ORIGINAL ARTICLE

CORRELATION OF SKELETAL AGE USING GREULICH AND PYLE ATLAS WITH CHRONOLOGICAL AGE IN BALOCHISTAN POPULATION

Palwasha Gul, Imran Masoud, Kaukab Javed, Nazia Dildar, Tanzila Parveen, Samia Rauf

Department of Radiology, Combined Military Hospital (CMH), Quetta, Pakistan.

PJR July - September 2018; 28(3): 200-208

ABSTRACT

INTRODUCTION: Determination of bone age is a clinical procedure used in pediatric radiology to evaluate skeletal maturity on the basis of bone growth in the left hand and wrist, as seen on a radiograph. The determination of skeletal maturity (also referred to as bone age) plays an important role in the diagnosis and treatment of endocrinologic abnormalities and growth disorders in children. In clinical practice, the method most commonly used to assess bone age is matching of a radiograph of the left hand and wrist with the Greulich and Pyle atlas. Aim of this study was to determine skeletal age by Greulich-Pyle atlas of the hand-wrist and its difference from chronological age in males and females aged 0-19. **MATERIAL AND METHODS:** This was a cross sectional study conducted at Department of Diagnostic Radiology, Combined Military Hospital, for a period of six months (01.01.2017 - 30.06.2017). Hackman study had a total of 406 left hand / wrist radiographs (157 females and 249 males) were age assessed using GP atlas. Analysis showed a strong correlation between CA and estimated age (females R^2 = 0.939, males R^2 = 0.940) with a p<0.001. R^2 = 0.945, CI=95%, then the estimated sample size will be at least n=50. We used non-probability consecutive sampling. **RESULTS:** Total of 100 patients with left or right hand-wrist x-rays were included (50 males, 50 females). The age groups were broken down into year cohorts and compared with GP atlas. The males showed over-estimation of age in 11-19 years. It was 0.15-3.76 months. Females showed overestimation of 0.84-2.43 in 1-15 years age. Males had underestimation of age at 0-10 years of 3.17-3.31 months. Females age showed underestimation of 0.9-11.6 months. T-test was applied (males t=0.25, females t=1.09).Analysis showed a strong correlation between CA and SA with R^2 = 0.98 and regression coefficient=0.96 with p<0.05. The difference between SA and CA is less than ± 2 SD. **CONCLUSION:** The study showed that the GP atlas can be used for age estimation and applied to Balochistan population but would recommend that any analysis takes into account the potential for over- and under-aging as shown in the study.

Key words: Greulich-Pyle Atlas, Chronological age, Skeletal Age, Tanner-Whitehouse

Introduction

Determination of bone age is a clinical procedure used in pediatric radiology to evaluate skeletal maturity on the basis of bone growth in the left hand and wrist, as seen on a radiograph. The determination of skeletal maturity (also referred to as bone age) plays an important role in the diagnosis and treatment of endocrinologic abnormalities and growth disorders in children. In clinical practice, the method most commonly used to assess bone age is matching of

Correspondence : Dr. Palwasha Gul,
Department of Radiology,
Combined Military Hospital (CMH),
Quetta, Pakistan.
Email: gul_1123@yahoo.com

Submitted 27 April 2018, Accepted 18 May 2018

a radiograph of the left hand and wrist with the Greulich and Pyle atlas.[1]

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. These assessments involve comparing the skeletal age of a test population against established standards. The most commonly used standards are those published in the Radiographic atlas of skeletal development of the hand and wrist by Greulich and Pyle.[2]

Skeletal maturity is the only developmental indicator that remains at hand from birth to adulthood. Skeletal Age (SA) affords an excellent estimate of the growth, development, health and nutrition of a child. This makes accurate SA assessment invaluable for diagnostic and therapeutic decisions in paediatrics, endocrinology, orthopaedics and orthodontics. In medico-legal cases, SA is regarded the most convincing estimate of age; its estimation assumes critical bearing for ascertaining criminal liability, especially in developing countries where properly maintained birth records are often lacking.[3]

Literature shows that the atlas consistently over-ages females by 0.20 and 5.73 months. For males, there is a varying tendency to over-age and under age individuals. The atlas consistently over-ages boys from 1.62 months and 11.05 months.[4]

There is evidence that skeletal maturation may vary between different ethnic and socioeconomic groups of children or among children living in various geographical locations.[5]

In this study, we investigated as to what extent the GP method is adequate for determining the skeletal age of children in contrast to chronological age in our setup. Balochistan is a province where births are not routinely registered. This study will help in age assessment not only to ensure that children and juveniles are identified and treated appropriately but also from forensic point of view to prevent adult criminals from getting juvenile rights and vice versa. Unfortunately in Pakistan the studies done are limited to the time of appearance and fusion of ossification centres.[6]

The present cross sectional study will provide preliminary observations on bone maturity in Balochistan where no such information is at present available.

