ASSESSMENT OF RELIABILITY OF GREULICH AND PYLE (GP) METHOD FOR DETERMINATION OF AGE OF CHILDREN AT DEBRE MARKOS REFERRAL HOSPITAL, EAST GOJJAM ZONE

MSc THESIS SUBMITTED TO ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES, DEPARTMENT OF ANATOMY FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN ANATOMY.

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LIST OF ABBREVIATIONS/ACRONYMS

AAU……………………………………………..Addis Ababa University
CA……………………………………………..Chronological Age
DDX……………………………………………..Differential Diagnosis
DMRH…………………………………………..Debre Markos Referral Hospital
FDRE……………………………………………..Federal Democratic Republic of Ethiopia
GP………………………………………………Greulich and Pyle
RCPCH…………………………………………Royal College of Pediatrics and Child Health
SA……………………………………………….Skeletal Age
SES……………………………………………..Socioeconomic status
SPSS…………………………………………....Statistical Package for Social Science
TW2………………………………………………Tanner and Whitehouse (2nd ed.)
ABSTRACT

Introduction: The significance of chronological age has gained salience in response to the development of laws and policies that rely on age as a marker or boundary. Skeletal age, or bone age, is the most common measure for biological maturation of the growing human. Greulich & Pyle and Tanner-Whitehouse (TW2) are the most prevalently employed skeletal age techniques today. However, the applicability of the Greulich & Pyle standards to populations which differ from their reference population is often questioned.

Objectives: To assess reliability of Greulich and Pyle (GP) method for determination of age of children at Debre Markos referral hospital, East Gojjam Zone, Ethiopia.

Subjects and methods: Hospital based cross sectional study design was applied to children coming to Debre Markos referral hospital from May to October 2015 GC who are fulfilling inclusion criteria of the study. The data was analyzed using SPSS version 20 and medcalc version 15 software. Significance was set at α= 0.05.

Results: A total of 108 radiographs were analyzed. Mean skeletal age values are generally less than the corresponding chronological age. 64% of males and 63% of females sample CA were under estimated. The mean under-estimation was 11.8 months in the female sample and 8.7 months in the male sample. Although significant correlations were found to exist between SA and CA ($p = .000$), there was a significant difference between CA and SA (male, $p = 0.0196$; female, $p = 0.0029$). These differences occurred at 14, 19, 20, 21 and 22 years of age in females and 21 and 22 years of age in males. GP became inapplicable for the sample at 16 years for females and 16.5 years for males and later. Delay in skeletal maturation was observed in both sexes but the females in the sample are maturing earlier than the males.

Conclusion: The findings of this study suggest against the applicability of GP atlas and may not be directly applicable to an East Gojjam zone population.

Recommendation: Large scale researches should be planned and nationwide guideline and atlas which can easily be used throughout the country should be developed.

Key words: chronological age, bone age, Greulich & Pyle
1. INTRODUCTION

1.1 Measures of Age: Chronological, Biological, Social and Developmental Age

Chronological age is the actual age in years determined from the child’s birth date. It has gained salience in response to the development of laws and policies that rely on age as a marker or boundary. Biological age is defined by an individual’s present position with respect to his or her potential life span. An individual may be younger or older than his or her chronological age. Social age is defined by an individual’s roles, responsibilities and habits with respect to other members of the society of which he or she is a part. Psychological age is defined by the behavioral capacities of individuals to adapt to changing demands. It includes the use of adaptive capacities of memory, learning, intelligence, skills, feelings, motivations and emotions for exercising behavioral control and self-regulation (Settersten and Mayer, 1997).

The most common methods of carrying out assessments of chronological age include a range of medical, physical, and psycho-social assessments, as well as approaches to determine age that make use of existing local knowledge. Most experts agree that age assessment is not a determination of chronological age but an educated guess, and can only ever provide an indication of skeletal or developmental maturity from which conclusions about chronological age may be inferred (Crawley et al., 2007).

In the evaluation of physical development in children, variations in maturation rate are poorly described by chronological age. Measures of height, weight, and body mass index, although closely related to biological maturation, are not sufficiently accurate due to the wide variations in body size. Similarly, the large variations in dental development have prevented the use of dental age as an overall measure of maturation, and other clinically established techniques such as the age at menarche and determinations of sexual development using the Tanner classification are of limited value (Gilsanz and Ratib, 2005).

Skeletal age, or bone age, the most common measure for biological maturation of the growing human, derives from the examination of successive stages of skeletal development, as viewed in hand-wrist radiographs (Gilsanz and Ratib, 2005). Estimation of skeletal age is a means of assessing development and the process of skeletal maturation in children and adolescents for clinical or forensic purposes (Schmidt et al., 2007). These assessments involve comparing the
skeletal age of a test population against established standards (Dembetembe and Morris, 2012). It involves measuring and observing skeletal features. For this purpose various techniques are available, which are based on the understanding of the patterns of skeletal development (Dembetembe, 2010).

Greulich & Pyle, (1959) and Tanner-Whitehouse (TW2), (1975) are the most prevalently employed skeletal age techniques. Despite their differing theoretical approaches, both are based on the recognition of maturity indicators, i.e., changes in the radiographic appearance of the epiphyses of tubular bones from the earliest stages of ossification, expansion of epiphysis, capping until fusion with the diaphysis, or changes in flat bones until attainment of adult shape (Gilsanz and Ratib, 2005).

1.2 Bones of Hand

The hand is the region of the upper limb distal to the wrist joint. It is subdivided into three parts: the wrist (carpus); the metacarpus; and the digits (five fingers including the thumb) (Drake, 2009). The wrist, or carpus, is composed of eight carpal bones (carpals) arranged in proximal and distal rows of four (Moore, 2010).

From lateral to medial, the four bones in the proximal row of the carpus are:

- Scaphoid: a boat-shaped bone that articulates proximally with radius and has a prominent scaphoid tubercle; it is the largest bone in the proximal row.
- Lunate: a moon-shaped bone between the scaphoid and the triquetral bones; it articulates proximally with the radius and is broader anteriorly than posteriorly.
- Triquetrum: a pyramidal bone on the medial side of the carpus; it articulates proximally with the articular disc of the distal radio-ulnar joint.
- Pisiform a small, pea-shaped sesamoid bone that lies on the palmar surface of the Triquetrum.

From lateral to medial, the four bones in the distal row of carpus are:

- Trapezium: a four-sided bone on the lateral side of the carpus; it articulates with 1st and 2nd metacarpals, scaphoid, and trapezoid bones.
- Trapezoid: a wedge-shaped bone that resembles the trapezium; it articulates with 2nd metacarpal, trapezium, capitate, and scaphoid bones.
- Capitate: a head-shaped bone with a rounded extremity and the largest bone in the carpus; it articulates primarily with the 3rd metacarpal distally and with trapezoid, scaphoid, lunate, and hamate.
- Hamate: a wedge-shaped bone on the medial side of the hand; it articulates with 4th and 5th metacarpal, capitate, and triquetral bones; it has a distinctive hooked process, the hook of the hamate, that extends anteriorly.

There are five metacarpal bones, each of which has a base, a shaft, and a head. The first metacarpal bone of the thumb is the shortest and most mobile. It does not lie in the same plane as the others but occupies a more anterior position. The bases of the metacarpal bones articulate with the distal row of the carpal bones; the heads, which form the knuckles, articulate with the proximal phalanges. The shaft of each metacarpal bone is slightly concave forward and is triangular in transverse section. Its surfaces are posterior, lateral, and medial. There are three phalanges for each of the fingers but only two for the thumb (Snell, 2012).

