number of unidentified human remains being received at FPS Medico-legal Mortuaries (Steyn *et al*., 1997). During the year 1995, a substantial amount of bodies received at these medico-legal facilities were unidentified, although it was noted that this number included both fully fleshed individuals and not only skeletonized human remains (Steyn *et al*., 1997). More recently, Evert (2011) conducted a study noting the number of unidentified deceased individuals received at the Pretoria FPS Medico-legal Mortuary over a four year period (2005 – 2008). The author concluded that on average, 10.0% of people being sent to this medico-legal mortuary, during this time frame, were unidentified and thus buried as paupers (Evert, 2011). Within South Africa, the National Health Act (2003) defines a body that has remained unidentified, from the date of admission into a medico-legal facility, for a period of 30 days or longer as that of a pauper. Individuals who are deemed paupers will then be buried by the local municipality in state graves (National Health Act, 2003).

This rise in unidentified deceased individuals being sent to FPS Medico-legal Mortuaries across South Africa is compounded by numerous factors, some of which include the illegal immigration of people into this country without valid identification, the lack of individuals seeking public medical and dental health services, and the influx of people into major cities leaving their families in rural areas in search of jobs (Steyn *et al*., 1997; L'Abbè & Steyn, 2012).

Forensic anthropologists in South Africa of late are being utilized more and more during appropriate cases and their expertise is being sought by relevant parties, including the South African Police Services (SAPS) and Forensic Pathologists or Medical Practitioners. The Forensic Anthropology Research Centre (FARC) at the University of Pretoria has received a total of 419 forensic anthropological cases in just under a decade (L'Abbè & Steyn, 2012). Worldwide, there has been an increase in relevant cases sent for anthropological assessment, where many of these individuals are estimated to be young to middle-aged adults (Bass & Driscoll, 1983; L'Abbè & Steyn, 2012). Although there is an abundance of literature of differing techniques that can be used by forensic anthropologists in South Africa, minimal amounts of this research focus on the estimation of population affinity and age-of-death (L'Abbè & Steyn, 2012).

South Africa is a developing country and faces many social, economic and political challenges. Although living conditions have improved within South Africa, more than half of the country's population is classified as living in poverty (Statistics South Africa, 2017). This unfortunately brings along an inherent set of exogenous factors, such as poor diet and living conditions, malnutrition of individuals and poor or no access to medical care – which are some of the challenges faced by South Africans. The exact effect of how one of these factors or a combination of these factors may possibly affect the skeleton is complex and relatively understudied. Also how much of the skeleton is influenced by genetic factors is unknown. The only reference materials to date that may be able to prove an insight into this are longitudinal growth studies assessing skeletal growth. Thus it is difficult to state exactly which one or what impact all of these social-economic or environmental factors has on fusion rates in populations world-wide. The one common theme noted throughout previous studies on the spheno-occipital synchondrosis, medial end of the clavicle, and sacral elements one and two is the need for the development of age ranges for the ossification and fusion time of each of these skeletal elements for the specific population in question. This is due to numerous different reasons such as the effects of social-economics, health statuses, and environmental factors (some of these factors have been mentioned previously) – all of which can affect the fusion rates of these skeletal elements (Bassed *et al*., 2010; Shirley & Jantz, 2011).

Alternative influences, which could lead to the variation in the time of complete fusion noted between populations as suggested by Singh and Chavali (2011), can be attributed to the different geographical locations in which individuals live. If population-specific data already exists for a country, this data may need to be updated due to the effects of secular trends within populations. This was an important fact noted by Introna & Campobasso (2006) as well as Shirley & Jantz (2011).

Within South Africa, no current population-specific data exists regarding the age of fusion of these three skeletal elements. This indicates the importance of this research to determine the age at which ossification and complete fusion of the spheno-occipital synchondrosis, the medial end of the clavicle, and sacral elements one and two occur in a South African Black male and female sample.

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Chapter 3

SPHENO-OCCIPITAL SYNCHONDROSIS: EXAMINING THE DEGREE OF FUSION IN A SOUTH AFRICAN BLACK SKELETAL SAMPLE

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Abstract

Estimating age-at-death is one of the many biological demographics that a forensic anthropologist needs to determine for a set of unknown skeletal remains. A useful skeletal developmental marker, which can aid in estimating age in sub-adult remains, is the state of fusion of the spheno-occipital synchondrosis. This study aimed to determine the repeatability of a three-stage scoring method and the age at which the spheno-occipital synchondrosis begins and completes fusion in a Black South African sample. A total of 147 male and female skeletal individuals aged between 12 – 30 years from the Raymond A. Dart Collection of Human Skeletons were included. The mean age-at-death noted for the commencement of fusion at the spheno-occipital synchondrosis was 16.2 (±2.9) years in females and 16.7 (±1.2) years in males, with females displaying signs of fusion taking place approximately three years before their male counterparts. Complete fusion of this skeletal developmental marker was observed in 100% of the sample over the age of 20 years, regardless of sex. A Kappa value of 1.0 was achieved when the repeatability and reproducibility of the three-stage scoring method was tested. Complete fusion of this synchondrosis occurred slightly later in this South African sample than that noted in previous literature. This finding may be attributed to the differences in health and socio-economic statuses between these different population groups, reiterating the need for population-specific data to be developed. The importance of noting the state of fusion of the spheno-occipital synchondrosis during the examination of relevant forensic cases may aid in the formation of a narrower age-at-death range for sub-adult and young adult skeletal remains.

Key words:

Forensic Anthropology Population Data; Age-at-death estimation; Spheno-occipital synchondrosis; South Africa.

3.1 Introduction

Age can be one of the most problematic biological features for a physical/forensic anthropologist to assess. It forms an essential component of a biological profile by aiding in the narrowing down of possible missing persons [1-5]. Establishing an age range of a set of skeletal remains is often compounded by numerous factors, such as the experience of the individual conducting the analysis and the type and state of preservation of the remains presented for examination [2,3]. The spheno-occipital synchondrosis is an important skeletal developmental marker that can be used in estimating the age-of-death in sub-adult skeletal remains [6]. This is due to complete fusion of this synchondrosis occurring much later than other aging indicators in sub-adult individuals [7-11].

Internationally, more and more data regarding the age at which this skeletal developmental marker commences and completes fusion has been obtained using dry bone samples, radiographic images, computed tomography (CT) scans, magnetic resonance imaging (MRI), or a combination of these techniques [6-10,12-20]. Due to the vast range of techniques and staging methods used to estimate the age at which this synchondrosis fuses in populations worldwide, it is hypothesized by the authors that comparisons between the age-at-death ranges can only be made if the same method of examination is used between the published articles' reference sample and that of the forensic case. Thus, age-at-death ranges obtained using reference samples from radiographic imaging origin can only be compared to and used if the relevant forensic case in question also consists of radiographic images. Reasons for these conflicting age ranges include the use of different methods and the fact that visual details of bone development seen on radiographic images, CT scans, or MRIs are different to what can be seen on dry bones [5,9].

As mentioned above there is a plethora of literature regarding the age at which this skeletal developmental marker completely fuses. However, the one noticeable discrepancy among these studies is the immense variation in reported ages in studies both between populations and within a single population group [6-10,12-20]. These variations in the age at which the spheno-occipital synchondrosis begins and completes fusion could be attributed to the technique, method and sample sizes used in these studies, the inclusion of both or a single sex, the differences in the population groups studied, as well as secular trends within one population group.

No information vis-á-vis the age at which the spheno-occipital synchondrosis fuses has been noted in a South African population. This study therefore aimed to develop population-specific data in this geographical region for the age at which this synchondrosis commences and completes fusion, and to test the repeatability of a three-stage scoring method designed by Shirley & Jantz [10].

3.2 Materials and Methods

The sample consisted of 147 randomly selected South African Black individuals from both sexes (almost equal portions of males (n=74) and females (n=73)) between the ages of 12 to 30 years from the Raymond A. Dart Collection of Human Skeletons (commonly referred to as the Dart Collection). The mean age-at-death for male and female individuals was 21.9 (± 5.2) years and 23.7 (± 4.5) years respectively. Although the year-of-death of the sample population ranged from 1925 to 2000, the average year of death of the individuals was 1952. The Dart collection is stored in the School of Anatomical Sciences at the University of the Witwatersrand, South Africa. Within this skeletal collection, South African Black individuals are composed of a number of different ancestral groups [21]. However, De Villiers [22] notes that the cranial elements of these different ancestral groups do not morphologically differ extensively from each other and can thus be clustered together into a single population group.

The following exclusion criteria were applied: signs of trauma, pathological conditions or damage to the inferior aspect of the cranium, and unknown individuals. A non-invasive, macroscopic three-stage scoring method developed by Shirley & Jantz [10] was implemented to describe the fusion of this synchondrosis. Numerical values were then assigned to each of the three staging scores for statistical purposes. Table 3.1 details the scoring method and assigned numerical values.

Repeatability and reproducibility of staging and scoring of the spheno-occipital synchondrosis was assessed by randomly selecting 30 individuals from the sample. Intra-observer error was established after the principle investigator staged and scored the synchondrosis a second time. Inter-observer error was established using an independent individual who has a moderate understanding of osteology and forensic anthropology. All demographic data were recorded as either numerical values or frequencies. Box plots were used, which allowed for the median, and upper and lower interquartile ranges to be displayed graphically. An unpaired t-test was used to compare the mean age at which fusion begins between males and females in order to establish if any significant differences between the sexes existed. Correlation between age and the staging score assigned was determined through the use of a Spearman’s ranked correlation coefficient. All statistical analyses were tested at the 5.0% level of significance (α=0.05). Statistical analysis was done using Graphpad Instat® 3.10 and PAST: Paleontological Statistics Software Package for Education and Data Analysis [23]. Both observer errors were determined statistically with the use of a Cohen’s Kappa test; which was conducted using Microsoft Excel® 2013 with an add-in feature analysis-it®.

Table 3.1: Description of the staging categories used to evaluate the degree of fusion of the spheno-occipital synchondrosis, and the numerical values used

Staging categories	Description	Numerical values assigned
Unfused	A noticeable opening or space can be observed at the spheno-occipital synchondrosis (Figure 3.1)	1
Fusing	Active fusion can be seen between the basilar portion of the occipital bone and the body of the sphenoid bone. However, slight spaces or openings are still observable on the ectocranial surface (Figure 3.2)	2
Fused	No openings or spaces are observable at this synchondrosis. If a fusion scar was noted at the sight of the two joining bones, it was placed within this category (Figure 3.3)	3

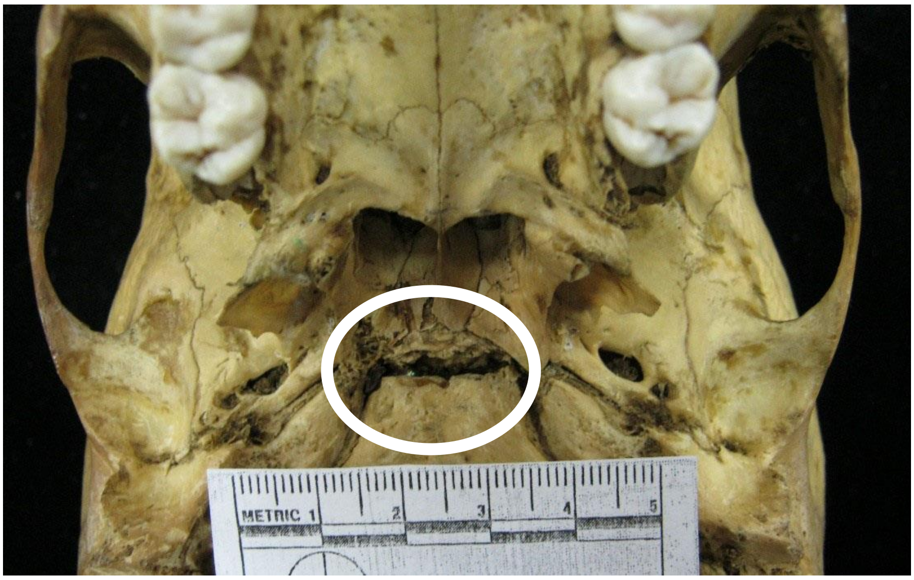

Figure 3.1: Specimen A1244 showing an unfused spheno-occipital synchondrosis

3.3 Results

Perfect repeatability and reproducibility of this method were established, as a Kappa value of 1.0 was obtained when both observer errors were statistically evaluated. A summary of the descriptive statistics of the individuals included and the degree of fusion of the synchondrosis by age can be found in Table 3.2 and Table 3.3.