Greulich Pyle is the most frequently used as it is easy and fast to use. In this method a radiograph of the left hand is compared with a series of radiographs of known age. These standards were compiled from research conducted on normal white children in the United States during the 1930s. The applicability of the GP method beyond populations dissimilar to its own has been shown to be variable.[7]

OPERATIONAL DEFINITION:

Skeletal age: It is the assessment of skeletal maturity undertaken on the basis of degree of maturation of epiphysis (at birth, epiphyses usually present at the ends of the long bones are not present. As a child grows the epiphyses appear and become calcified and fuses which appears on the x-rays) to normal age-related standards advised by GP atlas.

Chronological age: Age of a person measured in years, months, and days from the date the person was born. It would be investigated from the guardian/ parent by asking for birth certificate.

Material and Methods

Study Design: Cross sectional study

Setting: Diagnostic Radiology department of Combined Military Hospital, Quetta.

Duration of Study: Six months (01.01.2017-30.06.2017)

Sample size: The sample size was calculated by using WHO sample size calculator. Hackman[4] study had a total of 406 left hand / wrist radiographs (157 females and 249 males) were age assessed using GP atlas. Analysis showed a strong correlation between CA and estimated age (females R^2 = 0.939, males R^2 = 0.940) with a $p<0.001$. R^2 = 0.935, CI=95%, then the estimated sample size was at least n=50.

Sample Technique: Non-probability Consecutive sampling

Sample Collection:

The inclusion criteria: The participants of the study were both boys and girls having chronological age less than 19 years determined by their birth certificate, visiting to the radiology department of Balochistan origin.

The exclusion criteria: (1) Those with gigantism (macrodactyle), Turner's syndrome (short 4th meta-carpal), Klinefelter's syndrome (angulation at an interphalangeal joint affecting 5th digit), Marfan's syndrome (spider fingers), Hurler's disease (pointing of proximal metacarpals), Achondroplasia (short hands with stubby fingers, separation between the middle and ring fingers). (2) Patients of other than Balochistan origin and refugees. These are excluded to remove bias from the results.

DATA COLLECTION PROCEDURE: A total of 100 participants of Balochistan including 50 boys and 50 girls were included in the study. The participants were the children of 0 to 19 years of age visiting outpatients and Diagnostic Radiology department of Combined Military Hospital, Quetta fulfilling the inclusion criteria. Approval of the study was taken from the hospital ethical committee. Informed written consent was taken from the guardian of the children before including in the study. The whole study sample was divided into 4 subgroups (0 to 4 years, 5 to 9 years, 10 to 14 years and 15 to 19 years). The implementation of the Patient administration system (PAS) was prospectively selected for inclusion. Radiographs were unrevealed, with the omission of information on age and sex of the individual. The estimated bone ages from plain Radiography and hand wrist x-ray results interpreted by use of Greulich pyle atlas were correlated by the radiologist for estimation of skeletal age. The Mean differences between SA and CA was documented and differences in months were assessed in all age groups between males and females using Greulich pyle atlas as a reference. The atlas of Greulich and Pyle, offering standards of skeletal development of white North American children, was used as reference and the accuracy was assessed in contemporary sample from Balochistan population. The results acquired were annotate on the ossification process in girls and in boys and also documented which gender was earlier than other. In general, the highest agreement between bone age and chronologic age was for the ossification centers of the distal epiphysis of the ulna and radius and for those of the metacarpal bones. Observer was blinded to information on CA at the time of assessment of the radiographs to avoid bias of the results. The relationship between CA and SA was examined for each one year age group, to determine the applicability of the method to Balochistan population. Basic demographic information including name, age, gender, CA and estimated age by Greulich pyle Atlas was recorded on a predesigned Proforma.