![Radiograph of a normal hand and wrist joint (anteriorposterior view) showing bones of carpus, metacarpals and phalanges](image)

Figure 1: Radiograph of a normal hand and wrist joint (anteriorposterior view) showing bones of carpus, metacarpals and phalanges (Drake, 2009 page 1068)
1.3 Indicators of Skeletal Maturity in Children and Adolescents

Greulich and Pyle defined “maturity indicators” are the features which can be used as indicators of advancement toward maturity. These features were used because they “tend to occur regularly and in a definite and irreversible order” (Greulich & Pyle, 1959) and are visible using radiography techniques (Dembetembe, 2010). These features vary with the age of the child. In younger children the presence or absence of certain carpal or epiphyseal ossification centers are often pointers for the physician about the skeletal age of a child. In older children the shape of the epiphyses and the amount of fusion with the metaphysis are a good indicator of skeletal age (Spampinato, 1995).

In the majority of healthy children, there is an established sequence of ossification for the carpal, metacarpal and phalangeal bones, which is remarkably constant and the same for both sexes. Overall, the first ossification center to appear in hand and wrist radiographs is the capitate, and the last is, most often, the sesamoid of the adductor pollicis of the thumb (Gilsanz and Ratib, 2005).
The capitate begins to ossify in the second month, the hamate at the end of the third month, the triquetrum in the third year, the lunate, scaphoid, trapezium and trapezoid in the fourth year in females and fifth year in males (Standring, 2008). However, according to Srivastav (et al., 2004) the trapezium, trapezoid and scaphoid ossify in the females and males at the age of 8 to 9 and 9 to 10 years, respectively. The pisiform begins to ossify in the ninth or tenth year in females, and the twelfth year in males. The order varies according to sex, nutrition and, possibly, race (Standring, 2008).

The first epiphyseal center to appear is that of the distal radius, followed by those of the proximal phalanges, the metacarpals, the middle phalanges, the distal phalanges, and, finally, the ulna (Gilsanz and Ratib, 2005). According to Srivastav et al., (2004) the centre for lower end of radius ossify at the age of 1 to 2 and 2 to 3 years in the males and females, respectively, while ossification starts, at the lower end of ulna at 5 to 6 years of age in both sexes and at the age of 8 years and above it will present in all children. There are, however, two main exceptions to this sequence: the epiphysis of the distal phalanx of the thumb commonly appears at the same time as the epiphyses of the metacarpals, and the epiphysis of the middle phalanx of the fifth finger is frequently the last to ossify (Gilsanz and Ratib, 2005).

Each metacarpal ossifies from a primary centre for the shaft and a secondary centre which is in the base of the first metacarpal and in the heads of the other four. Ossification begins in the midshaft about the ninth week. Centres for the second to fifth metacarpal heads appear in that order in the second year in females, and between 1½ to 2½ years in males. They unite with the shafts about the 15th or 16th year in females, 18th or 19th in males. The first metacarpal base begins to ossify late in the second year in females, early in the third year in males, uniting before the 15th year in females and 17th in males. The thumb metacarpal ossifies like a phalanx (Standring, 2008).

Phalanges are ossified from a primary centre for the shaft and a proximal epiphyseal centre. Ossification begins prenatally in shafts as follows: distal phalanges in the eighth or ninth week, proximal phalanges in the tenth, middle phalanges in the 11th week or later. Epiphyseal centres appear in proximal phalanges early in the second year (females), and later in the same year (males), and in middle and distal phalanges in the second year (females), or third or fourth year (males). All epiphyses unite about the 15th to 16th year in females, and 17th to 18th year in males (Standring, 2008).
1.4 Greulich and Pyle Skeletal Age Estimation Standards (GP)

GP standards are the most widely used age estimation standards all over the world. These standards were derived from a longitudinal study carried out in 1931, in children of North European ancestry with high socioeconomic status who were born in the United States of America with sample population comprised of 1000 children (Greulich & Pyle, 1959).

In 1929 preliminary studies were started at the Western Reserve University School of Medicine in Ohio. These studies were the base for a long-term investigation of human growth and development. A large number of children of different ages were enrolled in the study. These children had radiographs taken of their left shoulder, elbow, hand, hip and knee. In the first postnatal year an examination was conducted every three months, from twelve months to five years they were examined each 6 months and annually thereafter. In total the study ran from 1931 until 1942 (Spampinato, 1995).

In 1937 an atlas, “Atlas of Skeletal Maturation of the Hand”, was published by Todd. This atlas was based on a part of the data collected in the study. Greulich and Pyle based their atlas partly on the atlas by Todd. Since their atlas was first published in 1950 they were able to use all the radiographs obtained in the original study. In total they had at their disposal from two to twenty-one hand radiographs made at successive examinations of each of 1000 children. In their method for each of these bones an elaborate description of its developmental stages is included. The descriptions are more a general guideline to the development of each bone in the hand than an instruction on how to rate bone (Spampinato, 1995).

In order to determine the skeletal age using the modified Greulich and Pyle method one uses the atlas that they have developed. The atlas is divided into two parts, one for the male client and one for the female client. Each part contains standard radiographic images of the left hand of children arranged by chronological age. The first step in an analysis is to compare the given radiograph with the image in the atlas that corresponds closest with the chronological age of the patient. Next one should compare it with adjacent images representing both younger and older children. When comparing the radiograph against an image in the atlas there are certain features, that vary with the age of the child, a physician should use as maturity indicator (Spampinato, 1995).
1.5 Statement of the Problem

The applicability of the Greulich–Pyle standards to populations which differ from their reference population is often questioned. This skepticism is because, by its nature, a standard is based on the results of a specific study performed on a specific population at a specified point in time (Loder et al., 1993). Current age estimation using bone development in the hand and wrist is based on the standards developed by Greulich and Pyle in 1959. However; these skeletal age estimation standards are based on a study of wrist radiographs of Euro-American children.

Differences in growth rate and maturation which were noted when the Greulich & Pyle standards were applied to contemporary populations, have been attributed to secular trends and differences in genetic origin, health status and economic status (Loder et al., 1993; Zhang et al., 2009). These factors influence growth and skeletal development, causing varying effects on populations, which thereby affect the direct applicability of the Greulich & Pyle standards to various populations (Dembetembe and Morris, 2012).

Comparative studies on various populations including those of African biological descent have shown that these standards tend to under- or over-estimate biological age in these populations. There have been many studies of the GP methods and standards, and most of these identify significant discrepancies and variations. For example; Ontell et al., (1996) conclude that using the GP standards to determine bone age must be done with reservations, particularly in black and Hispanic girls and in Asian and Hispanic boys in late childhood and adolescence when bone age may exceed chronologic age by 9 months to 11 months and 15 days.

Similarly, Mora et al., (2001) concluded that new standards are needed to make clinical decisions that require reliable bone ages and accurately represent a multiethnic pediatric population. Zhang et al., (2009) showed that ethnic and racial differences in growth patterns exist at certain ages; which are not recognized in the Greulich and Pyle atlas and atlas can be improved by considering the subject's ethnicity. For both the GP and TW2 methods, it has generally been accepted that bone maturity is affected by racial, socio-economic and nutritional factors (Crawley et al., 2007).

In addition, evidence suggests that children are developing earlier today than in the 1930’s when the method was developed, so that many subjects will be younger than the apparent age of the skeleton indicates, particularly in the case of girls (Save the Children Norway, 2006:02 as cited by
Smith & Brownlees, 2011). The Royal College of Pediatrics and Child Health (RCPCH) states that a boy’s skeleton today is fully developed at the age of 16 to 17 and girls at 15 to 16 years. This differs in both cases by two to three years from the GP atlas (Smith & Brownlees, 2011).

1.6 Literature Review

In 1895 Wilhelm Roentgen used the X-rays from a cathode ray tube to expose a photographic plate and produce the first radiographic exposure of his wife's hand. Over the past 30 years there has been a revolution in body imaging, which has been paralleled by developments in computer technology (Drake, 2009).