The youngest examined female and male individuals showing signs of fusion taking place at the spheno-occipital synchondrosis were 12 and 15 years of age respectively. However, the oldest male and female individuals to still demonstrate these signs of fusion were both 19 years of age. The mean age-at-death determined for South African Black males was 16.7 (±1.2) years with an age range of 16.0 – 17.8 years (Figure 3.4). A strong correlation ($r_s = 0.70$) was noted between age and the staging score assigned for included male individuals. South African Black females were noted to have a mean age-at-death of 16.2 (±2.9) years and an age range of 13.5 – 19.0 years (Figure 3.4). A moderate correlation ($r_s = 0.49$) was obtained between the age and the staging score allocated for females included.

Table 3.2: Descriptive statistics of South African individuals included per staging category

Staging category	**N**	**Percentage of sample (%)**	**Mean (years)**	**SD**	**Median (years)**	**Min**	**Max**	**Range**
Unfused	9	6.1	14.3	± 1.9	14.0	12	17	5
Fusing	17	11.6	16.5	± 1.8	16.0	12	19	7
Fused	121	82.3	24.3	± 4.0	24.0	15	30	15

No statistically significant differences were observed between the mean age at which the spheno-occipital synchondrosis starts to fuse in this sample of males and females ($p = 0.8$). Thus a mean age and an age-at-death range for the commencement of fusion between the basilar part of the occipital and the body of the sphenoid in this South African sample was established as 16.5 (±1.8) years and 15.6 – 17.5 years respectively. A strong correlation ($r_s = 0.63$) was noted between the age and the staging score assigned when both males and females were assessed.

In this study sample, the youngest male to show complete fusion of the spheno-occipital synchondrosis was 15 years of age. A 16-year-old female was noted to be the youngest female to display complete union at this synchondrosis. Complete fusion of the spheno-occipital synchondrosis, irrespective of sex, was noted in 100% of the sample in all individuals over the age of 20 years.

Figure 3.2: Specimen A151 showing a fusing spheno-occipital synchondrosis.

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Figure 3.3: Specimen A184 showing a completely fused spheno-occipital synchondrosis

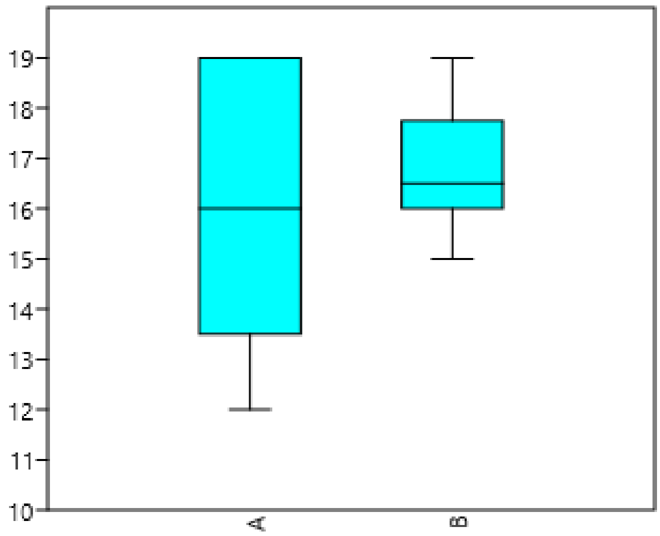

Figure 3.4: Box plot showing the median and quartile ranges for South African female and male individuals showing signs of a fusing spheno-occipital synchondrosis. Key: Females (A) and Males (B).

Table 3.3: The degree of fusion of the spheno-occipital synchondrosis in South African males and females by age

Age (years)	Staging categories					
	Unfused		Fusing		Fused	
	M	F	M	F	M	F
12	-	1	-	1	-	-
13	3	-	-	-	-	-
14	1	1	-	-	-	-
15	-	-	2	1	1	-
16	1	-	4	1	1	1
17	2	-	3	-	2	-
18	-	-	2	-	5	2
19	-	-	1	2	-	3
20	-	-	-	-	4	4
21	-	-	-	-	3	5
22	-	-	-	-	6	4
23	-	-	-	-	4	6
24	-	-	-	-	3	7
25	-	-	-	-	3	6
26	-	-	-	-	4	6
27	-	-	-	-	4	4
28	-	-	-	-	3	7
29	-	-	-	-	5	4
30	-	-	-	-	7	7

3.4 Discussion

Multiple methods of age estimation must always be employed during relevant forensic anthropological cases in order to obtain a more accurate estimation of biological age [4,5,24,25]. Noting the age at which the spheno-occipital synchondrosis starts and completes fusion is only one of numerous methods that can be utilised during the formation of an age range. The biggest limitation for estimating age in skeletal remains is the lack of well-defined scoring systems that can be used with ease during skeletal analysis [1,10]. Scheuer & Black [9] and Webb & Suchey [26] observed that when more categories of staging are present within a single method, there is an increase in the error rate between observers. Thus having a well-defined scoring method is crucial for judicial purposes. A three-stage scoring method designed by Shirley & Jantz [10] was used to determine the ectocranial state of fusion of the spheno-occipital synchondrosis. These authors state that they had yet to statistically test the accuracy for this method, both within and between observers [10]. This study observed that there was perfect repeatability and reproducibility between both the principle investigator and an independent observer, thus allowing for this staging method to be used with ease within a laboratory or field setting.

It is important to note that only published literature that estimates the age of ectocranial closure of the spheno-occipital synchondrosis on dry bone were reviewed for this study. Although all of these studies were conducted on different skeletal collections worldwide; some authors have noted that using skeletal collections as reference samples can be problematic [7-10,21,27,28]. This is due to the estimation of some of the ages of the individuals included in these skeletal collections as the chronological age of the individual is not known. This highlights one of the biggest hurdles in forensic anthropology when estimating the age-at-death using information obtained from skeletal collections [21,27,28]. In spite of these concerns, however, studies conducted on dry bones have also been described as the most accurate way of evaluating this skeletal developmental marker [10].

One of the earliest published attempts to determine the age at which the spheno-occipital synchondrosis fuses was conducted by McKern & Stewart [7]. The authors concluded that complete fusion was observed as early as 17 years [7]. Almost two decades later, Redfield [8] noted that the spheno-occipital synchondrosis was completely fused by 20 – 29 years of age in a Yugoslavian population. Scheuer & Black [9] state that previous anatomical literature as well as studies analysing and detailing the growth of the cranium have overestimated the age at which this synchondrosis completes fusion. More recent age ranges of complete fusion between 11 – 16 years in females and 13 – 18 years in males have been suggested [9]. Shirley & Jantz [10], estimated the age at which the spheno-occipital synchondrosis fuses in a modern American population and concluded that this skeletal developmental marker commences closure as early as 11.4 years in females and 16.5 years in males [10].

The results of this study confirm what has previously been stated by Scheuer & Black [9] and Shirley & Jantz [10]. Fusion of this synchondrosis begins during adolescence and not at the period of young adulthood, as earlier established by McKern & Stewart [7] and Redfield [8]. However, it must be emphasised that the minimum age included in this study was 12 years (due to the unavailability of other samples matching the inclusion criteria), which makes direct comparisons of the age at which this synchondrosis begins fusion between studies difficult. Previous anatomical, embryological, and anthropological literatures have shown that females undergo pubescent changes approximately two years before males [9,25,29,30]. In the sample under review in this study, South African Black females demonstrated signs of fusion taking place at the spheno-occipital synchondrosis by up to three years earlier than their male counterparts, which is consistent with published literature.

No significant differences were observed between the age at which fusion commences at the spheno-occipital synchondrosis among males and females. Within this sample, female individuals demonstrated a weaker correlation between age and stage score assigned than male individuals. However, only 17 individuals showed signs of fusion taking place at this synchondrosis, with the number of males far out weighing the number of females. The inclusion of additional sub-adult or young adult individuals from other South African skeletal collections is recommended. Increasing the number of individuals could assist in establishing if there is in fact a significant difference between males and females and may strengthen the results of the correlation between age and staging score assigned. Skeletal collections around the world, however, have noted that juvenile and sub-adult individuals are underrepresented in these collections. This highlights just one of the many difficulties faced by physical/forensic anthropologists regarding establishing methods of estimating age in sub-adult individuals [5,9,21,27,30-37]. Furthermore, there is typically a skew distribution of sex within these skeletal collections. Conventionally, there are more males included in these collections than females.

Within this sample, it was observed that fusion at this synchondrosis may still persist into late adolescence, while complete ectocranial fusion of the spheno-occipital synchondrosis was observed in all individuals above the age of 20 years. This therefore aids in the establishment of an upper age-at-death range in sub-adult individuals and a lower age-at-death range in young adult individuals. This established upper age limit differs from the ranges obtained in previous publications [9,10]. Nevertheless, earlier studies conducted by McKern & Stewart [7] and Redfield [8] have indicated that complete fusion of this skeletal developmental marker only occurs during the second decade of life. These findings are consistent with the findings in this study.

Many of the skeletal individuals donated to the Dart Collection are cadaver-derived. These individuals are either unclaimed or unidentified deceased individuals from government hospitals or individuals who donated their bodies via a bequeathment programme [21]. Noticeable factors that may attribute to the variation in the age ranges observed between population groups include the differences in geographic location, diet, environmental factors, and socio-economic and health status among these collections [6,10,38]. This is however an inherent bias that exists when using skeletal collections as reference samples [21].

All previous studies have specified that complete fusion of this synchondrosis first occurs in female individuals and then in male individuals, which is different to the findings in this study [9,10]. An initial explanation may be due to the lack of females aged 13 – 15 years, as well as the underrepresentation of sub-adult female individuals within the study sample. Thus the assumption that the spheno-occipital synchondrosis in South African males fuses before their female counterparts cannot be made. Alternative reasons for this finding may be attributed to the differing cultural treatment of and behaviour towards males and females as South Africa is still very much a patriarchal society [39,40]. Recent studies have suggested that there are no observable differences in the amount of stunting (a growth and nutritional indicator) between South African children. The greatest factors affecting height-for-age is the cultural group and social upbringing of these individuals [41]. Further research still needs to be conducted on how this phenomenon may affect skeletal growth in a South African setting.

3.5 Conclusion

An easily repeatable and reproducible three-stage scoring method was used in this study, which is critical when dealing with forensically relevant cases. The state of fusion of the spheno-occipital synchondrosis can be used as a skeletal developmental marker to assist in the estimation of age-at-death from a set of unknown skeletal remains. Future studies, which include a larger sample of South African Black females (particularly within the 10 – 15 year age range) and a more modern contemporary skeletal population, are recommended by the authors.

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Chapter 4

UNION OF THE MEDIAL CLAVICULAR EPIPHYSIS IN A SOUTH AFRICAN BLACK SKELETAL SAMPLE

Article submitted to HOMO – Journal of Comparative Human Biology – Under consideration

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*Figure and Table numbers have been altered from the submitted manuscript to keep them in line with the University of the Witwatersrand requirements for an MSc dissertation.