DATA ANALYSIS PROCEDURE: Data analysis was performed using the (SPSS v 15) statistical software. Descriptive statistics were used to calculate mean and standard deviation for Quantitative variables like chronological age, skeletal age and difference in chronological age and skeletal age. The difference between CA and SA was calculated by subtracting the CA from the SA. A negative value indicated that the individual had been under aged, and a positive value indicated an individual who had been over aged using the GP atlas. Frequency with percentages were presented for Qualitative variables like gender and age groups.
Spearman correlations coefficient was applied to correlation chronologic age to skeletal age in both genders. P-value $\leq$ 0.05 was considered significant.

Results

This is a prospective study involving total of 100 children aged between 0 to 19 years old. There are 50 males and 50 female X- rays of hand and wrist. All the X-rays were studied and a skeletal age was assigned to them according to Greulich and Pyle (1959) atlas. Both right and left hand X-rays were included. In total left hand X-rays were 45 and right hand X-rays were 55. The question of concern is whether the degree of skeletal development is similar in each of the two hands or not. This is explained by study conducted by Dreizen et al. (1957) who found that the difference between skeletal ages of two hands exceeded 3 months in only 13% of the children and that it was more than 6 months in only 1.5% of the children which showed that the difference was negligible and insignificant. Therefore demarcation on the basis of right and left hand wrist X-rays was not done and were taken together.
The number of X-rays in both sexes were almost the same in all age groups except 16-20 years, where

only two x-rays were included (Fig. 1). X-rays from both right and left hands were included (Fig. 2).

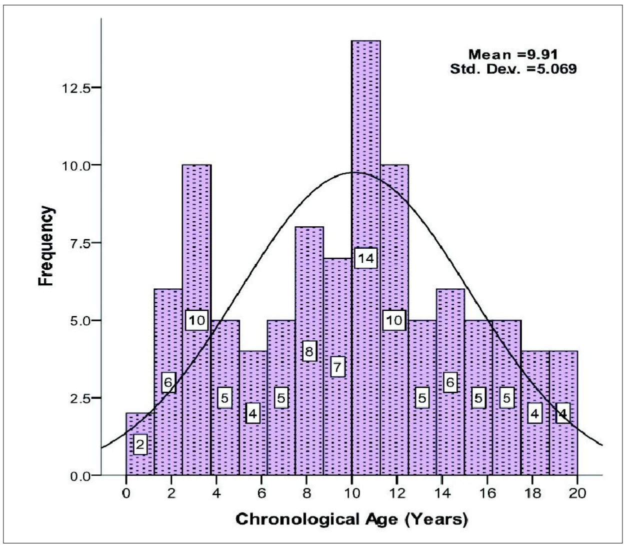

Figure 1: Histogram of age of the patients (n=100)

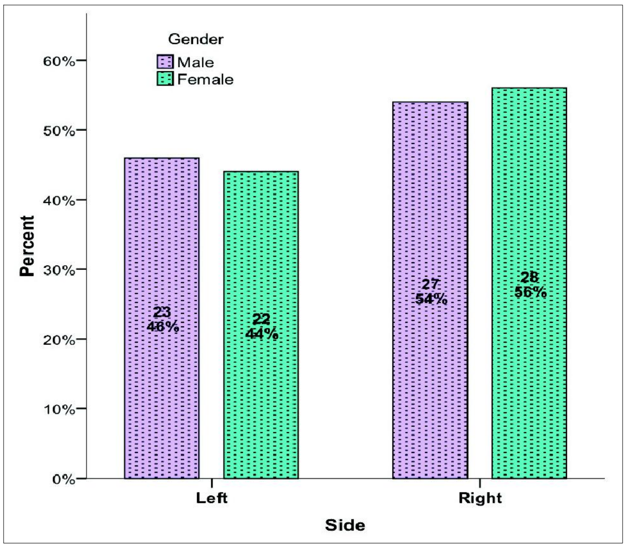

Figure 2: Site with respect to gender (n=100)

The (Tab. 1) represents the comparison of CA and SA. An under-aging of 0.25 months for females and an over-aging of 1.14 months for males is shown overall. The t-test value for males is -0.25 and for females is 1.09.