In plain radiography, the X-rays passing through the body are attenuated (reduced in energy) by the tissues. Those X-rays that pass through the tissues interact with the photographic film. In the body: air attenuates X-rays a little; fat attenuates X-rays more than air but less than water; and bone attenuates X-rays the most. These differences in attenuation result in differences in the level of exposure of the film. When the photographic film is developed, bone appears white and air appears dark on the film. As a result of the digital revolution, images can be obtained quickly and downloaded onto computer screens within seconds (Drake, 2009).

Plain radiography is useful to diagnose disease and injury such as pneumonia, heart failure, fractures, bone infections, arthritis, cancer, blockage of the bowel, and collapsed lung, etc. (O'Sullivan and Goergen, 2009). In addition to this it is also important for determination of skeletal age by using wrist and hand radiograph (Dembetembe, 2010).

Researches were done in different countries on peoples who are living in different socio-economic status with different ethnicity on the reliability of Greulich and Pyle method for determination of age.

In America

A study in America by Mora et al., (2001) showed significant differences in skeletal maturation between American children of European and African descent. They also reported significant differences in skeletal maturation after sexual maturity and advancement of the bone ages of postpubertal European American males by three months over those of African American males.
Similarly, Loder et al., (1993) found corresponding bone ages and chronologic ages for white girls of all ages. According to the study black girls were skeletally advanced, except during middle childhood. White boys were skeletally delayed during middle childhood and during late childhood, but they were advanced during the adolescent years. Black boys showed no difference except for the adolescent group, which was skeletally advanced. They concluded that the Greulich and Pyle atlas is not applicable to all children today, especially black girls.

Another study done by Zhang et al., (2009), on racial differences in growth patterns of children assessed on the basis of bone age, showed that bone age was significantly overestimated in Asian and Hispanic children than their African American and white peers in both sexes especially in girls aged 10–13 years and boys aged 11–15 years. They indicated that ethnic and racial differences in growth patterns exist at certain ages.

An investigation by Calfee et al., (2010), on American adolescents, demonstrated an advancement in mean skeletal age. Most of the girls had a more advanced skeletal age than chronological age. Females between the chronologic ages of 12 and 15 were demonstrated a discrepancy of at least 2 years between skeletal and chronologic age, while males demonstrated this potential throughout adolescence. Skeletal age and chronologic age were significantly correlated across the age spectrum.

In a Brazilian population bone and chronological ages were well correlated in both sexes. For the female sex, the evaluation of bone age was a mean of 8 months higher than the chronological age, whereas for the male sex, there was no significant difference. In general bone age was frequently higher than chronological age, particularly for the female sex. However, they proposed the method for estimating age and evaluating the patient's stage of development (De Sousa et al., 2015).

**In Asia**

Patil et al., (2012) in India revealed retardation of skeletal age by 0.7 years and by 0.33 years for males and females children respectively. According to the study male and female children were skeletally lag behind the American standard (GP Atlas) in all age groups except 12-13 years age group in which girls were accelerated by 0.22 years. At last they limited applicability of Greulich and Pyle atlas to Indian children of both sexes especially in middle and late childhood. Similarly, Rai et al., (2014) in India showed significant underestimation of SA for both sexes except in some
groups for boys who were slightly advanced. They also recognized early maturation of Indian females as compared to males. On the other hand, Mohamed et al., (2015) reported mild overestimation of skeletal age (SA) in girls (by 0.02 years) and underestimation of SA than CA by 0.23 years in boys South India.

In Taiwan Chiang et al., (2005) reported, underestimation of mean skeletal ages by 0.22 to 1.86 years in boys aged 2 to 12 years and overestimation of SA by 0.13 to 1.28 months in boys aged 13 to 18 years when compared to mean chronological ages. Mean skeletal ages were delayed by approximately 0.19 to 0.84 months when compared to mean chronological ages in girls aged 2 to 8 years and advanced by 0.18 to 1.48 months in girls aged 9 to 17 years. On the other hand a study in Korea on assessment of bone age in prepubertal healthy Korean children by Kim et al., (2015) showed underestimation of skeletal age in the whole group.

In Pakistan Awais, et al., (2014) found no statistically significant difference in mean CA and mean SA estimated by GP method for girls in a total of 283 children up to the age of 18 years. Another study in similar population by Zafar et al., (2010) on a total of 889 Hand-Wrist radiographs revealed a statistically significant mean differences of up to 13 months between SA and CA. In males, SA was advanced during early childhood, delayed during middle and late childhood and, again, advanced during adolescence. In females, the trend was similar except for advanced SA in late childhood.

On the other hand Mughal et al., (2014) reported significant underestimation of chronological age in Pakistani children between the ages of 54-113 months by 6.65 ± 13.47 months in females and 15.78 ± 12.83 months in males. They limit use of Greulich & Pyle Atlas in estimating chronological age for medico legal purposes but proposed for follow up use in growth disorder patients. Similar to the above researches done in Pakistan, Rikhasor et al., (1999) also showed retardation of skeletal age generally in all the bones of the hand. The majority of the male children showed complete maturity of the hand bones at the age of 18 years while most of the female children showed complete maturity of the hand bones at the age of 16 years.

In Europe

In Turkey reliability of GP method was assessed on 492 healthy children. The difference between chronological age and skeletal age determined by GP atlas was statistically significant for girls but
not for boys. There was a high correlation for girls and for boys. They reported advanced skeletal age almost for all ages for girls and in 15-17 years of age for boys (Büken et al., 2007). On the other hand Koc et al., (2001), on 225 Turkish boys, found retardation of mean skeletal ages than the mean chronological ages in the 7–13 years age groups and overestimation of skeletal ages in the 14–17 years age groups.

Hackman and Black (2013) in Scotland found no significance differences between chronological ages and estimated ages using the Greulich and Pyle atlas for both males and females. This study therefore supports the use of the Greulich and Pyle atlas when age estimating a child from this population. Similarly, Van Rijn et al., (2001) for Dutch Caucasian children found a strongly significant correlation between skeletal and chronological age. On average, chronological age preceded skeletal age by a small amount (1.7 months in girls and 3.3 months in boys). They proved applicability of Greulich and Pyle atlas in Dutch Caucasian children and adolescents. On the contrary Tisè et al., (2011) reported large margin of error in the determination of chronological age with the Greulich-Pyle method, particularly in the determination of the ages of 14 and 18 years in an Italian population sample with an age-range between 11 and 19 years. They prohibited usage of this method for determination of chronological age in forensic researches.

**In Africa**

According to a study in Malawi on 119 patients, the skeletal age was less than the chronological age in 85.6% patients. The mean discrepancy between the chronological and skeletal age was 20 months which was statistically significant; the mean age difference for girls was 18.6 months and for boys 20.7 months. They identified Poor nutrition and chronic diseases such as malaria and diarrhea to be contributing factors for the low skeletal age. However they failed to show any correlation between the reduction in body mass index in the sample and the degree of retardation of skeletal age (Lewis et al., 2002).

In South Africa Dembetembe (2010) done research on 163 individuals of African descents. He revealed underestimation of skeletal age (SA) than chronological age (CA) for a large proportion of the sample. For females the mean difference was 12 months and for the males it was about 6.8 months when using the CA as the “gold standard”. At ages 17 years for the male sample and 15 years for the female sample, GP became inapplicable. Only 23% of 19 years old individuals had undergone complete epiphyseal fusion and attained full skeletal maturity.
Similarly, Dembetembe and Morris (2012) reported underestimation of skeletal age for approximately 74% of the sample on average by 6 months and overestimation of skeletal age for 26% of the sample. Skeletal maturity occurred approximately 2.1 years later than Greulich and Pyle’s estimate of 19 years which indicates progression of skeletal maturation. They recommended formulation of skeletal age estimation standards specific to South African populations (Dembetembe and Morris 2012). In Ethiopia research on reliability of Greulich and Pyle method was not done, but the method is utilized for determination of age.