Abstract

Current research points to an increase in interest and focus on the age at which the medial end of the clavicle begins and completes union in population groups worldwide. As it currently stands, no information detailing the age at which this skeletal developmental marker commences or completes union within a South African population exists. The aim of this study was to establish the age at which partial and complete union occurs and to note if there is any correlation between age and staging score assigned in a South African Black skeletal sample. Paired clavicles of 211 male and female individuals with ages ranging between 12–45 years from the Raymond A. Dart Collection of Human Skeletons were included. A strong correlation between age and the staging score assigned was observed for both males and females ($r_s = 0.73$). No statistically-significant differences between the stage of union at the left and right clavicle were observed ($p=0.9$). In this sample, partial union was commonly observed in individuals in their second decade of life; while complete union occurred more often in individuals older than 30 years of age. Assessing and noting the degree of union at the medial end of the clavicle can be a helpful additional age-at-death indicator to use when establishing an age-at-death range. However, overlap and variability between the ages of individuals categorised as either displaying signs of partial or complete union were evident.

4.1 Introduction

Over the last decade, there has been a significant increase in the number of publications detailing the age at which the medial clavicular epiphysis begins and completes union. This can be attributed to a new paradigm shift within the field of forensic anthropology that focuses on the estimation of age in living individuals. Among other recommendations noted by the Study Group on Forensic Age Diagnostics (*Arbeitsgemeinschaft für Forensische Altersdiagnostik* [AGFAD]), a radiographic image of the medial end of the clavicle should be taken in order to assist with estimating if an individual is above or below 21 years of age (Schmeling et al., 2003; Schmeling et al., 2004a; Garamendi et al., 2005; Schulz et al., 2005; Mühler et al., 2006; Schmeling et al., 2006; Schulze et al., 2006; Schmidt et al., 2007; Schmeling et al., 2008; Schulz et al., 2008a; Schulz et al., 2008b; Quirmbach et al., 2009; Garamendi et al., 2011; Hillewig et al., 2013; Işcan and Steyn, 2013; Schulz et al., 2013). This guideline has led to an increase in studies detailing the age at which this skeletal developmental marker begins and completes fusion by using numerous imaging techniques in populations worldwide (Kreitner et al., 1998; Schmeling et al., 2004b; Schulz et al., 2005; Schulze et al., 2006; Schmidt et al., 2007; Schulz et al., 2008b; Quirmbach et al., 2009; Kellinghaus et al., 2010; Bassed et al., 2011; Garamendi et al., 2011; Hillewig et al., 2013; Işcan and Steyn, 2013; Schulz et al., 2013; Milenkovic et al., 2014; Tangmose et al., 2014; Pattamapaspang et al., 2015; Zhang et al., 2015).

Academics have long been interested in the clavicle and its association with age. The clavicle is the first bone to start to ossify in the fetal skeleton and is the last bone to complete fusion in the adult skeleton (Stevenson, 1924; Todd and D’Erric, 1928; McKern and Steward, 1957; Webb and Suchey, 1985; Kreitner et al., 1998; Black and Scheuer, 1996; Scheuer and Black, 2000; Meijerman et al., 2007; Auerbach and Raxter, 2008; Quirmbach et al., 2009; Langley-Shirley and Jantz, 2010; Singh and Chavali, 2011; Garvin et al., 2012; Işcan and Steyn, 2013). The length of time taken for complete fusion of this skeletal element assists with age estimation in unknown skeletal remains. The delay is caused by the slow growth, which takes place at the medial end of the clavicle, and results in complete fusion only occurring during the third decade of life. This skeletal element is a helpful additional age-at-death indicator as fusion begins and completes long after growth has ceased at other epiphyses (Stevenson, 1924; Todd and D’Erric, 1928; McKern and Steward, 1957; Webb and Suchey, 1985; Kreitner et al., 1998; Black and Scheuer, 1996; Scheuer and Black, 2000;

Meijerman et al., 2007; Auerbach and Raxter, 2008; Quirmbach et al., 2009; Langley-Shirley and Jantz, 2010; Singh and Chavali, 2011; Garvin et al., 2012; Işcan and Steyn, 2013).

As previously mentioned, there is a wealth of literature regarding the age at which the medial end of the clavicle begins and completes fusion in a variety of different population groups. No information regarding the age at which this skeletal developmental marker commences or completes fusion in a South African population has been documented. The aim of this study was therefore three-fold: (i) to determine the age at which the medial end of the clavicle begins and completes union, (ii) to determine if there is a significant difference between the stage of union in the left and right clavicles, and (iii) to determine if there is a correlation between age and the stage of union at the medial end of the clavicle in a South African Black skeletal sample.

4.2 Materials and Methods

The paired clavicles of 211 South African Black male (n = 101) and female (n = 110) individuals with ages ranging from 12 – 45 years from the Raymond A. Dart Collection of Human Skeletons were included in this study. The mean age-at-death for males was 29.7 (±9.1) years; while females had a mean age-at-death of 29.7 (±7.9) years. Within the Dart collection, South African Blacks comprise a number of different ancestral groups, with the largest group represented by the Zulu tribe (Dayal et al., 2009). However, these ancestral groups are not statistically different from each another and can be classified as one homogenous population (Lundy, 1983).

Any clavicles that showed signs of trauma, pathology, damage, or were from an unknown individual were excluded. A three-stage scoring method, noted in Table 4.1, was used to score both the left and right clavicles. This method involved the direct macroscopic inspection of the medial end of the clavicle. After the clavicle was placed into one of the three-staging categories, a numerical value was allotted for statistical purposes. It is important to note that if paired clavicles were observed to be in different stages of union, an overall score of the side which displayed the least developed medial epiphysis was then also categorized for statistical purposes. Inter- and intra-observer errors were tested for the three-stage scoring method described above. The principle investigator assessed and scored 30 randomly selected clavicles; while a blind test with the same 30 clavicles was carried out by an independent observer, who has a good understanding of human osteology.

Statistical analysis was assessed using both Graphpad Instat® 3.10 and PAST: Paleontological Statistical Software Package for Education and Data Analysis (Hammer et al., 2001). The median, upper, and lower interquartile ranges were displayed graphically using a box plot and demographic data was recorded as either a frequency or numerical value. A Wilcoxon-paired test was used to establish any existing significant differences between the stage of union at the left and right clavicle. A Mann-Whitney test was used for all comparisons; while a relationship between age and staging score assigned was tested using a Spearman's ranked correlation coefficient and a Kruskal-Wallis test. All statistical analyses were tested at the 5.0% level of significance (α=0.05). A Cohen's Kappa was used to determine both observer errors.

Table 4.1: The descriptions used to assess the degree of fusion at the medial end of the clavicle, as well as the numerical values allotted.

Staging categories	**Description**	**Allotted values**
No union	No signs of fusion or the development of a medial epiphyseal flake. Burrows and furrows can be observed on the medial end of the clavicle (Figure 4.1).	1
Partial union	A medial epiphyseal flake is observable, although burrows and furrows are still evident in some areas. The medial epiphyseal flake has yet to completely surround and fuse to the medial end of the clavicle i.e. complete capping of the medial end of the clavicle has yet to occur (Figure 4.2).	2
Complete union	Complete capping of the medial end of the clavicle by the epiphyseal flake. No burrows and furrows can be seen at this stage. [If an epiphyseal scar is observed it was placed into this category] (Figure 4.3)	3

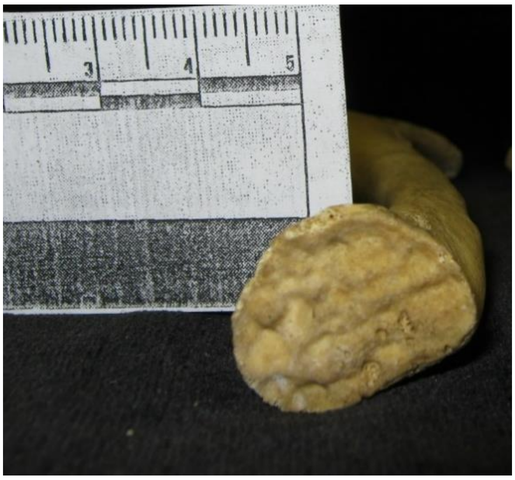

Figure 4.1: Specimen A151 – non-union nor development of a medial epiphyseal flake. Note the burrows and furrows evident.

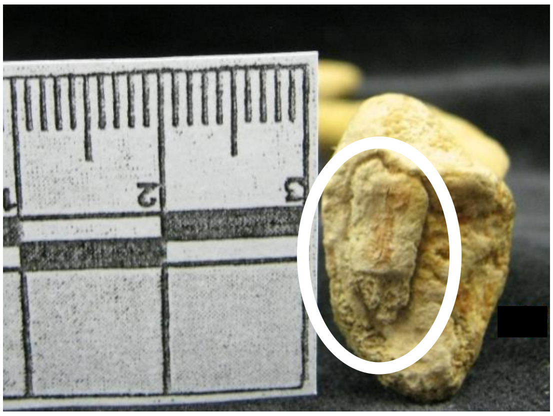

Figure 4.2: Specimen A592 - partial union at the medial end of the clavicle. Observe the medial epiphyseal flake which has begun to form (circle).

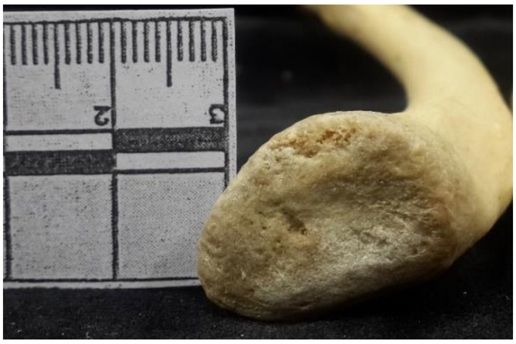

Figure 4.3: Specimen A3863 - complete union. This is due to the complete capping of the medial epiphyseal flake and in addition neither burrows nor furrows can be seen.

4.3 Results

No statistically-significant differences were detected between the stage of union at the left and right clavicles ($p=0.9$). Only 7.1% ($n=15$) of the paired clavicles assessed were observed to be in different stages of union to each other. Females displayed different stages of union at the medial epiphysis twice as often when compared to their male counterparts. Very good agreement was obtained for intra-observer error ($k=0.91$); whereas a moderate ($0.60 \geq k \leq 0.79$) agreement was noted for inter-observer error ($k=0.78$). A summary of the degree of union of this skeletal developmental marker by sex and age can be found in Table 4.2.

Overall, males and females displayed a strong correlation between age and staging score assigned ($r_s=0.73$); although males ($r_s=0.75$) did exhibit a slightly stronger association than females ($r_s=0.70$) when assessing the correlation between age and staging score by sex. Significant differences between all three staging categories and age were observed in both males and females ($p=<0.001$). No significant differences were observed between the mean age of males and females for all three staging categories ($p=0.09$, $p=0.85$, and $p=0.18$ respectively). A summary of the descriptive statistics of the South African female individuals included in this study can be found in Table 4.3 and Figure 4.4; while Table 4.4 and Figure 4.5 present a summary of the descriptive statistics of South African male individuals included in this study.

Table 4.2: The degree of union of the medial end of the clavicle in South African males and females by age

Age	Staging categories					
	No union		Partial union		Complete union	
	M	F	M	F	M	F
12	-	1	-	-	-	-
13	1	-	-	-	-	-
14	1	1	-	-	-	-
15	2	1	-	-	-	-
16	3	1	1	-	-	-
17	4	-	-	-	-	-
18	5	1	-	-	-	-
19	-	5	-	-	-	-
20	4	3	-	2	-	-
21	1	3	2	1	1	1
22	1	-	-	-	2	2
23	1	1	1	2	1	2
24	-	1	2	3	1	1
25	-	1	1	-	-	3
26	-	-	1	3	4	1
27	-	-	1	3	2	2
28	-	-	2	3	1	4
29	-	-	1	-	2	4
30	1	1	-	1	4	4
31	-	-	-	-	2	4
32	-	-	1	-	2	5
33	-	-	-	1	4	4
34	-	-	-	-	1	1
35	-	-	-	-	4	3
36	-	-	1	-	2	5
37	-	-	-	-	4	4
38	-	-	-	-	2	4
39	-	-	-	-	3	2
40	-	-	1	-	8	5
41	-	-	-	-	2	-
42	-	-	-	1	2	3
43	-	-	-	-	2	2
44	-	-	-	-	3	3
45	-	-	-	-	3	1

Non-union was most commonly seen in individuals 25 years of age and younger, although one male and one female, who were both 30 years of age, showed no signs of a developed medial epiphyseal flake. The mean age-at-death of South African male and female individuals included in this sample for this staging category was 18.3 years (±3.5) and 19.8 years (±4.0) respectively. The age-at-death range for females was observed to be 18.3 – 21.0 years; while the age-at-death range noted for males was 16.0 – 20.0 years.