(Tab. 2) summarizes the mean, range and the standard deviation of the residual age (months) for females in four divided groups. Differences between chronological and skeletal age were explored further by calculating mean differences in months separately

Gender	Chronological Age	Skeletal Age	Difference	t-test	P-Value
Male (n=50)	130.16 ± 66.95	130.41 ± 64.83	-0.25 ± 7.12	-0.25	0.80
Female (n=50)	108.62 ± 53.69	107.48 ± 54.80	1.14 ± 7.36	1.09	0.27

Paired t test applied

Table 1: Comparison of chronological age and skeletal age for male and female

Age Groups	N	Mean Residual difference	Range of residual in months	Standard deviation
0-5 Years	13	0.84	9.7, -5.4	4.07
6-10 years	15	2.43	15.7, -11.6	8.43
11 -15 Years	20	2.06	13.4, -0.9	6.29
16 – 19 years	2	-15.77	-22.8, -8.8	9.91

Table 2: Mean, range and (SD) of residual age (months) in female by 5 years age groups

on the basis of year cohort for both male and female subjects. For females, age was found to be consistently over-estimated in all four age groups. With regard to these over-estimations, these are found to vary from a minimum of 0.84 months to a maximum of 15.77 months. The under-estimations were found to vary from a minimum of 0.9 months to a maximum of 11.6 months. For males, over-estimations were found among those aged 11-20 years. These over-estimations were found to vary from a minimum of 0.15 months to a maximum of 3.76 months. Additionally, under-estimations were found among those aged 0-10 years. These under-estimations were found to vary from a minimum of 3.17 months to a maximum of 3.31 months. (Fig. 3) presents box plots and whiskers of actual age discrepancy male subjects.

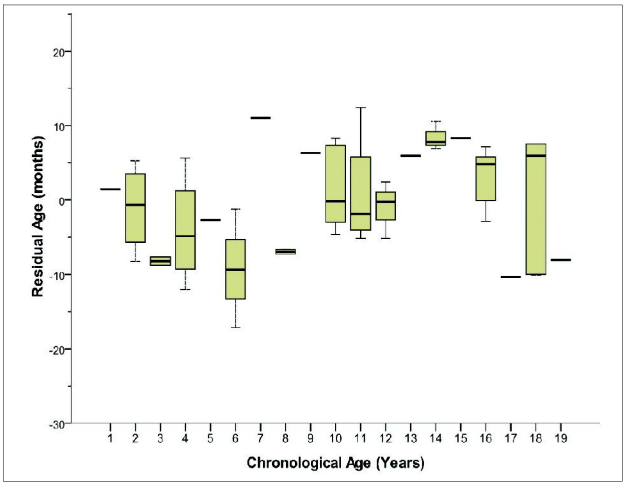

Figure 3: Box and whiskers plot between chronological age in years and residual age in months for male patients (n=50)

It further confirm the results just presented in that age, discrepancy tends to centre around zero in both cases. Specifically, while the following two figures indicate some variation in prediction accuracy on the basis of subject age (i.e., variability is higher among female subjects aged 8, 9 and 11, while variability is higher among male subjects aged 4, 6 and 11).
The scatter plot indicated in (Fig. 4) represent a positive linear association between skeletal and chronological age for females subjects. Pearson's correlation coefficient was also conducted separately for female and male subjects between skeletal and chronological age. A strong correlation coefficient of 0.99 was found between skeletal and chronological age for both female and male subjects ($p < 0.05$).

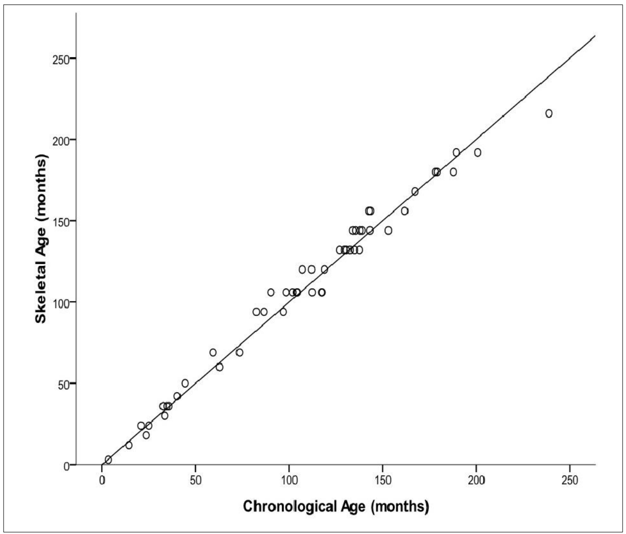

Figure 4: Scatter plot between chronological age and skeletal age in months for female patients (n=50)

Linear regression was conducted which included skeletal age as the predictor and chronological age as the dependent variable. (Tab. 3) presents the results of these analyses, with separate regressions conducted on the basis of subject sex. The R^2 value for both females and males was found to be 0.98 ($p < 0.05$).