1.7 Significance of the Study

In the living age determination is the most important issue to the court and to the common citizens as well. It is essential to establish the identity of a person at the time of admission to schools, colleges, institutes, or while competing in sports tournaments at regional, state or national levels. It is also important while taking consent or in cases relating to juvenile offenders, rape, kidnapping, employment in government competency as a witness, attainment of majority, marriage, fixation of criminal responsibility, etc. (Bokariya et al., 2010).

In law the crime and punishment are entirely based on criminal responsibility and this is in turn dependent on the age of the person. In the modern society, crimes committed against children and by children are increasing. Thus pediatric age group has got significant medico-legal importance (Srivastav et al., 2004).

In Ethiopia, the Labor Proclamation prohibits the employment of a child under 14 years of age (art. 89 (2). Children between 14 and 18 years of age are categorized as young workers and the Proclamation provides that this category of children can work under strict conditions. According to the family Code of Ethiopia (art. 7 (1)), a man or woman who have not attained the full age of 18 years cannot contract marriage.

In Ethiopia, school certificates and birth certificates offered by religious institutions and municipalities are those usually used as evidence of the age of an individual which, however, are often liable to forgery. Hence, child offenders are required to pass through relevant medical examinations to ascertain their actual age (Tadele, 2000). For these purposes, normal standards of bone age should accurately represent chronological age. An over or under-estimation of bone age can result in the inappropriate diagnosis and treatment of growth disorders, unjust punishment, misplacement in a new school or undue advantage in competitive sports (Mughal et al., 2014). Accurate age estimation is also vital to ensure that local authorities fulfill their obligations in
providing support and services to vulnerable groups such as unaccompanied minors less than 18 years of age (Abbing, 2011).
2. OBJECTIVES

2.1 General Objective

- To assess reliability of Greulich and Pyle (GP) method for determination of age of children at Debre Markos referral hospital, East Gojjam Zone, Ethiopia

2.2 Specific Objectives

- To determine skeletal age of children using Greulich and Pyle method
- To assess whether the estimated SA correlates with the CA.
- To determine the timing of wrist epiphyseal closure
- To assess factors that affect bone maturity like age and sex and by reviewing different articles
3. METHODOLOGY

3.1 Study Design

Hospital based cross sectional study design was applied and conventional Plain radiographs of the hands and wrists were obtained from people that fit the study criteria. The atlas of Greulich and Pyle comprises a large series of standard anteroposterior radiographs of the hand. Each radiograph is assigned to a specific age in years and months and the patient’s skeletal age was determined by comparing his or her radiographs with the standard in the atlas for the appropriate gender.

3.2 Study Area and Time

Debre Markos, the capital of East Gojjam Administrative Zone is located in the north west of the capital city of the F.D.R.E. of Ethiopia, Addis Ababa at a distance of 300Kms and 265 kms to the capital of Amhara Nation Regional State Bahir Dar. Debre Markos Referral Hospital is found in this town. It was established in 1957 E.C by Emperor H/Selassie on the area of 30,020 m². Debre Markos Referral Hospital provides health service to more than 3.5 million populations. Currently about 100 health centers and four district hospitals are available in the catchment area of the referral hospital. There are 109 Nurse, 19 midwives, three health officer, 16 General practitioners, one emergency surgeon, six specialists including a radiologist, and radiology technicians. The hospital provides age determination service at the radiology unit. It is highly crowded during belig and bega seasons to fulfill the criteria of wedding ceremony asked by the police in their district to prevent early marriage.

The study was conducted from May, 2015 to October 30, 2015 GC

3.3 Source Population

All patients coming to radiology department for x-ray service

3.4 Study Population

All patients coming to the hospital at radiology unit for wrist and hand x-ray
3.5 Eligibility Criteria

3.5.1 Inclusion criteria

All patients 10 to 22 years of age having wrist radiography at inpatient ward, and all patients at outpatient department who were ordered for wrist and hand x-ray.

3.5.2 Exclusion criteria

Patients with congenital anomaly over the hand and wrist, medico-legal issues, radiographs with poor clarity, patients who didn’t know their chronological age and any severe fracture over the hand and wrist that hinder determination of age were excluded.

3.6 Sample Size and sampling method

Purposive sampling technique was applied. All children coming to the hospital and fulfill inclusion criteria of the study from May to October, 2015 were taken as total sample size. The minimum required sample size was calculated using medcalc by setting \( \alpha = 0.05 \) and correlation coefficient from a study in South Africa.

3.7 Variables of the Study

3.7.1 Independent variables

Sex, chronological age

3.7.2 Dependent variable

Skeletal age determined by Greulich & Pyle method

3.8 Data Collection Procedure

Chronological ages of the children were asked from their parents or taken from the patient card. The radiograph films were collected from the technicians and were viewed using a standard light box in manual x-ray machine. From the digital x-ray machine the images were automatically saved and seen from the screen at the department of Radiology at DMRH. The skeletal ages were determined using Greulich and Pyle atlas.
3.9 Data Quality Assurance

In order to maintain the quality of the data the investigator was trained by the professionals on how to determine age. Furthermore, the investigator worked under the supervision of the radiologist and other radiography technicians, along with the radiologist and the radiology technicians which were well experienced in determination of age. The films that were clear and not vague were selected. In order to avoid bias chronological ages of the patient is blinded while SA was determined using Greulich and Pyle method.

3.10 Data Analysis

Data analysis was performed using the SPSS statistical software (version 20) and medcalc version 15. Tests of frequency was done to evaluate overall skeletal age and chronological age as well as means at each age group. A correlation between the chronological age and the GP skeletal age was performed using a non-parametric Spearman Rank Analysis. The strength of agreement between the chronological age and skeletal ages were tested using the Bland and Altman plot (Bland and Altman, 1986). The results of all the analyses were summarized and then compared to the results from other populations on which the GP method was applied. Significance was set at $\alpha = 0.05$.

3.11 Ethical Consideration

Ethical clearance was obtained from IRB of the college of Health Sciences, Addis Ababa University.
4. RESULTS

4.1 General Distribution of the Sample

A total of 108 children, male 65 (60.2%) and female 43 (39.8%), from 10 to 22 years were included in this study. Samples were grouped according to GP atlas and the general distribution is shown in table 1.01. The left hand and wrist was studied. Individuals represented by both left and right hand radiographs were counted as single individuals and age estimation analysis was performed on the left hand unless it was too damaged or the radiographs were unclear or incomplete.

4.1.1 Age and Sex Distribution of the Sample

Table 1.01 below shows the age distribution of the study sample. The age groups used follow those used in the Greulich and Pyle Radiographic Atlas of Skeletal Development of the Hand and Wrist which has whole and half year category. However, in the current sample there is no half year category since there are no subjects that fall in the category. Patient’s age was approximated to the whole numbers. For example if the CA of the patient is 10.6 years, it is taken as 11 years of age; if it is 10.3 years, taken as 10 years and the like.

From table 1.01, it is evident that there were notably more male than female research subjects. $\chi^2$ Test for Proportions was done. It was found that the proportion of males compared to females, was significant at the 0.05 level ($\chi^2=103.9$; degrees of freedom =1; $P < 0.0001$) meaning that there were significantly more males than females.
Using a Histogram the recorded data in the above table can be evaluated visually whether the data are distributed symmetrical, Normal or Gaussian or whether the distribution is asymmetrical or skewed for both male and female samples. The youngest and oldest age was 10 and 22 for both males and females. As seen from the histogram 22 years age group for both males and females has highest samples.
Figure 3: Age distribution histogram of the male sample. Youngest age was 10 years and the oldest age was 22 years.