Table 4.3: Descriptive statistics of South African Black females included in this study

Staging category	**N**	**Percentage of sample (%)**	**Mean (years)**	**SD**	**Median (years)**	**Min**	**Max**	**Range**
No union	20	18.2	19.8	± 4.0	19.5	12	30	18
Partial union	20	18.2	26.4	± 5.0	26.0	20	42	22
Complete union	70	63.6	33.4	± 6.2	33.0	21	45	24

The youngest South African male in this sample to demonstrate signs of partial union at the medial end of the clavicle was 16 years of age. A 20-year-old was the youngest female to be categorized with partial union. The mean age established for this staging category for South African males was 26.7 (±6.0) years, with an age-at-death range of 23.0 – 29.0 years. The mean age obtained for South African females was 26.4 (±5.0) years, with an age-at-death range of 23.3 – 28.0 years.

Table 4.4: Descriptive statistics of South African Black males included in this study

Staging category	**N**	**Percentage of sample (%)**	**Mean (years)**	**SD**	**Median (years)**	**Min**	**Max**	**Range**
No union	24	23.8	18.3	± 3.5	18.3	13	30	17
Partial union	15	14.9	26.7	± 6.0	26.7	16	40	24
Complete union	62	61.4	34.8	± 6.6	34.8	21	45	24

Complete union was more commonly seen in individuals older than 30 years of age. The youngest South African male and female to exhibit complete capping at the medial end of the clavicle were both 21 years of age. However, it must be noted that three male individuals and three female individuals, who were 30 years and older, still showed signs of partial union taking place at this epiphysis. A mean age of 33.4 years (±6.2) years and an age-at-death range of 29 – 38 years were established for South African females included in this sample; while a mean age of 34.8 (±6.6) years and an age-at-death range of 30.0 – 40.0 years were observed for South African males included in this sample.

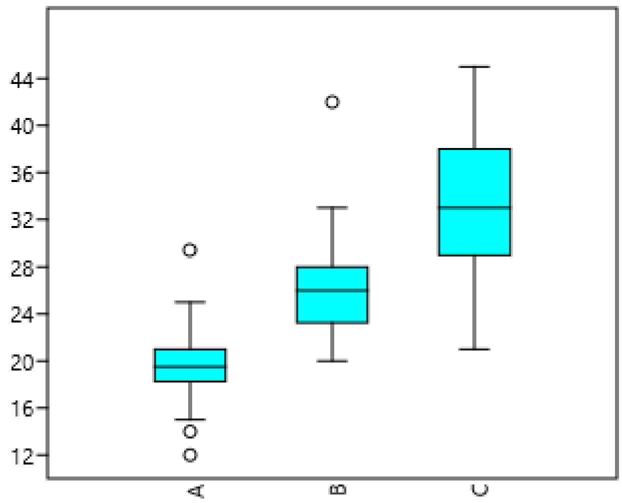

Figure 4.4: Box plots showing the median age and age-at-death range for South African Black female individuals in this study sample for all three stages of union. Key: No Union (A); Partial Union (B); Complete Union (C). Outliers are denoted with a circle

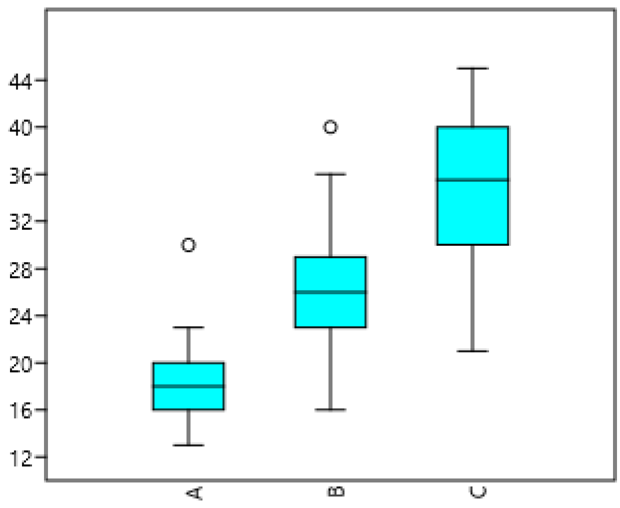

Figure 4.5: Box plots showing the median age and age-at-death range for male skeletal individuals from the South African Black sample for all three staging categories. Key: No Union (A); Partial Union (B); Complete Union (C). Outliers are denoted in the circles

4.4 Discussion

Moderate repeatability and reproducibility were achieved in this study using a three-stage scoring method. Assessing the rate of error for any stage-scoring method is vital in the field of forensics due to the legal requirements and ramifications faced by the fields' practitioners (Ritz-Timme et al., 2000; Calarusso, 2016). The greatest discrepancy between observers was found to occur more when categorizing the clavicles into either the partial and/or complete union stages. This disagreement can easily be explained due to one observer perceiving that recent complete capping at the medial end of the clavicle has taken place; while the other observer assumed that the epiphyseal flake was still fusing to the medial end of the clavicle. When assessing the medial epiphysis of the clavicle, a great deal of care needs to be taken when evaluating if there are any signs of recent union so as to avoid misclassification. The results of the observer agreement obtained in this study are comparable to other published studies (Langley-Shirley and Jantz, 2010; Milenkovic et al., 2013). The three-stage scoring method described in this research can therefore be used during the examination of forensically relevant cases.

Comparisons were only made between this study and previously-published studies examining the degree of union at the medial end of the clavicle on dry bone samples (skeletal or autopsy collections). This is due to the recognized visual variations that exist when examining the degree of fusion with the use of different radiological techniques and that of dry bone (Scheuer and Black, 2000; Cardoso, 2008; Milner and Boldsen, 2012). Statistically significant differences were not noted between the stage of union and the left and right clavicles. This finding is consistent with those of McKern & Stewart (1957) and Singh & Chavali (2011). If paired clavicles were observed to be in different stages, there was never more than a single stage difference noted between them. However, females did demonstrate more variability than their male counterparts, as different stages of union at the medial epiphysis were observed twice as often in females.

No union at the medial end of the clavicle was noticed in two individuals who were 30 years of age in this sample. Both these individuals were classified as outliers in this staging category. Explanations for this finding may be due to the cleaning process before these remains were added to the skeletal collection. As with all skeletal collections, the process of cleaning the remains is laborious and often involves the use of physical maceration among other techniques (Dayal et al., 2009). Small bones, such as a medial epiphyseal flake, may unintentionally become lost during this cleaning method if they had not yet completely fused. However, it must be understood that normal biological and anatomical variations do exist and these two individuals could possibly be an exception in this population group.

The ages obtained in this study for partial union at the medial end of the clavicle are similar to those noted by Todd and D’Errico (1928), Webb & Suchey (1985), Black &

Scheuer (1996), Langley-Shirley & Jantz (2010), and Milenkovic et al. (2013). In this sample, partial union at the medial end of the clavicle was observed more often in individuals 23 – 28 years of age. However, partial union was noted in a single individual who was documented to be only 16 years of age. This finding of partial union occurring before the second decade of life is not unusual and has been highlighted in previous studies (Webb and Suchey, 1985; Langley-Shirley and Jantz, 2010). It is important to emphasize that variability in the age at which fusion is actively taking place at the medial end of the clavicle did exist within this sample, as six individuals 30 years of age and older were classified into the partial union category.

Although complete union at the medial end of the clavicle was noted in two individuals as young as 21 years of age, it was more commonly observed in persons 30 years and older. Within this sample, complete union was achieved at a slightly later age than that documented in several previously-published studies when comparing mean ages (Langley-Shirley and Jantz, 2010; Singh and Chavali, 2011). This finding can be attributed to the differences between the population groups, diet, and socio-economic and health statuses of the individuals under study (Redfield, 1970; Singh and Chavali, 2011; Shirley and Jantz, 2011).

Overlap in the ages of partial and complete union is evident within this sample. Numerous reasons may account for this finding. It is well known that the ages for some individuals included in skeletal collections are not the actual (chronological) ages of these persons, which is problematic when these collections are being used to establish age ranges (Hunt and Albanese, 2005; Dayal et al., 2009; Rissech et al., 2013). This limitation will always exist when using skeletal collections as reference samples. However, the analysis of skeletal collections as reference samples remains a researcher's best source of gathering data, especially since direct comparisons between dry bone collections and forensically relevant cases can be made. Skeletal collections often consist of an assortment of unclaimed and bequeathed individuals with varying degrees of socio-economic and health statuses (Hunt and Albanese, 2005; L'Abbé et al., 2005; Dayal et al., 2009). Both these factors have been known to affect the rate at which fusion occurs in different skeletal elements and may account for the overlap in the ages of partial and complete union observed in this skeletal sample (Bassed et al., 2010; Shirley and Jantz, 2011; Singh and Chavali, 2011). An alternative reason for this variability could be attributed to what has already been highlighted by Stevenson (1924), Todd & D'Errico (1928) and Cardoso (2016). These authors have stated that immense individual variation exists at this epiphysis and it may be difficult to define an upper and lower age-at-death limit (Stevenson, 1924; Todd and D'Errico, 1928; Cardoso, 2008).

Despite these obvious variabilities seen for the age of partial and complete union at the medial end of the clavicle, strong correlation between age and staging score assigned does exist. Additionally, statistically-significant differences between all three staging categories and the age of both males and females were achieved. Therefore, assessing and classifying the degree of union at the medial end of the clavicle can be a useful additional age-at-death indicator during the analysis of unknown skeletal remains.

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Chapter 5

THE USE OF VENTRAL FUSION BETWEEN SACRAL ELEMENTS S1 AND S2 AS AN ADDITIONAL AGE-AT-DEATH INDICATOR IN A SOUTH AFRICAN BLACK SKELETAL SAMPLE

Article submitted to Forensic Science International – Under consideration

Article follows the format and referencing style of the journal to which it was submitted

*Figure and Table numbers have been altered from the submitted manuscript to keep them in line with the University of the Witwatersrand requirements for an MSc dissertation.

Abstract

Despite indicating numerous demographic features such as sex, stature, and age; the sacrum remains a relatively insufficiently researched skeletal element. A set pattern of ossification and fusion of the sacrum makes it a useful bone for estimating age-at-death in unknown skeletal individuals. The aims of this study, which examined a South African Black sample, were to establish if fusion correlated to age and to estimate the age at which fusion between the first two sacral vertebrae began and ended. A total of 316 male (n=149) and female (n=167) sacra from the Raymond A. Dart collection of Human Skeletons were assessed, with ages ranging from 13 – 60 years and 12 – 60 years respectively. A three-stage scoring method was used to categorise the degree of fusion of each sacrum. In comparison to females (rs=0.59), males demonstrated a higher correlation between age and staging score assigned (rs=0.68). It was observed that the age of partial and complete fusion was highly variable. However, it was noted that partial fusion occurred more often in individuals younger than 30 years, while complete fusion was observed commonly in both sexes above the age of 35 years. Despite this variability, the sacrum can be used as an additional age-at-death indicator.

5.1 Introduction

Forensic Anthropology, as a subfield of Physical Anthropology, deals with skeletal remains in a medico-legal setting by helping to establish osteo-demographic profiles (Snow, 1982; Işcan, 1988; Ubelaker, 1996). The biological profile consists of estimating sex, population affinity, age, and stature for a set of remains (Sauer & Lackey, 2000; Dirkmaat *et al*, 2008; Boyd and Boyd, 2011). This is usually done by using numerous techniques both metrically and morphologically (Sauer & Lackey, 2000).