Gender	Correlation Coefficient	Regression Coefficient	R^2 Value	t-value	P-value
Male	0.995	0.96	0.989	66.84	0.0005
Female	0.991	1.011	0.982	51.24	0.0005

Dependent variable is chronological age and predictor is skeletal age.
Spearman and Pearson correlation coefficient are same

Table 3: Correlation and regression coefficient for both male and female for the age estimation with chronological and skeletal age

Following this, sample paired t-tests were conducted comparing chronological and skeletal age on the basis of subject sex. No statistical significance was achieved in regards to females however statistical significance was indicated with respect to male subjects ($p < 0.05$).
The results of the Pearson's correlations were being conducted. Strong correlations were found in all cases, with statistical significance also being found in all four cases ($p < 0.05$). Additionally, the 95% confidence intervals calculated for each of these four correlations also indicated positive correlations being present across these confidence intervals.

Discussion

In the last few decade scholars debate on the effect of puberty, nutrition, socioeconomic status, geographic location and ethnicity on skeletal maturation and skeletal age in both sexes. Age has been indicated as one of the most essential factors for establishment of the identity of an individual and determination of the growth factors related to the individual. The question raised over past decades in multiple studies conducted worldwide and is to whether the Greulich and Pyle standards set in 1959 are still applicable on current population and also whether the population skeletal maturation used at that time is different to current population. To answer these questions a multi-centre longitudinal study needs to be undertaken[8,9] including children of all races and ethnicity and taking into account different geographic locations. As this is a set standard and is used in important fields of life like medical field for assessing growth abnormalities and response to treatments, forensic, criminal and legal cases for trafficking victims and illegal immigrants. it is important that this technique should be continuously checked and investigated especially in developing world.
The current study is based on contemporary Balochistan population and assessing the applicability of Greulich and Pyle (1959) atlas on our population. In current study the maximum numbers of subjects were seen in 2-4 and 8-12 year old. High number of subject are seen in same age group in the study of Nahid et al. (2009) and is further supported by Mora et al.

(2001) and Griffith et al. (2007) who also had adequate number of X-rays in their pre pubertal groups. The reason could be as this particular age group is involved more in sports and recreational activities and so more presentations to Emergency Department with trauma.

It is important to have an adequate number of X-rays in pubertal age groups which is seen in our study and study of Beunen et al,[10] Murata et al,[11] Loder et al[8] and Hackman et al[4] also indicated the importance of having good number of participants in these age groups so that skeletal difference might come out clearly because of the expected variation brought out due to the pubertal change. Smaller number of X-rays is seen in age group 0 in both sexes. The reason might be the avoidance of unnecessary X-ray exposure due to its hazardous effects. Small number in early childhood years was seen in study of Ontell et al,[9] Hackman et al[4] and Paxton et al.[6]

Greulich and Pyle (1959) did not consider different ethnicities when standardizing the atlas for skeletal development of children and adolescents. Sutow[12] mentioned in the study about the effects of ethnicity causing retardation of skeletal age. Sutow[12] discussed racial differences to be among the causes of retardation of skeletal maturation among the Japanese children. Sutow[12] sought a sample of 898 children of the Japanese origin from San Francisco, California. These were aged between 5 and 18 years. Boys between 13 and 17 and girls between 10 and 17 showed comparative acceleration. This is as contrasted to Greulich and Pyle 5-7 year age group (Greulich and Pyle, 1959). Sutow[12] attributed the trend to the fact that the children from the Japanese descendent did not have favourable environmental and nutritional conditions.

The current study showed that the difference between skeletal and chronological in more than 95% sample is less than the ± 2 SD advised in the Greulich and Pyle (1959) atlas, with few outliers. According to the assumption made by Greulich and Pyle (1959) atlas "that one standard deviation above and below the skeletal age corresponding to the child's chronological age will include approximately two third of white children in this country who are adequately nourished and in good health; that two standard deviations will include about 90% of all such children, in addition to some whose skeletal development is delayed as result of disease of nutritional inadequacy; and that a difference of more than two standard deviations above or below the mean would make it highly probable that the child is abnormally advanced or delayed". Van Rijn et al[13] and Moradi et al[14] found that the difference between chronological and skeletal age was less than the ± 2 SD that Greulich and Pyle (1959) mentioned supporting the findings in current study.