Figure 4: Age distribution histogram of the female sample. Youngest age was 10 years and the oldest age was 22 years.
4.2 Skeletal Age Analysis

4.2.1 Difference between Skeletal Age and Chronological Age for the Whole Hand

Once the radiographs had been aged using the GP method, the estimated Skeletal Age (SA) was compared to the known chronological age (CA). This was done for the samples grouped according to the SA as determined by the GP method which records a maximum age of 18 years for females and 19 years for males using the bones of the hand and wrist. Skeletal ages for the known CA’s of each of the SA single year categories were averaged and the results are presented below in Table 1.02 for males and 1.03 for females.

Table 1.02 Chronological Age Grouped by Skeletal Age for males

<table>
<thead>
<tr>
<th>Chronological age (CA)</th>
<th>Skeletal age (SA)</th>
<th>Difference</th>
<th>CA-SA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>9.875</td>
<td>1.9311</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>10.000</td>
<td>0.0000</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>11.000</td>
<td>*</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
<td>12.250</td>
<td>0.3536</td>
</tr>
<tr>
<td>14</td>
<td>3</td>
<td>14.333</td>
<td>1.0408</td>
</tr>
<tr>
<td>15</td>
<td>3</td>
<td>15.667</td>
<td>2.0817</td>
</tr>
<tr>
<td>16</td>
<td>7</td>
<td>16.571</td>
<td>0.7868</td>
</tr>
<tr>
<td>17</td>
<td>4</td>
<td>16.750</td>
<td>1.7078</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>16.938</td>
<td>1.4745</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>18.500</td>
<td>0.7071</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>18.727</td>
<td>0.6467</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>19.000</td>
<td>0.0000</td>
</tr>
<tr>
<td>22</td>
<td>14</td>
<td>19.000</td>
<td>0.0000</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>16.746</td>
<td>2.9845</td>
</tr>
</tbody>
</table>

*Values not available due to single observation; CV Coefficient of Variation; SD Standard Deviation
Table 1.03 Chronological Age Grouped by Skeletal Age for females

<table>
<thead>
<tr>
<th>Females Chronological age (CA)</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>Difference CA-SA</th>
<th>CA-SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1</td>
<td>8.00</td>
<td>*</td>
<td>*</td>
<td>2.0</td>
<td>24</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>10.00</td>
<td>*</td>
<td>*</td>
<td>1.0</td>
<td>12</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>10.00</td>
<td>*</td>
<td>*</td>
<td>2.0</td>
<td>24.0</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
<td>13.00</td>
<td>*</td>
<td>*</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>18.00</td>
<td>*</td>
<td>*</td>
<td>-4.0</td>
<td>-48</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td>13.50</td>
<td>*</td>
<td>*</td>
<td>1.5</td>
<td>18</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>16.00</td>
<td>1.00</td>
<td>6.2%</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>16.20</td>
<td>1.0954</td>
<td>7.0%</td>
<td>0.8</td>
<td>9.6</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>17.71</td>
<td>0.756</td>
<td>4.5%</td>
<td>0.3</td>
<td>3.6</td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>18.00</td>
<td>0.00</td>
<td>0.0%</td>
<td>1.0</td>
<td>12.0</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>18.00</td>
<td>0.00</td>
<td>0.0%</td>
<td>2.0</td>
<td>24.0</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>18.00</td>
<td>*</td>
<td>*</td>
<td>3.0</td>
<td>36.0</td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>18.00</td>
<td>0.00</td>
<td>0.0%</td>
<td>4.0</td>
<td>48.0</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>16.94</td>
<td>2.215</td>
<td>13.7%</td>
<td>0.98</td>
<td>11.8</td>
</tr>
</tbody>
</table>

*Values not available due to single observation; CV Coefficient of Variation; SD Standard Deviation

Table 1.02 and 1.03 above showed that the mean SA values are generally less than the corresponding CA. Thus the GP method is underestimating age for all age groups except 14, 15, 16 years in the male sample and 14 years in the female sample. The GP method was over-estimating age by between 4.0 and 6.0 months and underestimating age ranged from 1.5 to 12.7 months between the ages of 10 and 19 years for the males. On the other hand it overestimates the age of female subject by 4 years, but must be seen with caution since the female sample size is small in this case 1, and underestimating by between 3.6 and 24 months (seen with caution for the small sample size) in the female sample between the ages of 10 and 18 years.

However, after CA of 18 years and 19 years in the female and male samples respectively, the GP method became inaccurate by 1 year in females and 1.3 years in males and this under estimation
increased as CA increased. The mean under-estimation was 11.8 months in the female sample and 8.7 months in the male sample.

Furthermore table 1.02 and 1.03 show the measures of variability, indicated by the coefficient of variation (CV). It is relatively consistent for the female sample, showing higher variability at age between 16 to 18. (Taking note of the small sample sizes in each age group). In the male sample age group 10 shows the highest variability then from 14 to 18 years of age but the older age groups show a more consistent level of variation in the average SA. A comparison of the variability of the two samples confirms that the females show more consistency (13.7 %) in the under estimation of SA than the male sample (18.0%).

Significant correlations were found to exist between Skeletal Age (SA) estimated using the GP method, and the Chronological Age (CA). The correlation coefficients as measures of association were recorded using the Spearman Rank Order Correlation; $r_s=0.912, p = .000$ for males and $r_s=0.761, p = .000$ for females. These values are significant at $\alpha$ level 0.05 and show strong, positive correlation between CA and SA which means both are measuring an increase in age. But the SA underestimates the CA and this difference is consistent in the female sample.

The presence or absence of significant difference between the CA and SA as determined by the GP age estimation method was assessed by Mann-Whitney test. For the male sample ($U = 1616.00, p = 0.0196$) was recorded indicating that there was a significant difference between CA and SA which demonstrates that there is a mismatch between SA and CA. The female sample also recorded a significant difference between CA and SA ($U = 593.50, p = 0.0029$).

A multiple comparison performed for each age group using the Kruskal-Wallis test showed that the significant age differences occurred at 14,19, 20, 21 and 22 years of age in females ($P = 0.000325$), and 21 and 22 years of age in males($P < 0.000001$). This is expected as the GP skeletal age estimation method identifies the attainment of maturity as age 19 years for males and 18 years for female and does not continue beyond this age.

Further analyses to investigate the extent of the difference in SA and CA were performed.

Figures 5 for males and figure 6 for females plot below, show the magnitude of the difference in years between CA and SA for individuals in the sample. The scatter plots show an over- or under-estimation of age according to the GP skeletal age estimation. Most of the points in the scatter plot
lies above y=0 line, the line by which all the points would lie if SA was accurately estimating CA at all ages. Points falling below the y=0 line signify an over-estimation of chronological age by using the GP skeletal age estimation. 83% of males and 68% of females sample CA was underestimated.

Figure 5: Scatter plot illustrating the difference between CA and SA for the male data against CA.

R^2 = 0.83
Figure 6: Scatter plot illustrating the difference between CA and SA for the female data against CA.

The regression line in the above figures increase in gradient as CA age group increase which implies that the difference between CA and SA increase. This shows that GP skeletal age estimation method becomes less accurate in older individuals in both female and male samples.

Bland-Altman (1986) plot was utilized to assess the agreement between the two methods as shown in Figure 7 for males and Figure 8 for females below, plotting the average of the two measurements (SA and CA) against the difference between them (CA-SA). These plots show the number of individuals for whom the difference between CA and the GP skeletal age estimate differed by more than 2 standard deviations.

Bland Altman Plot of males (Figure 7) below shows a mean difference of 1.12 years between chronological age and bone age. 95% of the points lie between -1.9 years and +4.1 years. For females (Figure 8) a mean difference was 1.6 years and 95% of the points lie between -1.9 years and +5.0 years.
Figure 7: Bland-Altman (1986) Plot showing the difference between SA and CA for the male sample plotted against the average age given by the two methods.

Figure 8: Bland-Altman (1986) Plot showing the difference between SA and CA for the female sample plotted against the average age given by the two methods.
The above figures also show the point at which GP became inapplicable for the sample at 16 years for females and 16.5 years for males characterized by the increased number of estimates falling outside of the two standard deviation limits.