One of the more challenging parameters to estimate is age (Sauer & Lackey, 2000; Baccino & Schmitt, 2006; Cunha *et al*., 2009). Traditional markers such as the sternal ends of ribs (Oettlè & Steyn, 2000), pubic symphysis (Brooks & Suchey, 1990), and auricular surface of the sacrum (Buckberry & Chamberlain, 2002) can assist in the formation of an age-at-death range. However, due to their porous nature, these markers are often damaged or lost during the process of decomposition and processing or collection techniques (Nawrocki, 1995; Galloway *et* al., 1997; Sauer & Lackey, 2000; Stojanowski *et al*., 2002). Furthermore, the use of each of the above skeletal features have been criticised for various reasons, including their fluctuating degrees of accuracies as well as the large age ranges suggested (Garvin *et al*., 2012; Milner & Boldsen, 2012). Multiple age-at-death methods should always be employed when dealing with skeletal remains to ensure that accurate age ranges are established (Ritz-Timme *et al*., 2000; Schmeling *et al*., 2007; Konigsberg *et al*., 2008; Cunha *et al*., 2009; Franklin, 2010; Garvin *et al*., 2012; Milner & Boldsen, 2012; Rissech *et al*., 2013). This is not only limited to the use of several of the previously mentioned methods, but also includes assessing additional skeletal elements that may aid in narrowing down these age ranges. Examination of other skeletal features, such as dental wear, osteophyte development, and the degree of fusion of the spheno-occipital synchondrosis, medial end of the clavicle, and sacral elements S1 and S2, are a few additional criteria which, when assessed, could help in forming an upper or lower limit of an age range (Krogman & Işcan, 1986; White & Folkens, 2005; Işcan & Steyn, 2013). It is observable that further research on the sacrum, which is often found in forensically-relevant cases, needs to be conducted (Belcastro *et al*., 2008; Ríos *et al*., 2008; Passalacqua, 2009; Karakas *et al*., 2011).

Sex, stature, and age-at-death can be estimated from the sacrum through the use of both morphological traits and metric analysis and it is thus a helpful skeletal element to assess in forensically-relevant cases (Krogman & Işcan, 1986; Scheuer & Black, 2000; Işcan & Steyn, 2013). Despite ossification and fusion of the sacrum being extremely complex; it does display a seemingly uniformed sequence of development (Scheuer & Black, 2000; Belcastro *et al*., 2008). Before the commencement of puberty, both the lateral elements and the anterior bodies begin to fuse in a caudocranial direction resulting in the bodies of S1 and S2 being the last sacral elements to completely fuse (McKern & Stewart, 1957; Scheuer & Black, 2000). The sacrum is important in forensic anthropological cases when it comes to age-at-death assessment due to its late fusion, which usually occurs during young adulthood (McKern & Stewart, 1957; Scheuer & Black, 2000; Belcastro *et al*., 2008; Ríos *et al*., 2008). However, considering this, the time of commencement and complete fusion between sacral elements S1 and S2 is sparsely researched (McKern & Stewart, 1957; Broome *et al*., 1998; Belcastro *et al*., 2008; Ríos *et al*., 2008; Passalacque, 2009). None of this research has been conducted on a South African sample, highlighting the necessity for the development of population-specific data for this geographic area.

Within South Africa, a significant portion of individuals received by various Forensic Pathology Services (FPS) Medico-Legal facilities nationwide are unidentified. This means that they are buried as paupers (National Health Act, 2003; Steyn *et al*., 1997; Evert, 2011). These individuals are sent to various medico-legal facilities due to their deaths being classified as unnatural in accordance with the National Health Act (2003). Forensic Anthropologists may be called upon by the South African Police Services (SAPS) or the Medico-Legal Practitioners at these facilities to aid in establishing an osteo-demographic profile of some of these advanced decomposed and unidentified remains. Nationwide, over a third of the documented unnatural deaths sent to these FPS Medico-Legal facilities comprise of individuals aged between 15 – 40 years (National Injury Mortality Surveillance System, 2012). Thus assessing the degree of fusion of the sacrum, in addition to the use of more traditional age-at-death markers, could be a helpful indicator when dealing with this essential age range. Thus, the aim of this study was two-fold: (i) to determine if fusion of these sacral elements correlates with age and (ii) to estimate the age at which sacral bodies of S1 and S2 start and complete fusion in a South African Black skeletal sample.

5.2 Materials and Methods

The sacra of 316 South African Black male (n=149) and female (n=167) individuals, with ages ranging from 12 to 60 years were included in this study. The mean age-at-death for males and females was 32.4 (± 12.0) years and 35.5 (±12.4) years respectively. These individuals were randomly selected from the Raymond A. Dart Collection of Human Skeletons. The South African Black population is the most abundant population group within this skeletal collection and comprises numerous tribal groups (Dayal *et al*., 2009). Lundy (1983) noted that these different groups do not post-cranially differ (either morphologically or metrically) extensively from each other and can thus be clustered into one population group. Unconditional ethical approval was obtained for this study from the Human Research Ethics Committee – Medical, University of the Witwatersrand (M140246).

Fusion between sacral elements S1 and S2 was noted using a three-stage scoring method, which involved the direct macroscopic assessment of the ventral aspect of the sacrum. Thereafter, numerical values were allotted to each of these categories for statistical analysis. This is depicted in Table 5.1. The sacrum usually consists of five sacral segments, but normal anatomical variations of four and six vertebral segments have also been documented (McKern & Stewart, 1957; Broome *et al*., 1998; Scheuer & Black, 2000; White & Folkens, 2005; Belcastro *et al*., 2008; Drake *et al*., 2010). All individuals, regardless of the number of sacral segments, were assessed and included in this study. The distribution of sex and age of the individuals included can be found in Table 5.2.

Table 5.1: The description of the staging categories used to evaluate the degree of fusion between sacral elements S1 and S2 and the numerical values assigned.

Staging categories	**Description**	**Numerical values assigned**
Unfused or open	A noticeable space or gap is evident between sacral bodies S1 and S2 (Figure 5.1). [If the first sacral element has yet to fuse to the second sacral element (either dorsally and/or ventrally), it was placed into this staging category].	1
Partially Fused	A gap is still present between the bodies of S1 and S2. However, some areas on the ventral aspect show signs of partial and/or complete fusion (Figure 5.2).	2
Completely Fused or closed	No space or gap between sacral bodies S1 and S2 can be observed (Figure 5.3).	3

Intra-observer error was assessed by randomly choosing 30 sacral elements whereupon the principle investigator repeated the staging and scoring of these skeletal elements. Inter-observer error was established by means of a blind test in which an independent person, a postgraduate student, who has knowledge of human osteology and experience in forensic anthropology, assessed and staged the same 30 randomly selected sacra.

Figure 5.1: Unfused or open. This figure shows a space between sacral elements S1 and S2 with no signs of fusion evident.

Figure 5.2: Partially fused sacra. This figure shows a variety of different sacra which were classified as demonstrating signs of fusion still taking place between the bodies of S1 and S2.

All statistical analyses were tested at the 5.0% level of significance (α=0.05) using both Graphpad Instat® 3.10 and PAST: Paleontological Statistics Software Package for Education and Data Analysis (Hammer *et al*., 2001). Numerical values were used to record all demographic data and graphical representations of the median, upper and lower interquartile ranges were noted using box plots. To test the normality of the sample, a Shapiro-Wilk test was run. A relationship between age and sacral fusion was assessed using a

Spearman's-ranked correlation coefficient. Nonparametric tests, such as the Krustal-Wallis and Mann-Whitney pairwise tests, were used to note if any significant differences between the ages of individuals and the staging score assigned existed. While a Chi² test was used to establish if any association between age and staging score assigned were observed, this test was calculated using the statistical software SAS 6.1. Both observer errors were determined with the use of a Cohen's Kappa test using Microsoft Excel® 2013 with an add-in feature known as analysis-it®.

Figure 5.3: Complete fusion between sacral bodies S1 and S2.

Table 5.2: The degree of fusion between sacral elements S1 and S2 separated by sex and age.

Age (year)	Total		Staging categories					
			Unfused		Partially fused		Completely fused	
	M	F	M	F	M	F	M	F
12	-	2	-	2	-	-	-	-
13	3	-	3	-	-	-	-	-
14	1	1	1	1	-	-	-	-
15	3	1	2	1	1	-	-	-
16	6	2	6	2	-	-	-	-
17	5	-	3	-	1	-	1	-
18	6	1	5	1	1	-	-	-
19	1	4	1	2	-	2	-	-
20	3	5	2	2	1	2	-	1
21	4	4	2	2	1	1	1	1

22	6	4	3	-	2	1	1	3
23	4	6	1	3	2	2	1	1
24	4	7	-	1	3	5	1	1
25	2	6	1	-	1	4	-	2
26	4	6	-	1	1	2	3	3
27	4	4	-	1	2	3	2	-
28	3	8	-	1	2	3	1	4
29	6	3	-	-	2	1	4	2
30	6	6	-	-	4	4	2	2
31	5	4	1	-	-	1	4	3
32	4	5	-	-	1	2	3	3
33	5	6	-	-	3	-	2	6
34	3	2	-	-	1	1	2	1
35	4	4	-	-	-	1	4	3
36	4	5	-	-	3	4	1	1
37	4	5	-	-	-	1	4	4
38	2	3	-	-	-	1	2	2
39	2	2	-	-	-	-	2	2
40	9	5	1	-	2	1	6	4
41	3	0	-	-	-	-	3	-
42	1	4	-	1	1	-	-	3
43	1	3	-	-	-	1	1	2
44	3	4	-	-	-	2	3	2
45	3	2	1	-	1	-	1	2
46	4	3	-	-	1	-	3	3
47	1	3	-	-	-	-	1	3
48	2	5	-	-	-	-	2	5
49	1	2	-	-	-	-	1	2
50	5	4	-	-	-	-	5	4
51	0	3	-	-	-	-	-	3
52	2	3	-	-	-	-	2	3
53	1	2	-	-	-	1	1	1
54	2	3	-	-	-	-	2	3
55	2	2	-	-	-	1	2	1
56	1	3	-	-	-	-	1	3
57	1	1	-	-	-	-	1	1
58	2	4	-	-	-	-	2	4
59	0	2	-	-	-	1	-	1
60	1	3	-	-	-	-	1	3

5.3 Results

Strong agreement was found for both intra-observer and inter-observer error, as values of k=0.90 and k=0.84 were obtained respectively. The results for South African males and females are discussed separately. Due to a skew distribution observed within the sample, the median age of unfused, partially fused, and completely fused are noted.

Black males

Descriptive statistics of males included in this study are noted in Table 5.3 and Figure 5.4. The youngest male to show signs of partial fusion between S1 and S2 was 15 years of age. However, two individuals aged 40 and 45 years had yet to display any signs of fusion between these sacral elements. A 17-year-old male was the youngest individual categorised with complete fusion. Five males 40 years or older were still exhibiting signs of partial fusion between these two sacral elements.

A correlation between age and staging score assigned and an association between the category of fusion of the sacrum and age were observed and noted (Table 5.5). The Kruskal-Wallis and the Mann-Whitney pairwise tests showed significant differences between the staging categories and the age of male individuals (p= <0.001 and p = <0.001 respectively). Male individuals were more likely to show no signs of fusion between sacral elements S1 and S2 until 20 years of age. The median age at which partial fusion was observed was 29 years, with an age-at-death range of 25 – 32 years. Complete fusion between these two sacral elements were more commonly noted in male individuals above the age of 35 years.

Table 5.3: Descriptive statistics of South African Black males per staging category.