For the current study, the difference between skeletal and chronological age is found to be less when compared to the previously attempted studies conducted worldwide. The standard deviation for the females in entire sample was 4.07-6.29 (considering the maximum groups) and 5.71-7.91 for males whereas the range noted in the Greulich and Pyle (1959) atlas for females was between 0.72 to 7.31 and in males was 0.6 to 13.0 months as per Brush foundation study and 2.7 to 11.2 and 2.1 to 15.4 for females and males as per their own standards derived from study in Boston. These results support the clinical applicability of the Greulich and Pyle (1959) method for Balochistan children and adolescents as long as the standard deviations are under ± 2 SD as suggested by Greulich and Pyle (1959). The gap between chronological and skeletal age though small for most of the data but the outliers in the current study should be considered when assigning age in forensic use due to the fact that in clinical situations the date of birth is provided but in forensic situations the history and background for age estimation is not known in most of the cases. The gap can still be justified with the fact that maturation changes with respect to time. According to Himes,[15] there is 0.22-0.66 years increase in skeletal maturation per year/decade with changes seen more in ages of fusion.

All the outliers were included in the study as it is true representation of the data. The reason for the few outliers may be due to the systematic error on the assessor's part for example a bone can be over-aged or under-aged than its actual value causing variations in the results. However it should be noted that it is random and could increase the variation when being considered on its own but balancing the error when taken in group. The variability in assessing the skeletal age is lower for the carpal bones as compared to other bones of the hand-wrist.[16,17] This could increase the changeability of the skeletal ages noticed for these bones. The other possible reason could be that at the time of presenting to Emergency Department with trauma there might have been an underlying pathology which was not diagnosed or was not apparent at that point of time.

The results in (Tab. 5) showed that the correlation coefficient between skeletal and chronological age of both female and male subjects is 0.99. Büken et al18 found high correlation with r = 0.88 for girls and r = 0.90 for males. The study indicated for both females and males a R^2 value 0.98 and regression coefficient of 0.96 for males and 1.011 for females ($p < 0.05$) indicated by the observer. The variation is high at 98% indicating that the relationship between the chronological and skeletal age is positive and highly correlated. Hackman et al[4] showed an R2 value of 0.93 and 0.94 with regression coefficient of 0.89 and 0.97 for females and males ($p < 0.001$) respectively. This study also supports that chronological age is closely related to skeletal age. The coefficient obtained in both studies was comparatively lower than the current sample study.

It is noted that skeletal maturation is sex specific and different in males and females respectively.[19] In the current study, both sexes show different pattern of results yet still within the range of Greulich and Pyle (1959) standard deviations. The results were separate for females and males. This study found out that there has been a general mixed pattern for both under-aging and over-aging in both sexes. The over-aging ranged from 0.84 to 2.43 months from the age 1 to 15 years in females, showing that girls are over-estimated. This over-estimation in ages 1 to 15 years can be due to the fact that girls in general are skeletally 4-6 weeks more mature than boys as suggested by Marshall et al.[19] Also it can be due to the fact that girls reach the stages of skeletal maturation earlier in comparison to the male population. For males over-aging ranged from 0.15 to 3.76 months in age groups 11 to 19 years. The pattern for males has been consistent for under-aging from 0 to 10 years old. This pattern of under-aging in males before puberty and over-aging after puberty is seen in other studies conducted by Ontell et al,[9] Koc et al,[20] Büken et al,[18] Nahid et al,[21] Zafar et al[3] and Hackman et al.[4] In both males and females19 years showed an over-aging and could be due to the end of the atlas series at this age group. In comparison, the Hackman et al[4] study showed a general mixed pattern of under-aging and over-aging. It was seen that for females over-aging occurred at 1, 3, 6, 7 and 9-16 years old with over-aging from 0.20 to 5.73 months.

In the current study this general pattern of under-estimation of males prior to puberty and over-estimation after puberty and in female's over-estimation before and somewhat under-estimation after puberty is seen. The differences amongst girls and boys can also be due to the effect of puberty and genetics on skeletal maturation. Girls enter puberty before boys with ages 11 years versus 13 years[22] and complete pubertal stages earlier in comparison to boys who complete the puberty stages later (onset of growth spurt occurs later and slow progression to completion) and continue to show advanced bone age even after the girls have achieved skeletal maturity. According to Carpenter and Lester,[23] after puberty skeletal age advanced in both females and males while in current sample, only in case of males this finding is supported, however in females it is supported only by age groups 12 years. In addition these differences can be due to influence of genetics on skeletal maturation and its predisposition can range from 41 to 71%.[24,25] Also this could be the result of the change of population and geographical location different from the one on which Greulich and Pyle (1959) study was based. According to Schmeling et al26 economic progress and modernization in medicine have an impact on the ossification of hand and wrist resulting in high ossification rates.