4.2.2 Termination of Growth and Attainment of Maturity

GP conclude that male individuals have reached full skeletal maturity at 19 years of age and females at 18 years age, as characterized by complete epiphyseal fusion in the hand and wrist. Table 1.04 below shows individuals that had reached full skeletal maturity before 19 years of age for males and 18 years of age for females and those who don’t reach full maturity.

Table 1.04 Percentage of Skeletally Mature Individuals per Chronological Age Group

<table>
<thead>
<tr>
<th>Males</th>
<th>CA</th>
<th>N</th>
<th>Individuals With Complete Epiphyseal Fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>17</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>20</td>
<td>11</td>
<td>9</td>
<td>82</td>
</tr>
<tr>
<td>21</td>
<td>4</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>22</td>
<td>14</td>
<td>14</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Females</th>
<th>CA</th>
<th>N</th>
<th>Individuals With Complete Epiphyseal Fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>18</td>
<td>7</td>
<td>6</td>
<td>85.7</td>
</tr>
<tr>
<td>19</td>
<td>6</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>21</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>22</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

As shown in the table above, those individuals who are 19 chronologically in male sample are reached in skeletal maturity in 50 % of the group. The remaining individuals had not yet attained maturity and are below 19 years of age which indicates delay in skeletal maturation. At 20 years of age 82% of individuals reach maturity and 100% at 21 years of age. The females in the sample are maturing earlier than the males. At 18 years of age 85.7% individuals are reached full maturity; at 19 being 100%.
4.2.3 Sample Radiographs and Matching Procedures

The following figures (Figures 9 to 12) present illustrations of the process of matching a radiograph to the closest representative in the GP Atlas.

In order to determine the skeletal age using the modified Greulich and Pyle method one uses the atlas that they have developed. The sex of the patient is one of the most important pieces of information, because females develop quicker than males. The atlas is divided into two parts, one for the male patients and one for the female patients. Each part contains standard radiographic images of the left hand of children ordered by chronological age. The first step in an analysis is to compare the given radiograph with the image in the atlas that corresponds closest with the chronological age of the patient. Next one should compare it with adjacent images representing both younger and older children.

When comparing the radiograph against an image in the atlas there are certain features a physician should use as maturity indicators. These features vary with the age of the child. In younger children the presence or absence of certain carpal or epiphyseal ossification centers are often pointers for the physician about the skeletal age of a child. In older children the shape of the epiphyses and the amount of fusion with the metaphysis is a good indicator of skeletal age. Once the atlas image that most resembles the radiograph is found the physician should conduct a more detailed examination of the individual bones and epiphyses. When the physician is sure that the matching radiograph has been found, she/he can find the skeletal age printed at the top of the page.
Figure 9: Left hand radiograph of male individual with CA of 18 years of age (A) whose age is underestimated as compared with GP standard radiograph for 18 years old male (B) but comparable with 15 years old male of GP standard radiograph (C). Note that Epiphyseal fusion is still active in individual with radiograph (A) but more advanced in individual with radiograph (B) who has the same CA with (A). On the other hand radiograph “A” is better represented by radiograph “C” who has lower CA (i.e.15 years).
Figure 10: Left hand radiograph of male individual with CA of 17 years of age (A) whose age is overestimated as compared with GP standard radiograph for 17 years old male (B), but comparable with 19 years old male of GP standard radiograph (C). Note that Epiphyseal fusion is completed in individual with radiograph (A) but still active in individual with radiograph (B) who has the same CA with (A) and fused in radiograph (C). On the other hand Radiograph “A” is better represented by radiograph “C” who has higher CA (i.e. 19 years).
Figure 11: Left hand radiograph of female individual with CA of 12 years of age (A) whose age is underestimated as compared with GP standard radiograph for 12 years old female (B), but comparable with 10 years old female of GP standard radiograph (C). Note that capping and epiphyseal fusion is still active in both individuals with radiograph (A) and (B) but more advanced in individual with radiograph (B) who has the same CA with (A). Similarly the gap between epiphysis and diaphysis of the ulna is wide in individual with radiograph (A) as compared to radiograph (B). On the other hand radiograph “A” is better represented by radiograph “C” who has lower CA (i.e. 10 years).
Figure 12: Right hand radiograph of male individual with CA of 20 years of age (A) whose age is underestimated as compared with GP standard radiograph for 19 years old male (B), but comparable with 17 years old male of GP standard radiograph (C). Note that full epiphyseal closure is expected at 19 years of age according to GP standard radiographic atlas. On the other hand epiphyseal fusion is still active in individual with radiograph (A) but has fused in individual with radiograph (B) who has the lower CA than (A). Hence radiograph “A” is better represented by radiograph “C” who has lower CA (i.e. 17 years).
5. DISCUSSION

The estimation of skeletal age is a means of assessing development and the process of skeletal maturation in children and adolescents for clinical or forensic purposes (Schmidt et al., 2007). These assessments involve comparing the skeletal age of a test population against established standards (Dembetembe and Morris, 2012). Greulich & Pyle and Tanner-Whitehouse (TW2) are the most prevalently employed skeletal age techniques today (Gilsanz and Ratib, 2005).

The applicability of the GP standards to modern day populations has been tested over the past few decades. Various international studies have reported different results regarding the applicability of the Greulich & Pyle Atlas for estimation of chronological age.

Differences in growth rate and maturation which were noted when the Greulich & Pyle standards were applied to contemporary populations, have been attributed to secular trends and differences in genetic origin, health status and economic status (Loder et al., 1993; Zhang et al., 2009). These factors influence growth and skeletal development, causing varying effects on different populations, which thereby affect the direct applicability of the Greulich & Pyle standards to various populations (Dembetembe and Morris, 2012).

The present study examined the applicability of the Greulich and Pyle method of age determination in the context of Ethiopia, East Gojjam zone and had found the results mentioned in part 4 of the thesis.

The total sample size was 108; male (65) and female (43) ranged from 10 to 22 years of age. This was done in order to ascertain the earliest age at which full skeletal maturity characterized by complete epiphyseal closure could be observed and the latest age at which incomplete epiphyseal fusion was observed. According to Dembetembe, (2010) the oldest age at which non-fusion was observed was 21 years in the male sample and earliest age at which complete fusion was observed was 14.6 years in the female sample. In India epiphyseal fusion was not completed at 20 years of age in both sexes and the earliest age by which completed epiphyseal fusion seen was 17 years of age (Patil et al., 2011). In the current sample the earliest age at which complete fusion was observed was 14 years in the female sample and the oldest age at which non-fusion was observed was 20 years in the male sample.
Significant correlations were found to exist between Skeletal Age (SA) estimated using the GP method, and the Chronological Age (CA) (Büken et al., 2007; Dembetembe, 2010; Dembetembe and Morris, 2012; Mughal et al., 2014; de Sousa Dantas et al., 2015 and Mohamed et al., 2015). Similar to the above findings, significant correlations exist in the current sample and confirmed a positive linear correlation which indicates that CA varies as SA varies.

5.1 Difference between Chronological Age and Skeletal Age

Lewis et al., (2002); Dembetembe, (2010); Dembetembe and Morris, (2012) and Kim et al., (2015) showed that skeletal ages determined using the Greulich & Pyle method were lower than the chronological ages for a large proportion of the sample both in males and females and the method underestimates CA. Although the difference is not significant, Paxton et al., (2013) in Australia reported overall underestimation of BA by 2.2 months; 1.5 months for females and 3.7 months for males.

Patil et al., (2012) concluded that the method was not applicable to the Indian children of both sexes and records a difference of 0.7 years for males and 0.33 years for females. Similarly, Mohamed et al., (2015) records a difference of 0.02 years for girls and 0.23 years for boys. Zafar et al., (2010) reported a mean differences of up to 13 months in Pakistan. While Lewis et al., (2002) reported a discrepancy of up to 20 months in a Malawian sample. Ghotbi et al., (2010), in Iran showed a difference of 2 to 21.6 months in boys and 6.6 to 11.9 months in girls aged from 7-14 years and concluded the possibility of different pattern of skeletal maturation among Kurdish children than the reference.