Staging category	**N**	**Mean (years)**	**SD**	**Median (years)**	**Min**	**Max**	**Lower confidence interval**	**Upper confidence interval**
Unfused/open	33	19.7	± 7.0	18	13	45	16	20
Partially fused	37	29.1	± 7.6	29	15	46	25	32
Completely fused	79	39.3	± 10.2	39	17	60	35	41

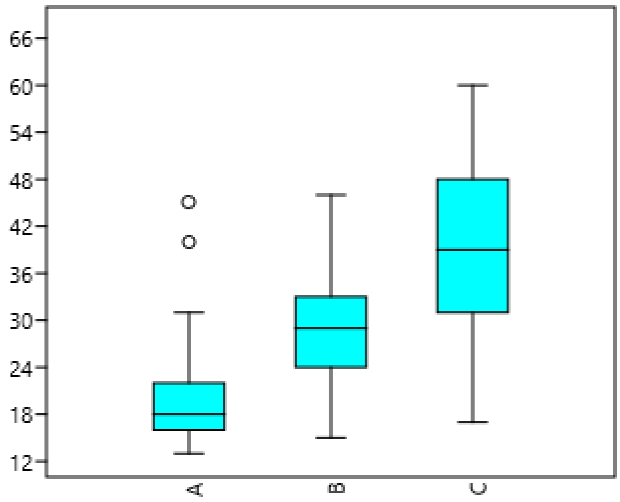

Figure 5.4: Box plots showing the median age and age-at-death range for male skeletal individuals from the South African sample for all three staging categories. Key: Unfused or Open (A); Partially fused (B); Completely fused (C). Outliers are denoted by the circle.

Black females

Descriptive statistics of females included in this study are noted in Table 5.4 and Figure 5.5. The youngest South African female to show signs of partial fusion between S1 and S2 was 19 years of age. One individual, aged 42 years, showed no signs of fusion. A 20-year-old female was the youngest individual categorised with complete fusion. However, seven females 40 years or older were still exhibiting signs of partial fusion between these two sacral elements.

In addition to noting the correlation between age and the staging category (Table 5.5), an association between the categories of fusion of the sacrum and the age of individuals was obtained. Significant differences between the three staging categories and the age of female individuals were observed when both a Kruskal-Wallis test and a Mann-Whitney pairwise test were run ($p = <0.001$ and $p = <0.001$ respectively). Sacral elements S1 and S2 are more likely to remain unfused in females, who are younger than 22 years of age. The median age of females, who exhibited partial fusion, was 28 years with an age range of 25 – 30 years. Complete fusion was more evident in female individuals above the age of 36.5 years.

Table 5.4: Descriptive statistics of South African Black females per staging category

Staging category	**N**	**Mean (years)**	**SD**	**Median (years)**	**Min**	**Max**	**Lower confidence interval**	**Upper confidence interval**
Unfused/open	21	20.9	± 6.7	20	12	42	17	22
Partially fused	48	30.7	± 9.2	28	19	59	25	30
Completely fused	98	41.0	± 11.2	41	20	60	36.5	45

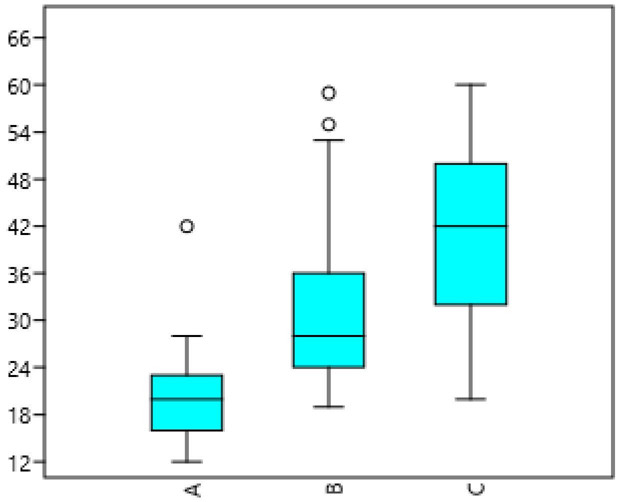

Figure 5.5: Box plot showing the median age and age-at-death range for female skeletal individuals from the South African Black sample for all three staging categories. Key: Unfused or Open (A); Partially fused (B); Completely fused (C). Outliers are signified by the circles

Table 5.5: Spearman's ranked correlation co-efficient and Chi² test for association between the three staging categories and the age of individuals by sex

	Statistic	**p-value**
Males		
Spearman's (rs)	0.68	<0.001
Chi²	148.62	<0.001
Females		
Spearman's (rs)	0.59	<0.001
Chi²	147.82	<0.001

5.4 Discussion

This study focused solely on the fusion between sacral elements S1 and S2. According to the author's knowledge, this is the only study to do so within this particular population group. Although there is a wealth of literature focusing on establishing a demographic profile for South African skeletal remains, a negligible amount of this literature centres on estimating age in this population group (L'Abbè & Steyn, 2012).

A high repeatability rate was noted for the three-stage scoring method utilised in this study. The assessment of error rates for any method used in age-at-death estimation is paramount for legal purposes (Ritz-Timme *et al*., 2000; Colarusso, 2016). In forensic anthropology, estimating age for a set of unknown skeletal remains is often centred on using stage, phase, and/or component based methods (Shirley & Montes, 2015). These approaches are critiqued on usability and repeatability. Despite numerous researchers focusing on simplifying and better defining published methods, a perfect repeatability rate is often not achieved. The more categories added in a single method; the higher the rate of error noted between observers and within a single observer (Webb & Suchey, 1985; Scheuer & Black, 2000; Shirley & Montes, 2015). The authors in this study recommend using this three-stage scoring method for assessing the sacrum, which incorporates clear and unambiguous categories.

A noticeable concern throughout published literature is the variability in the time of fusion between sacral elements S1 and S2 (Scheuer & Black, 2000; Belcastro *et al*., 2008; Ríos *et al*., 2008; Passalacqua, 2009). Variability was also noted in this study, as a number of outliers were observed. Correlation and association between the age of an individual and the staging score assigned were noted and in agreement with data observed by Ríos *et al*. (2008). Belcastro *et al*. (2008) obtained a greater correlation between age and stage allotted when compared to the results obtained in this study. However, the total sample of skeletal individuals included in the Belcastro *et* al. (2008) study was almost three times greater than the samples used in this study, which may account for this discrepancy. South African Black males demonstrated a stronger correlation when compared to their female counterparts. A reason for this result could be attributed to females showing greater variability in the age at which partial and complete fusion was noted to occur in certain individuals. Very few studies have focused on establishing reasons accounting for this variability. McKern & Stewart (1957) attributed the discrepancy in complete fusion of this skeletal element to two hypotheses. One was based on the concept of prolonged terminal ossification or lapsed union as suggested initially by Todd & Lyon (1924). The other hypothesis stated that the gap between sacral elements S1 and S2 is different for each individual (McKern & Stewart, 1957). Consequently, a narrow gap (when compared to a large gap between these sacral elements) would result in complete fusion between S1 and S2 occurring more quickly (McKern & Stewart, 1957). Hence it was suggested that one be aware that fusion between sacral elements S1 and S2 may not actually show a relationship to age due to these diverse individual variations.

As previously mentioned, all individuals, regardless of the number of sacral elements, were included in the study sample. This may account for the high number of males and females demonstrating unfused and partial fusion of this skeletal element well into their third, fourth, and fifth decade of life. This approach was taken to form a more holistic view of potential cases that may be presented to a forensic anthropologist for analysis. However, more research into what effect the number of sacral segments has on the age at which fusion commences and concludes between sacral elements S1 and S2 is required.

McKern & Stewart (1957) have suggested that when partial fusion between the bodies of sacral elements S1 and S2 is evident, then the individual is more likely to be in their mid-twenties or younger. Yet if complete fusion of these two sacral bodies is seen, then the individual may be 25 years of age or older (McKern & Stewart, 1957). This suggestion did not apply to the South African skeletal sample used in this research. If no fusion is observed between these skeletal developmental markers, then the individual is more likely to be younger than 20 years of age (if male) and 22 years of age (if female). Partial fusion between the ventral aspect of S1 and S2 in males and females was seen more often in individuals younger than 30 years of age. In both sexes, complete fusion of this skeletal element was frequently noted in individuals over 35 years of age. Therefore, the age of fusion for this skeletal element in this South African sample occurred later than what has been recorded in previous publications. Consequently, this highlights the need to develop geographical specific data (McKern & Stewart, 1957; Belcastro *et al*., 2008; Ríos *et al*., 2008; Passalacqua, 2009).

Despite the evident variability of ventral fusion in this skeletal developmental marker, statistically significant differences, clear correlation, and association were noted (in both males and females) between age and staging score allotted. Thus the results in this study confirm that classifying the degree of fusion of this skeletal element can aid in placing an unknown skeletal individual into an adult age group (young/middle/older adult) as suggested by Ríos *et al*. (2008). However, to obtain an accurate estimation of age, a multifactorial approach is still stressed when assessing cases in a forensic setting (Scheuer, 2002; Lewis & Flavel, 2006; Garvin *et al*., 2012; Milner & Boldsen, 2012).

5.5 Conclusion

Ventral fusion between sacral elements S1 and S2 can be used as an additional age-at-death indicator in a South African skeletal sample. However, it must be used with caution as variability between the age and state of fusion is evident, especially among females. A low error rate was obtained for the three-stage scoring method used, making it applicable for use in forensically-relevant cases.

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Chapter 6

CONCLUDING REMARKS

This study aimed to determine the age at which three skeletal elements began and completed fusion in a South African Black skeletal sample. From the results obtained, a number of conclusions can be drawn which are listed below:

Spheno-occipital synchondrosis

1. The perfect repeatability and reproducibility of Shirley and Jantz (2011) three-stage scoring method makes it a valuable tool during the analysis of forensically relevant cases.
2. No statistically significant differences were noted between males and females for the age at which the spheno-occipital synchondrosis begins fusion in a South African Black sample. However, there was a skew distribution of sex within the study and future studies with larger sample sizes would be greatly beneficial.
3. A moderate correlation between age and staging score assigned was noted.
4. All individuals above the age of 20 years, regardless of sex, demonstrated complete fusion of this skeletal developmental marker.

Medial end of the clavicle

1. No statistically significant differences were observed between the degree of union at the left and right clavicles. Thus, if only a single clavicle is received during the analysis for a set of skeletal remains the medial end of that clavicle can be categorised to assist in age estimation.
2. Strong correlation between age and staging score assigned was achieved in this sample.
3. No statistically significant differences were noted between males and females in this sample population for all three staging categories.
4. Substantial overlap between the ages of individuals, and categories of partial and complete union was observed in this sample. However, partial union was frequently observed in individuals between the ages of 20 to 30 years. While complete union was more commonly seen in individuals over 30 years of age.

Sacral elements S1 and S2

1. Variability in the age at which fusion began and completed in both males and females for this skeletal developmental marker was observed.
2. Statistically significant differences were noted between the staging categories and age of the individuals assessed in both males and females.

3. Males demonstrated a greater correlation between age and the staging score assigned than females within this study sample.
4. Age estimation based on the degree of fusion between skeletal elements S1 and S2 should be used with caution when assessing a forensically relevant case. However, it can be a useful tool when assigning a large age group (young/middle/older adult) to a set of unknown skeletal remains.