When four age cohorts were taken for both males and females it showed that in males an under-age of 12 months and an over-age of 5.6 months was seen in ages 0 to 5 years and in females similar group was under-aged by 5.4 months, with SD value of 4.07 for females and 5.73 for males, which is small compared to other age groups. The age groups 16 to 19 years in females and 6 to 10 in males show a larger SD value compared to other age groups (9.91 for females and 7.91 for males). The smaller range of under and over-aging in age group 0 to 5 years is also seen in study conducted by Hackman et al[4] who appeared to have an under-age of 15 months and an over-age of 14 months in same age group. Studies conducted by Loder et al,[8] Ontell et al,[9] Rikhasor et al[7] and Mora et al[27] also agreed to smaller difference between chronological age and skeletal age in young age groups. This smaller difference noted in these younger cohorts could be due to the small gap of 3 months given in taking X-rays under 1 year of age followed by a gap of 3 and 6 months until the age of 5 years. However a gap of 12 months was given thereafter which could be contributing to the larger differences seen in older age groups.

The reliability and the accuracy of the Greulich and Pyle atlas (1959) method is not 100% because it brings out the mixed patterns of the difference between the chronological and skeletal age among both sexes. Tisè et al[28] in their study where they assessed the applicability of the Greulich and Pyle (1959)[29] method in the assessment of the age for the Italian sample in the legal practice found out that the method was not 100% reliable in determine the true age of the study participants. Tisè et al[28] indicated that the true ages of the subjects were either over-estimated or under-estimated and therefore posing a challenge to the legal practice. But one should be aware of the fact that in order to prove 100% efficacy of the method of age assessment ideal situation is required where there is no deviation of the individuals from the population upon which the method of assessing the skeletal age is based. Considering these points our study could not prove the efficacy to be 100% assuming the different gene pool and also the environmental and geographic factors were different to the one in 1930. However 98% of the variation in the chronological age being interpreted on the basis of skeletal age for both subjects ($p < 0.05$) was achieved in our study.

Conclusion

The application of the Greulich and Pyle (1959) atlas technique in this study to assess the skeletal and chronological age among the study subjects was undertaken using the cross-sectional study design.

The main objective was to quantify the difference, if any between the ages; it was found that this error was small. The study found that there is strong correlation between skeletal age and chronological age among the study participants after applying the Greulich and Pyle (1959) atlas method. Current study has not found any evidence against the applicability of Greulich and Pyle (1959) atlas on the basis of changes in specific bony maturation patterns despite of variability in maturation rates. The results suggest that application of the Greulich and Pyle (1959) atlas method should be encouraged among the assessors and researchers who want to determine the relationship between the chronological age and the skeletal age in the field of medicine. The current study supports the use of Greulich and Pyle (1959) method in the age estimation among Balochistan children as long as differences highlighted in our study are taken in to consideration.

References

1. Zhang A, Sayre JW, Vachon L, Liu BJ, Huang HK. Racial differences in growth patterns of children assessed on the basis of bone age. Radiol. 2009; **251(1):** 228-235.

2. Dembetembe KA, Morris AG. Is Greulich-Pyle age estimation applicable for determining maturation in male Africans. S Afr J Sci. 2012; **108(9-10):** 1-6.

3. Zafar AM, Nadeem M, Husen Y, Ahmad MN. An appraisal of Greulich-Pyle atlas for skeletal age assessment in Pakistan. J Pak Med Assoc. 2010; **60(7):** 552-5.

4. Hackman L, Black S. The reliability of the Greulich and Pyle atlas when applied to a modern Scottish population. J Forensic Sci.2013; **58(1):** 114-9.

5. Ghotbi N, Afkhamzadeh A, Salehi MG, Elyasi A. Assessment of bone age in Kurdish children in Iran. Pak J Med Sci. 2010; **26(1):** 36-9.