In South Africa, Braude et al., (2007), showed that the average difference between the chronological and skeletal ages was 0.5 years with a maximum under prediction of 2.4 years and maximum over prediction of 0.9 years among male adolescents. Dembetembe, (2010); Dembetembe and Morris, (2012), reported a significant difference. For females the mean difference was 12 months and for the males it was about 6.8 months. The difference was highly significant for the male sample but not for female sample. This significant difference was occurred at 19, 20 and 21 years of age.

In this study overall results showed that skeletal ages determined using the Greulich & Pyle method were lower than the chronological ages for a large proportion of the sample both in males and
females and the method underestimates CA (i.e. SA is less than CA). The mean difference was 11.8 months for females and 8.7 months for males; SA was generally less than CA. The difference is significant for both male and female sample and this significant difference occurred at 14, 19, 20, 21 and 22 years of age in females ($P = 0.000325$), and 21 and 22 years of age in males ($P < 0.000001$).

Contrary to the above findings, a study by Hackman and Black, (2013) in Scotland; Awais et al., (2014) in Pakistan; Paxton et al., (2013) in Australia and Van Rijn et al., (2001) in Dutch found no significance difference between chronological age and estimated age using the Greulich and Pyle atlas for both males and females. This may be due differences in biological origin, health status and economic status (Loder et al., 1993; Zhang et al., 2009 and Crawly et al., 2007).

Bland-Altman (1986) plots for the male and female samples measures the agreement between the two methods by plotting the average of the two measurements (SA and CA) against the difference between them (CA-SA). These plots show the number of individuals for whom the difference between CA and the GP skeletal age estimate differed by more than 2 standard deviations. Van Rijn et al., (2009) published that at ages 17 years for the male sample and 15 years for the female sample, GP became inapplicable. These points were at age 16.5 for the male sample and 15.5 for the female sample in South Africa population (Dembetembe, 2010; Dembetembe and Morris, 2012). For the current sample of Ethiopia, these points are at age of 16.5 for the male sample and 16 for the female sample as characterized by the increased number of estimates falling outside of the two standard deviation limits.

5.2 Delayed Skeletal Maturation

The underestimation of chronological age by the Greulich & Pyle method by 11.8 months in the female sample and 8.7 months in the male sample reported here can be interpreted as a delay in skeletal maturation in present subjects compared with Greulich and Pyle’s reference population.

According to Büken et al., (2007) 78.3% of chronologically 17 years of age and 94.1% of 18 years of age in females attained full skeletal maturity. Where as in males 77.8% of chronologically 18 years of age and 95.7% of chronologically 19 years of age had reached full skeletal maturity among Turkish children. In India 87.5 % of chronologically 18-19 years of age and 100 % of 19-20 years of age in females showed complete epiphyseal fusion, while in males 96.5% of chronologically 19-
20 years of age completed epiphyseal closure (Patel et al., 2011). Dembetembe, (2010); Dembetembe and Morris, (2012) reported that individuals who were both chronologically and skeletally 19 years old, represent only 23% of the 19-year age group. Therefore 77% of 19-year-old individuals had not yet attained skeletal maturity in males and in females 50% of chronologically 17 and 67% of 18 has attained maturity. They also reported that more than three quarters of each sample CA was under estimated with recording scores of 78.1% and 74.0% for the female and male samples, respectively. On the contrary Büken et al., (2007) reported overestimation of skeletal age than the chronological age in 68% of cases.

In the current sample 50% of chronologically 19 years of age males reached full skeletal maturity. The remaining individuals had not yet attained maturity and are below 19 years of age which indicates delay in skeletal maturation. At 20 years of age 82% of individuals reached full skeletal maturity. The females in the sample were matured earlier than the males. At 18 years of age 85.7% individuals are reached full maturity at 19 being 100%. In this study 64% of males and 63% of females sample CA was under-estimated by GP method (1959) in comparison to 18% and 19% for male and female samples, respectively for which CA was over estimated.

5.3 Possible Causes of Delay in Skeletal Development

In the present study there were underestimation of chronological age by 11.8 months in the female sample and 8.7 months in the male sample. Individuals that are chronologically 19 years and above for males and 18 years and above for females that were showing active epiphyseal closure delay in epiphyseal closure (Greulich & Pyle, 1959). This may appear to be due to continuation of cartilage growth leading to increase in bone size or cease in the development of cartilage leading to delay in bone growth. However the first possibility is most unlikely because, when the bone age is delayed, growth typically continues longer than normal but are growing at a low-normal rate (Rogol, 2014).

On the other hand, a cease in pace of development of the bone is most likely. This leads to a marked height discrepancy during the early teenage years compared with their peers, but is followed by catch-up growth when they do enter puberty resulting in normal adult stature (Rogol, 2014). Delayed in skeletal development (bone age) does not necessary mean a diagnosis for short stature. For example; in familial type of short stature bone age is consistent with chronological age (Rogol, 2014). Delayed bone age is associated with constitutional delay, malnutrition, hypothyroidism, growth hormone deficiency etc. (Harding, 2015).
Differences in growth rate and maturation which were noted when the Greulich–Pyle standards were applied to contemporary populations, have been attributed to secular trends and differences in genetic origin, health status and economic status (Loder et al., 1993; Zhang et al., 2009; Crawley et al., 2007). These factors influence growth and skeletal development, causing varying effects on different populations, which thereby affect the direct applicability of the Greulich–Pyle standards to various populations (Dembetembe and Morris, 2012).

There is a large number of studies in the context of ethnic and racial impact, mainly conducted on populations of European Caucasians, North American Caucasians, other North American ethnic groups (including Hispanics and Blacks), different Mongoloid and Caucasian populations from Asia, and some incomplete studies on central and southern African Black populations.

The most recent studies in Europe appear to indicate that maturation rates for European Caucasians are close to those described in the GP systems or are slightly delayed or advanced in relation to them. Hackman & Black (2013) in Scotland and Van Rijn et al., (2001) for Dutch Caucasian found no significance difference between chronological age and estimated age using the Greulich and Pyle atlas for both males and females. However, in an Italian population sample with an age-range between 11 and 19 years, there was a large margin of error in the determination of chronological age with the Greulich-Pyle method, particularly in the determination of the ages of 14 and 18 years, so that this method was not indicated for helping to determine chronological age in forensic researches (Tisè et al., 2011).

In the U.S., studies indicate that Caucasian subjects either closely fit the GP and TW2 standards or often show a certain advance in maturation. For example; Loder et al., (1993) found corresponding bone ages and chronologic ages for white girls of all ages; Calfee et al., (2010) demonstrated an advancement in mean skeletal age among American adolescents.

There are also comparative studies on different races in America. Zhang et al., (2009) had studied racial differences in skeletal growth patterns of Asian, African American, white, and Hispanic children in the United States. They reported significant overestimation of bone age and early maturation of bone in Asian and Hispanic children than their African American and white peers in both male and females. Similarly, Ontell et al., (1996) showed overestimation of skeletal age in late childhood and adolescence in black and Hispanic females, Asian and Hispanic males. They advised to use GP with caution when examining populations of varying ethnicity.
On the other hand Mora et al., (2001) reported a significant differences in skeletal maturation between American children of European and African descent. According to the study the bone ages of postpubertal European American males were advanced by three months over those of African American males.


Despite the differences in the skeletal development rates of populations of varying ethnicities and biological origins reported by the above authors, Schmeling et al., (2000) reported that the apparent retardation in skeletal development in the non-European populations was influenced mainly by low socio-economic status and that the genetics played a lesser role. This is supported by Low, (1972) that noticed delayed in skeletal maturity of boys in China with low socioeconomic status.