It must be noted that the age and age ranges stated in this study were derived from individuals from the Raymond A. Dart Collection of Human Skeletons (also known as the Dart Collection). The Dart Collection comprises of over 2500 skeletons of individual who were either bequeathed to the University or individuals who were unclaimed from Provincial hospitals and then donated to the University for Medical Research (Dayal *et al*., 2009). It is one of the largest skeletal collection in the world and consists of individuals from numerous population groups including but not limited to South African Black, White, Coloured and Indian (Dayal *et al*., 2009). Several tribal groups, majority of which is represented by the Zulu tribe, make up the South African Black population within the Dart Collection (Dayal *et al*., 2009). De Villiers (1968) and Lundy (1983) assessed the cranial and post-cranial elements, respectively, of these different tribal groups using both metric and non-metric analysis. No statistically significant differences were noted by the authors between these tribal groups, although a few morphological and metric features were seen to be different between certain tribal groups (De Villiers, 1968; Lundy, 1983). Due to this finding of no statistical significant differences, De Villiers (1968) and Lundy (1983) suggested the idea of assessing these different tribal groups as a single South African Black population. Macho (1990) used multivariate statistical analysis using the femur to establish if there is any sexual dimorphic differences between these South African Black tribal groups and White South Africans within the Dart Collection. The author concluded that there is a difference between the sexes of each South African Black tribal group and South African Whites especially when shape is assessed (Macho, 1990). However, when canonical variates were tested using six, 11 and 18 different measurements the latter two tests produced no statistical significant differences between the South African Black tribes and South African Whites; it was concluded by the author that in two-dimensional space there is no sexual dimorphic differences between the sexes assessed (Macho, 1990). In addition to this South Africans are not classified by tribal groupings on official documentation but by population groups such as Black, White, Coloured, Indian or other. Thus classifying, assessing and establishing age ranges for each tribal group would be counterproductive in a forensic setting when minimal differences have previously been noted for these tribal groupings. This study assessed South African Blacks as one homologous population and included individuals from the Zulu, Xhosa, Sotho, Pedi, and Venda tribes to name a few.

Dayal *et al*. (2009), have previously mentioned that some of the ages of the individuals included in this collection have been estimated and are not the actual chronological age of the individual. This is unfortunately an inherent prejudice, which has been previously highlighted by several researchers, when using reference criteria derived from skeletal collections (McKern and Stewart, 1957; Redfield, 1970; Scheuer & Black, 2000; Shirley & Jantz, 2011; Hunt & Albanese, 2005; Dayal *et al*., 2009; Rissech *et al*., 2013). However, the use of dry bone collections worldwide have been recognised as the most accurate way of assessing certain skeletal developmental markers due to the inspection of the bone itself taking place (Shirley & Jantz, 2011). An additional advantage of assessing dry bones allows for direct comparisons to be made between the studies reference sample and that of a forensically relevant case.

An important point noted by Dayal *et al*. (2009) was that any individual whose age was documented to be between a 5 and a 10 year age range it was more likely to be the chronological age of that individual. This study included as many persons (with a one year interval) as possible encompassing all ages who matched the inclusion criteria for this study. Therefore, it could be assumed that the sample included in this research provides an adequate representation of individuals with known chronological ages. However, a greater sample size is needed before any inferences can be made regarding the age at which these three skeletal developmental markers begins and completes fusion in the South African Black population group as a whole. A larger sample size could be achieved through the inclusion of individuals from the Pretoria Bone Collection, the Cape Town Skeletal Collection and the Kirsten Collection in Stellenbosch. The addition of these different skeletal collections would also allow for comparisons to be made between different cultural and social groups within South Africa. These types of comparisons are vitally important, due to the fact that stunting is more affected by these above mentioned factors than the sex of an individual (Said-Mohammed *et al*., 2015). Thus, establishing a mean age and age-at-death range in a nationwide sample will be greatly beneficial to the field of forensic anthropology.

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Chapter 7

APPENDICES

APPENDIX A – Human Research ethics certificate

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140246

NAME: (Principal Investigator)	Ms Trisha-Jean Mahon
DEPARTMENT:	Forensic Medicine and Pathology Johannesburg Forensic Pathology Services
PROJECT TITLE:	Age determination, using the spheno-occipital Synchondrosis, Medical End of the Clavicle, and S1-S2 Fusion, on a South African Population
DATE CONSIDERED:	28/02/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Guinevere Gordon

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

DATE OF APPROVAL: 24/03/2014

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**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B – Letter from co-authors

Permission from co-authors letter

I, Trisha – Jean Mahon (student number: 361694), hereby declare that I contributed notably to the research and its findings in the accepted manuscript placed within Chapter 3 of this dissertation entitled "Spheno-occipital synchondrosis: Examining the degree of fusion in a South African Black skeletal sample". I was involved in the data collection, data analysis and the interpretation of the results. I also contributed sufficiently towards the final write up of this research.

The co-authors of this accepted manuscript agree to its use as part of the above mentioned students' dissertation.

Ms. T Mahon | Dr L.J Friedling | Dr G.M Gordon

APPENDIX C – Letter from co-authors

Permission from co-authors letter

I, Trisha – Jean Mahon (student number: 361694), hereby declare that I contributed significantly to the research and its findings in the manuscript placed within Chapter 4 of this dissertation entitled "Union of the medial clavicular epiphysis in a South African Black skeletal sample". This manuscript has been submitted to HOMO – Journal of Comparative Human Biology and is under consideration at present. I was involved in the conceptualisation of the study, data collection, data analysis and the interpretation of the results. I also contributed amply towards the final write up of this research.

The co-authors of this submitted manuscript agree to its use as part of the above mentioned students' dissertation.

Ms. T Mahon

Dr L.J Friedling

Dr G.M Gordon

APPENDIX D – Letter from co-authors

Permission from co-authors letter

I, Trisha – Jean Mahon (student number: 361694), hereby declare that I contributed significantly to the research and its findings in the manuscript placed within Appendix G of this dissertation entitled "The use of ventral fusion between sacral elements S1 and S2 as an additional age-at-death indicator in a South African Black skeletal sample". This manuscript has been submitted to Forensic Science International and is currently under review. I was involved in the data collection, data analysis and the interpretation of the results. I also contributed thoroughly towards the final write up of this research.

The co-authors of this submitted manuscript agree to its use as part of the above mentioned students' dissertation.

Ms. T Mahon   Dr L.J Friedling   Dr G.M Gordon

APPENDIX E – Turnitin Report

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Corresponding author:	**Ms. Trisha - Jean Mahon**
E-mail address:	**Trisha-jean.Mahon@wits.ac.za**
Journal:	**Forensic Science International**
Our reference	**FSI8873**
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DOI:	**10.1016/j.forsciint.2017.06.010**

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Forensic Anthropology Population Data

Spheno-occipital synchondrosis: Examining the degree of fusion in a South African Black skeletal sample☆

Trisha-Jean Mahon[a,*], Louise Jacqui Friedling[b], Guinevere Marianne Gordon[c]

[a] The Department of Forensic Medicine and Pathology, University of the Witwatersrand, 25 A Hospital Street, Braamfontein, 2195, South Africa
[b] The Department of Human Biology, University of Cape Town, Anzio Road, Observatory, 7925, South Africa
[c] Private Researcher

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ABSTRACT

Estimating age-at-death is one of the many biological demographics that a forensic anthropologist needs to determine for a set of unknown skeletal remains. A useful skeletal developmental marker, which can aid in estimating age in sub-adult remains, is the state of fusion of the spheno-occipital synchondrosis. This study aimed to determine the repeatability of a three-stage scoring method and the age at which the spheno-occipital synchondrosis begins and completes fusion in a Black South African sample. A total of 147 male and female skeletal individuals aged between 12–30 years from the Raymond A. Dart Collection of Human Skeletons were included. The mean age-at-death noted for the commencement of fusion at the spheno-occipital synchondrosis was 16.2 (±2.9) years in females and 16.7 (±1.2) years in males, with females displaying signs of fusion taking place approximately three years before their male counterparts. Complete fusion of this skeletal developmental marker was observed in 100% of the sample over the age of 20 years, regardless of sex. A Kappa value of 1.0 was achieved when the repeatability and reproducibility of the three-stage scoring method was tested. Complete fusion of this synchondrosis occurred slightly later in this South African sample than that noted in previous literature. This finding may be attributed to the differences in health and socio-economic statuses between these different population groups, reiterating the need for population-specific data to be developed. The importance of noting the state of fusion of the spheno-occipital synchondrosis during the examination of relevant forensic cases may aid in the formation of a narrower age-at-death range for sub-adult and young adult skeletal remains.

1. Introduction

Age can be one of the most problematic biological features for a physical/forensic anthropologist to assess. It forms an essential component of a biological profile by aiding in the narrowing down of possible missing persons [1–5]. Establishing an age range of a set of skeletal remains is often compounded by numerous factors, such as the experience of the individual conducting the analysis and the type and state of preservation of the remains presented for examination [2,3]. The spheno-occipital synchondrosis is an important skeletal developmental marker that can be used in estimating the age-of-death in sub-adult skeletal remains [6]. This is due to complete fusion of this synchondrosis occurring much later than other aging indicators in sub-adult individuals [7–11].

Internationally, more and more data regarding the age at which this skeletal developmental marker commences and completes fusion has been obtained using dry bone samples, radiographic images, computed tomography (CT) scans, magnetic resonance imaging (MRI), or a combination of these techniques [6–10,12–20]. Due to the vast range of techniques and staging methods used to estimate the age at which this synchondrosis fuses in populations worldwide, it is hypothesized by the authors that comparisons between the age-at-death ranges can only be made if the same method of examination is used between the published articles' reference sample and that of the forensic case. Thus, age-at-death ranges obtained using reference samples from radiographic imaging origin can only be compared to and used if the relevant

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* Corresponding author at: 25A Hospital Street, Braamfontein, 2001, South Africa.
E-mail addresses: Trisha-jean.Mahon@wits.ac.za (T. Mahon), jacqui.friedling@uct.ac.za (L.J. Friedling), Guinevere.Gordon@gmail.com (G.M. Gordon).

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forensic case in question also consists of radiographic images. Reasons for these conflicting age ranges include the use of different methods and the fact that visual details of bone development seen on radiographic images, CT scans, or MRIs are different to what can be seen on dry bones [5,9].

As mentioned above there is a plethora of literature regarding the age at which this skeletal developmental marker completely fuses. However, the one noticeable discrepancy among these studies is the immense variation in reported ages in studies both between populations and within a single population group [6–10,12–20]. These variations in the age at which the spheno-occipital synchondrosis begins and completes fusion could be attributed to the technique, method and sample sizes used in these studies, the inclusion of both or a single sex, the differences in the population groups studied, as well as secular trends within one population group.

No information vis-á-vis the age at which the spheno-occipital synchondrosis fuses has been noted in a South African population. This study therefore aimed to develop population-specific data in this geographical region for the age at which this synchondrosis commences and completes fusion, and to test the repeatability of a three-stage scoring method designed by Shirley and Jantz [10].

Fig. 1. Specimen A1244 showing an unfused spheno-occipital synchondrosis.

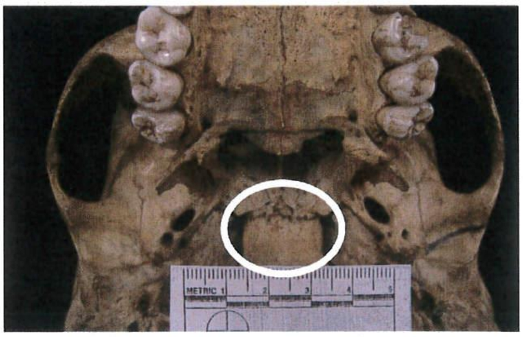

Fig. 2. Specimen A151 showing a fusing spheno-occipital synchondrosis.

2. Materials and Methods

The sample consisted of 147 randomly selected South African Black individuals from both sexes (almost equal portions of males (n = 74) and females (n = 73)) between the ages of 12–30 years from the Raymond A. Dart Collection of Human Skeletons (commonly referred to as the Dart Collection). The mean age-at-death for male and female individuals was 21.9 (± 5.2) years and 23.7 (± 4.5) years respectively. Although the year-of-death of the sample population ranged from 1925 to 2000, the average year of death of the individuals was 1952. The Dart collection is stored in the School of Anatomical Sciences at the University of the Witwatersrand, South Africa. Within this skeletal collection, South African Black individuals are composed of a number of different ancestral groups [21]. However, De Villiers [22] notes that the cranial elements of these different ancestral groups do not morphologically differ extensively from each other and can thus be clustered together into a single population group.

The following exclusion criteria were applied: signs of trauma, pathological conditions or damage to the inferior aspect of the cranium, and unknown individuals. A non-invasive, macroscopic three-stage scoring method developed by Shirley and Jantz [10] was implemented to describe the fusion of this synchondrosis. Numerical values were then assigned to each of the three staging scores for statistical purposes. Table 1 details the scoring method and assigned numerical values.