6. Paxton ML, Lamont AC, Stillwell AP. The reliability of the Greulich-Pyle method in bone age determination among Australian children. J Med Radiat Oncol. 2013; **57(1):** 21-4.

7. Rikhasor RM, Qureshi AM, Rathi SL, Channa NA. Skeletal maturity in Pakistani children. J Anat, 1999; **195:** 305-8.

8. Loder RT, Estle DT, Morrison K, Eggleston D, Fish DN, Greenfield ML, Guire KE. Applicability of the

Greulich and Pyle skeletal age standards to Black and White children of today. Am J Dis Child. 1993; **147:** 1329-33.

9. Ontell FK, Ivanovic M, Ablin DS, Barlow TW. Bone age in children of diverse ethnicity. Am J Roentgenol. 1996; **167:** 1395-8.

10. Beunen G, Lefevre J, Ostyn M, Renson R, Simons J, Van Gerven D. Skeletal maturity in Belgian youths assessed by the Tanner-Whitehouse method (TW2). Ann Hum Biol.1990; **17:** 355-76.

11. Murata M. Characteristics of pubertal growth in Japanese children from the standpoint of skeletal growth. Acta Paediatr Jpn. 1992; **34:** 240-2.

12. Sutow WW. Skeletal maturation in healthy Japanese children, 6 to 19 years of age. Comparison with skeletal maturation in American children. Hiroshima. J Med Sci. 1953; **2:** 181-93.

13. Van Rijn RR, Lequin MH, Robben SG, Hop WC, Kuijk C. Is the Greulich and Pyle Atlas still valid for Dutch Caucasian children today? Paediatr Radiol. 2001; **31:** 748-52.

14. Moradi M, Sirous M, Morovatti P. The reliability of skeletal age determination in an Iranian Sample using Greulich and Pyle method. Forensic Sci Int. 2012; **223(1-3):** 372.e1-4.

15. Himes JH. An early hand-wrist atlas and its implications for secular change in bone age. Ann Hum Biol. 1984; **11:** 71-5.

16. Thompson GW, Popovich F, Luks E. Sexual dimorphism in hand and wrist ossification. Growth. 1973; **37:** 1-11.

17. Roche AF, Davila GH. The reliability of assessments of individual hand-wrist bones. Hum Biol. 1976; **48:** 585-97.

18. Büken B, Safak AA, Yazici B, Büken E, Mayda AS. Is the assessment of bone age by the Greulich-Pyle method reliable at forensic age estimation for Turkish children? Forensic Sci Int. 2007; 173(2–3): 146–153.

19. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys, Arch Dis Child. 1970; **45:** 13-23.

20. Koc A, Karaoglanoglu M, Erdogan M, Kosecik M, Cesur Y. Assessment of bone ages: Is the Greulich-Pyle method sufficient for Turkish boys? Paediatr Int. 2001; **43(6):** 662-5.

21. Nahid G, Abdorrahim A, Gharib SM, Anvar E. Assessment of bone age in Kurdish children in Iran. Pak J Med Sci. 2010; **26(1):** 36-9.

22. Tanner JM, Whitehouse RH, Marshall WA. (1975). Assessment of Skeletal Maturity and Prediction of Adult Height (TW2 Method). London. Academic Press.

23. Carpenter CT, Lester EW. Skeletal age determination in young children: analysis of three regions of the hand/wrist film. J Paediatr Orthop. 1993; **13:** 76-9.

24. Tanner JM. (1955). Growth at Adolescence. UK. Blackwell.

25. Sinclair D. (1978). Human Growth after Birth. London. Oxford University Press.

26. Schmeling A, Reisinger W, Loreck D, Vendura K, Markus W, Geserick G. Effects of ethnicity on skeletal maturation: consequences for forensic age estimations, Int J Legal Med. 2000; **113(5):** 253-8.

27. Mora S, Boechat M, Pietka E, Huang HK, Gilsanz V. Skeletal age determinations in children of European and African descent: applicability of the Greulich and Pyle standards. Paediatr Res. 2001; **50(5):** 624-8.

28. Tisè M, Mazzarini L, Fabrizzi G, Ferrante L, Giorgetti R, Tagliabracci A. Applicability of Greulich and Pyle method for age assessment in forensic practice on an Italian sample. Int J Legal Med. 2011; **125:** 411-6.

29. Greulich WW, Pyle SI. Radiographic Atlas of Skeletal Development of the Hand and Wrist. 1959; Stanford California. Stanford University Press.