Rikhasor et al., (1999) in Pakistan showed underestimation of bone age in males from 1 year to 15 year and 2 year to 13 years in females. They postulated malnutrition, ill health or other environmental factors as a cause. Similarly, Chaumoitre et al., (2010) in Morocco investigated the relationship of socioeconomic status (SES) and body mass index (BMI) with skeletal maturation in children. They reported Global maturation delay in the sample and confirmed a significant relationship between skeletal maturation and BMI z-score among children but not for boys.

On the contrary, Freitas et al., (2004) evaluated variation in maturity associated with SES in Madeira and concluded that skeletal maturity was not related to SES in youths. Similar to Freitas et al., (2004), Mohamed et al., (2015) in India reported underestimation of skeletal age in boys though they maintained factors like biological ancestry, nutrition and socioeconomic status similar for all samples.

Different studies on applicability of Greulich and Pyle method of age determination can be summarized in the following table
TABLE 1.05 Comparison of Results of Relevant Age Estimation Studies.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Population Studied</th>
<th>Sample Size</th>
<th>Age Range (years)</th>
<th>Methodology</th>
<th>Reference Population</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loder et al., (1993)</td>
<td>American (black and white)</td>
<td>841</td>
<td>0-18</td>
<td>Retrospective</td>
<td>GP</td>
<td>No difference for white girls of all ages, Black girls were skeletally advanced except during middle childhood; White boys were skeletally delayed. Black boys showed no difference except for the adolescent group, which was skeletally advanced.</td>
</tr>
<tr>
<td>Mora et al., (2001)</td>
<td>North American (African American and European American)</td>
<td>534</td>
<td>0-19</td>
<td>Cross sectional New radiographs</td>
<td>GP</td>
<td>Significant differences in skeletal maturation between American children of European and African descent. The bone ages of postpubertal EA males were advanced by three months over those of AA males</td>
</tr>
<tr>
<td>van Rijn et al., (2001)</td>
<td>Dutch Caucasian</td>
<td>572</td>
<td>5-19.9</td>
<td>New radiograph</td>
<td>GP</td>
<td>GP is applicable</td>
</tr>
<tr>
<td>Lewis et al., (2002).</td>
<td>Malawian</td>
<td>139</td>
<td>1-28</td>
<td>Retrospective study using pre-existing radiographs and height weight data</td>
<td>GP</td>
<td>The mean discrepancy between the chronological and skeletal age was 20 months, statistically significant GP under estimated age</td>
</tr>
<tr>
<td>Chiang et al., (2005).</td>
<td>Taiwan</td>
<td>370</td>
<td>0-17.9</td>
<td>Retrospective</td>
<td>GP</td>
<td>Shows variation in age groups. It underestimates chronological age in some age group and overestimates in some age group</td>
</tr>
<tr>
<td>Büken et al., (2007).</td>
<td>Turkey</td>
<td>492</td>
<td>11-19</td>
<td>On new radiograph cross sectional</td>
<td>GP</td>
<td>SA was advanced almost for all ages and delayed at 11-14 ages for girls ; SA were significantly advanced in 15-17 ages but then delayed in 18-19 years of age (0.02-0.48) for boys</td>
</tr>
<tr>
<td>Zhang et al., (2009).</td>
<td>Asian, Hispanic and African American children</td>
<td>1390</td>
<td>0-18</td>
<td>New radiographs</td>
<td>GP</td>
<td>In both male and female subjects SA was overestimated in Asian and Hispanic children and mature sooner than their African American and white peers.</td>
</tr>
<tr>
<td>Ghotbi et al., (2010)</td>
<td>Kurdish children</td>
<td>228</td>
<td>7-14</td>
<td>Cross sectional, new radiograph</td>
<td></td>
<td>There is a discrepancy of more than one year between the chronological age and the measured bone age in some age groups GP Atlas is not completely applicable to Kurdish children</td>
</tr>
<tr>
<td>Dembetembe, (2010).</td>
<td>South Africa</td>
<td>163</td>
<td>13-22</td>
<td>Retrospective</td>
<td>GP</td>
<td>Individuals revealed that skeletal age (SA) as determined using the GP Atlas was less than the chronological age (CA) for a large proportion of the sample. GP under estimated age</td>
</tr>
<tr>
<td>Zafar et al., (2010).</td>
<td>Pakistan</td>
<td>889</td>
<td></td>
<td></td>
<td>GP</td>
<td>Mean differences of up to 13 months were observed between SA and CA which was statistically significant in all groups except adolescent males.</td>
</tr>
</tbody>
</table>
Calfee et al., (2010). American adolescents 138 12-18 Cohort GP Current American adolescents are significantly more mature by skeletal age, as determined by the Greulich and Pyle method, than their chronological age would suggest. The skeletal ages of females are most likely to markedly exceed chronologic age between the ages of 12-15 years.

Patil et al., (2012) India 375 0 to 19 GP Male and female children skeletally lag

Suri et al., (2013). Burlington Growth Centre 572 9 to 18 Retrospective cross sectional GP Showed wide ranges and distributions of differences at each yearly age group during the growth period from 9 to 18 years, even when mean differences were small.

Awais et al., (2014) Pakistan 283 0-18 Cross sectional GP GP was reliable in estimating SA in girls, but unable to accurately assess SA in boys

Mansourvar et al., (2014) Asian, African/ American, Caucasian and Hispanic 184 1-18 Cross sectional GP GP atlas is reliable for Caucasian and Hispanic ethnic groups but not for other ethnic groups for different ranges of age, especially in the sample of the male African/American group from 8 years to 15 years and Asian during childhood.

Kim et al., (2015). Korea 212 7-12 Cross sectional GP, TW3 and KS bone age measured by GP method tended to younger than chronological age in the whole group and the group of boys

6. LIMITATION OF THE STUDY

There are some limitations of this study that should be considered before drawing any implications from its findings.

- The fact that no study was conducted so far in Ethiopia on this topic; no literature was available to discuss in national context.
- Although assessment of, ethnicity as a factor, in determination of age was planned, different ethnic groups couldn’t be found in the area.
- Equal sample size for males and females was not achieved.
- Though most of the peoples today know their chronological age; even are celebrating their birth day, birth certificates was not found.
7. CONCLUSION

The findings of this study suggest against the applicability of GP atlas and may not be directly applicable to the East Gojjam zone population. Mean SA values are generally less than the corresponding CA. 64% of males and 63% of females sample CA was under estimated. The mean under-estimation was 11.8 months in the female sample and 8.7 months in the male sample.

Although significant correlations were found to exist between Skeletal Age (SA) estimated using the GP method, and the Chronological Age (CA), there was a significant difference between CA and SA. This differences occurred at 14, 19, 20, 21 and 22 years of age in females and 21 and 22 years of age in males. GP became inapplicable for the sample at 16 years for females and 16.5 years for males and later.

50%, 82% and 100% of male individuals reached full skeletal maturity at 19, 20 and 21 years of age, respectively. In females 85.7% of the individuals reached full skeletal maturity at 18 years of age and at 19 being 100%. Delay in skeletal maturation was observed in both sexes but the females in the sample are maturing earlier than the males. This findings are congruent with other studies done in different countries especially in Africa and Asia.
8. RECOMMENDATION

The following recommendations could be made from the present study:

- Large scale researches should be planned and nationwide guideline and atlas which can easily be used throughout the country should be developed.

- Bone age calculated by Greulich & Pyle Atlas may not be used for estimating chronological age of children in Ethiopia especially Gojjam in situations where high accuracy is required (e.g. medico-legal cases). But the method may be used with a cautious approach for follow up of a patient.

- Further studies should be conducted to investigate the reasons for delayed in skeletal maturation of the current subjects as compared to Euro-American from which Greulich & and Pyle atlases were developed.
9. REFERENCES


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