Repeatability and reproducibility of staging and scoring of the spheno-occipital synchondrosis was assessed by randomly selecting 30 individuals from the sample. Intra-observer error was established after the principle investigator staged and scored the synchondrosis a second time. Inter-observer error was established using an independent individual who has a moderate understanding of osteology and forensic anthropology. All demographic data were recorded as either numerical values or frequencies. Box plots were used, which allowed for the median, and upper and lower interquartile ranges to be displayed graphically. An unpaired t-test was used to compare the mean age at which fusion begins between males and females in order to establish if any significant differences between the sexes existed. Correlation between age and the staging score assigned was determined through the use of a Spearman's ranked correlation coefficient. All statistical analyses were tested at the 5.0% level of significance ($\alpha = 0.05$). Statistical analysis was done using Graphpad Instat® 3.10 and PAST: Paleontological Statistics Software Package for Education and Data

Table 1
Description of the staging categories used to evaluate the degree of fusion of the spheno-occipital synchondrosis, and the numerical values used.

Staging categories	Description	Numerical values assigned
Unfused	A noticeable opening or space can be observed at the spheno-occipital synchondrosis (Fig. 1)	1
Fusing	Active fusion can be seen between the basilar portion of the occipital bone and the body of the sphenoid bone. However, slight spaces or openings are still observable on the ectocranial surface (Fig. 2)	2
Fused	No openings or spaces are observable at this synchondrosis. If a fusion scar was noted at the sight of the two joining bones, it was placed within this category (Fig. 3)	3

Fig. 3. Specimen A184 showing a completely fused spheno-occipital synchondrosis.

Analysis [23]. Both observer errors were determined statistically with the use of a Cohen's Kappa test; which was conducted using Microsoft Excel® 2013 with an add-in feature analysis-it®.

3. Results

Perfect repeatability and reproducibility of this method were established, as a Kappa value of 1.0 was obtained when both observer errors were statistically evaluated. A summary of the descriptive statistics of the individuals included and the degree of fusion of the synchondrosis by age can be found in Tables 2 and 3.

The youngest examined female and male individuals showing signs of fusion taking place at the spheno-occipital synchondrosis were 12 and 15 years of age respectively. However, the oldest male and female individuals to still demonstrate these signs of fusion were both 19 years of age. The mean age-at-death determined for South African Black males was 16.7 (±1.2) years with an age range of 16.0–17.8 years (Fig. 4). A strong correlation (rs = 0.70) was noted between age and the staging score assigned for included male individuals. South African Black females were noted to have a mean age-at-death of 16.2 (±2.9) years and an age range of 13.5–19.0 years (Fig. 4). A moderate correlation (rs = 0.49) was obtained between the age and the staging score allocated for females included.

No statistically significant differences were observed between the mean age at which the spheno-occipital synchondrosis starts to fuse in this sample of males and females (p = 0.8). Thus a mean age and an age-at-death range for the commencement of fusion between the basilar part of the occipital and the body of the sphenoid in this South African sample was established as 16.5 (±1.8) years and 15.6–17.5 years respectively. A strong correlation (rs = 0.63) was noted between the age and the staging score assigned when both males and females were assessed.

In this study sample, the youngest male to show complete fusion of the spheno-occipital synchondrosis was 15 years of age. A 16-year-old female was noted to be the youngest female to display complete union at this synchondrosis. Complete fusion of the spheno-occipital synchondrosis, irrespective of sex, was noted in 100% of the sample in all individuals over the age of 20 years.

Table 3
The degree of fusion of the spheno-occipital synchondrosis in South African males and females by age.

Age (years)	Staging categories					
	Unfused		Fusing		Fused	
	M	F	M	F	M	F
12	–	1	–	1	–	–
13	3	–	–	–	–	–
14	1	1	–	–	–	–
15	–	–	2	1	1	–
16	1	–	4	1	1	1
17	2	–	3	–	2	–
18	–	–	2	–	5	2
19	–	–	1	2	–	3
20	–	–	–	–	4	4
21	–	–	–	–	3	5
22	–	–	–	–	6	4
23	–	–	–	–	4	6
24	–	–	–	–	3	7
25	–	–	–	–	3	6
26	–	–	–	–	4	6
27	–	–	–	–	4	4
28	–	–	–	–	3	7
29	–	–	–	–	5	4
30	–	–	–	–	7	7

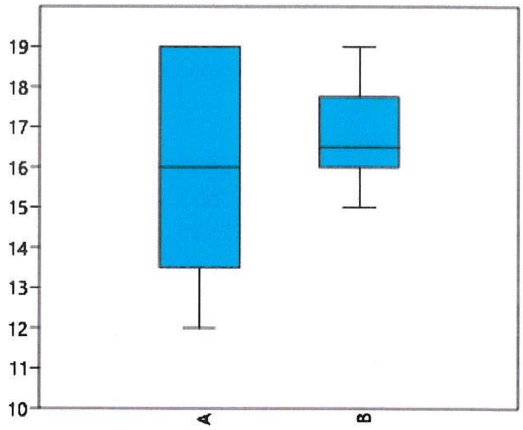

Fig. 4. Box plot showing the median and quartile ranges for South African female and male individuals showing signs of a fusing spheno-occipital synchondrosis. Key: females (A) and males (B).

4. Discussion

Multiple methods of age estimation must always be employed during relevant forensic anthropological cases in order to obtain a

Table 2
Descriptive statistics of South African individuals included per staging category.

Staging category	N	Percentage of sample (%)	Mean (years)	SD	Median (years)	Min	Max	Range
Unfused	9	6.1	14.3	±1.9	14.0	12	17	5
Fusing	17	11.6	16.5	±1.8	16.0	12	19	7
Fused	121	82.3	24.3	±4.0	24.0	15	30	15

more accurate estimation of biological age [4,5,24,25]. Noting the age at which the spheno-occipital synchondrosis starts and completes fusion is only one of numerous methods that can be utilised during the formation of an age range. The biggest limitation for estimating age in skeletal remains is the lack of well-defined scoring systems that can be used with ease during skeletal analysis [1,10]. Scheuer and Black [9] and Webb and Suchey [26] observed that when more categories of staging are present within a single method, there is an increase in the error rate between observers. Thus having a well-defined scoring method is crucial for judicial purposes. A three-stage scoring method designed by Shirley and Jantz [10] was used to determine the ectocranial state of fusion of the spheno-occipital synchondrosis. These authors state that they had yet to statistically test the accuracy for this method, both within and between observers [10]. This study observed that there was perfect repeatability and reproducibility between both the principle investigator and an independent observer, thus allowing for this staging method to be used with ease within a laboratory or field setting.

It is important to note that only published literature that estimates the age of ectocranial closure of the spheno-occipital synchondrosis on dry bone were reviewed for this study. Although all of these studies were conducted on different skeletal collections worldwide; some authors have noted that using skeletal collections as reference samples can be problematic [7–10,21,27,28]. This is due to the estimation of some of the ages of the individuals included in these skeletal collections as the chronological age of the individual is not known. This highlights one of the biggest hurdles in forensic anthropology when estimating the age-at-death using information obtained from skeletal collections [21,27,28]. In spite of these concerns, however, studies conducted on dry bones have also been described as the most accurate way of evaluating this skeletal developmental marker [10].

One of the earliest published attempts to determine the age at which the spheno-occipital synchondrosis fuses was conducted by McKern and Stewart [7]. The authors concluded that complete fusion was observed as early as 17 years [7]. Almost two decades later, Redfield [8] noted that the spheno-occipital synchondrosis was completely fused by 20–29 years of age in a Yugoslavian population. Scheuer and Black [9] state that previous anatomical literature as well as studies analysing and detailing the growth of the cranium have overestimated the age at which this synchondrosis completes fusion. More recent age ranges of complete fusion between 11–16 years in females and 13–18 years in males have been suggested [9]. Shirley and Jantz [10], estimated the age at which the spheno-occipital synchondrosis fuses in a modern American population and concluded that this skeletal developmental marker commences closure as early as 11.4 years in females and 16.5 years in males [10].

The results of this study confirm what has previously been stated by Scheuer and Black [9] and Shirley and Jantz [10]. Fusion of this synchondrosis begins during adolescence and not at the period of young adulthood, as earlier established by McKern and Stewart [7] and Redfield [8]. However, it must be emphasised that the minimum age included in this study was 12 years (due to the unavailability of other samples matching the inclusion criteria), which makes direct comparisons of the age at which this synchondrosis begins fusion between studies difficult. Previous anatomical, embryological, and anthropological literatures have shown that females undergo pubescent changes approximately two years before males [9,25,29,30]. In the sample under review in this study, South African Black females demonstrated signs of fusion taking place at the spheno-occipital synchondrosis by up to three years earlier than their male counterparts, which is consistent with published literature.

No significant differences were observed between the age at which fusion commences at the spheno-occipital synchondrosis among males and females. Within this sample, female individuals demonstrated a weaker correlation between age and stage score assigned than male individuals. However, only 17 individuals showed signs of fusion taking place at this synchondrosis, with the number of males far out weighing the number of females. The inclusion of additional sub-adult or young adult individuals from other South African skeletal collections is recommended. Increasing the number of individuals could assist in establishing if there is in fact a significant difference between males and females and may strengthen the results of the correlation between age and staging score assigned. Skeletal collections around the world, however, have noted that juvenile and sub-adult individuals are underrepresented in these collections. This highlights just one of the many difficulties faced by physical/forensic anthropologists regarding establishing methods of estimating age in sub-adult individuals [5,9,21,27,30–37]. Furthermore, there is typically a skew distribution of sex within these skeletal collections. Conventionally, there are more males included in these collections than females.

Within this sample, it was observed that fusion at this synchondrosis may still persist into late adolescence, while complete ectocranial fusion of the spheno-occipital synchondrosis was observed in all individuals above the age of 20 years. This therefore aids in the establishment of an upper age-at-death range in sub-adult individuals and a lower age-at-death range in young adult individuals. This established upper age limit differs from the ranges obtained in previous publications [9,10]. Nevertheless, earlier studies conducted by McKern and Stewart [7] and Redfield [8] have indicated that complete fusion of this skeletal developmental marker only occurs during the second decade of life. These findings are consistent with the findings in this study.

Many of the skeletal individuals donated to the Dart Collection are cadaver-derived. These individuals are either unclaimed or unidentified deceased individuals from government hospitals or individuals who donated their bodies via a bequeathment programme [21]. Noticeable factors that may attribute to the variation in the age ranges observed between population groups include the differences in geographic location, diet, environmental factors, and socio-economic and health status among these collections [6,10,38]. This is however an inherent bias that exists when using skeletal collections as reference samples [21].

All previous studies have specified that complete fusion of this synchondrosis first occurs in female individuals and then in male individuals, which is different to the findings in this study [9,10]. An initial explanation may be due to the lack of females aged 13–15 years, as well as the underrepresentation of sub-adult female individuals within the study sample. Thus the assumption that the spheno-occipital synchondrosis in South African males fuses before their female counterparts cannot be made. Alternative reasons for this finding may be attributed to the differing cultural treatment of and behaviour towards males and females as South Africa is still very much a patriarchal society [39,40]. Recent studies have suggested that there are no observable differences in the amount of stunting (a growth and nutritional indicator) between South African children. The greatest factors affecting height-for-age is the cultural group and social upbringing of these individuals [41]. Further research still needs to be conducted on how this phenomenon may affect skeletal growth in a South African setting.

5. Conclusion

An easily repeatable and reproducible three-stage scoring method was used in this study, which is critical when dealing with forensically relevant cases. The state of fusion of the spheno-occipital synchondrosis can be used as a skeletal developmental marker to assist in the estimation of age-at-death from a set of unknown skeletal remains. Future studies, which include a larger sample of South African Black females (particularly within the 10–15 year age range) and a more modern contemporary skeletal population, are recommended by the authors.

Conflict of interest

None declared.

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