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Applicability of T1-weighted MRI in the assessment of forensic age based on the epiphyseal closure of the humeral head

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Abstract

This work investigates the value of magnetic resonance imaging analysis of proximal epiphyseal fusion in research examining the growth and development of the humerus and its potential utility in establishing forensic age estimation.

In this study, 428 proximal humeral epiphyses (patient age, 12–30 years) were evaluated with T1-weighted turbo spin echo (T1 TSE) sequences in coronal oblique orientation on shoulder MRI images. A scoring system was created following a combination of the Schmeling and Kellinghaus methods.

Spearman's rank correlation analysis revealed a significant positive relationship between age and ossification stage of the proximal humeral epiphysis (all subjects: rho=0.664, $p < 0.001$; males: 0.631, $p < 0.001$; females: rho=0.651, $p < 0.001$). The intra- and inter-observer reliability assessed using Cohen's kappa statistic was κ=0.898 and κ=0.828, respectively. The earliest age of epiphysis closure was 17 years for females and 18 years for males.

MRI of the proximal humeral epiphysis can be considered advantageous for forensic age estimation of living individuals in a variety of situations, ranging from monitoring public health to estimating the age of illegal immigrants/asylum seekers, minors engaged in criminal activities and illegal participants in competitive sports, without the danger of radiation exposure.

Keywords age estimation, proximal humeral epiphysis, magnetic resonance imaging

Introduction

Forensic age estimation (FAE) is a proxy for the biological maturation of an individual, which is important for a variety of demographic, clinical and forensic purposes. Every skeletal part of the human body develops in a predictable way, which enables for the estimation of skeletal age, given specific markers [1]. Skeletal development is subject to a combination of genetic and environmental factors; thus, recording the process in different parts of the world is of crucial importance for public health. The level of skeletal maturation is often requested by clinicians, such as endocrinologists, in order to be compared with chronological age in suspected developmental disorders in children, to monitor response to hormone therapy or to estimate stature in healthy subjects [1-4]. Estimating the age of living individuals is an important requirement for the birth registration of unknown persons [5-7]. Moreover, the age of a living individual is required in cases concerning migration and legal responsibility. Legal systems in different countries stipulate different age limits for legal assessment, particularly for those aged 10–21 years, with 14, 16, 18, and 21 years as notable thresholds [7-9]. Age assessment plays a critical role in the definitions of the civil rights of refugees and those seeking asylum and in the specification of the age of marriage and adoption [7,8,10]. Last, FAE is crucial in establishing entry thresholds for participants in competitive sports [11].

The primary application area of radiological evaluations for skeletal maturation is the epiphyseal union. Although skeletal maturation is driven by a combination of intrinsic and extrinsic factors, it has been demonstrated that estrogen is primarily responsible for ultimate epiphyseal fusion [12]. In fact, it has been shown that prepubertal estrogen levels in girls are over seven-fold higher than in boys, which explains the five-fold more advanced skeletal maturation of girls in childhood [13]. It is thus possible that different estrogen levels between the sexes would impact the epiphyseal union time.

In long bones, the epiphyseal conversion to fatty marrow is noted within 6 months of the radiological appearance of the secondary ossification center. The fatty transformation continues in the diaphysis and moves toward the metaphysis. The last parts of the appendicular skeleton to convert are the proximal humeral and femoral metaphyses [14]. Epiphyseal closure has been studied to reveal minimal age limits for forensic age estimation using different articulations, imaging modalities and scoring systems [1,15-19]. The recommended methods for age estimation of living individuals by several forensic associations include physical and radiological examination of children and adolescents [6-7]. However, in recent years, radiation exposure in the pediatric age group has caused ethical concerns [20]. Therefore, developmental studies of living individuals have shifted to non-invasive modalities, such as ultrasonography [21-23] and magnetic resonance imaging (MRI) [15-17, 24-28].

MRI indeed offers a remarkable insight into the dynamic process of skeletal growth and maturation, as demonstrated by an increasing number of studies in children and adolescents in recent years [15-17, 24-40]. The vast majority of these studies focused on the clavicle, hand and wrist, producing large reference databases for different populations. A direct application of this body of work was the introduction of an MRI examination of the wrist for age verification in international youth competitions (under 17 years old) by the Federation Internationale de Football Association (FIFA) in Nigeria in 2009 [41]. Despite the encouraging results of the MRI imaging in the investigation of age estimation, only one study focused on the development of the proximal humeral epiphysis but presented several limitations in methodology and sample size [39]. The current study aims to address these issues by investigating the epiphyseal union of the proximal humerus in a large sample (N>400) with the aid of a hybrid staging system of previous methodologies. MRI imaging of the shoulder has a significant advantage in achieving detailed imaging of the non-ossified cartilaginous epiphysis, the secondary ossification centers, and the physis. MRI has also demonstrated to be highly efficient in detecting infections and tumors in young children or detecting sports-related injuries (intra-articular shoulder derangements and osseous abnormalities associated with shoulder dislocation) in older children and young adults [39]. The present work aims to evaluate the value of MRI analysis of the proximal epiphyseal fusion in the study of growth and development of the humerus and its potential utility in establishing FAE.

Materials and Methods

This cross-sectional retrospective study was performed at Bakırköy Dr Sadi Konuk Research and Training Hospital, İstanbul, Turkey from January 1, 2014 to January 1, 2016. We evaluated the left shoulder MRI of 449 patients who were 12-30 years of age and who were admitted to the radiology department with traumatic and ligamentous disorders after clinical evaluation. Five patients with traumatic shoulder bone fracture, 3 patients with operation history, and 13 patients with insufficient age and sex information were excluded from the study. A total of 428 patients were included in the study. All medical documents and information on patient age and sex were obtained from the data-processing center of the hospital. Data were anonymized to comply with medical ethics at our institution.

All MRI examinations were performed with a 1.5-T whole body scanner (Avanto; Siemens, Erlangen, Germany) with comparable imaging properties using an extremity coil. For the analysis of the scans, T1-weighted turbo spin echo (T1 TSE) sequences in coronal oblique orientation were used (TR, 500 ms; TE, 15 ms; 150 mm; FoV, 150 mm; slice thickness, 2-4 mm; voxel size, 0.5x0.5x3.5 mm, and scan time 1 min 44 s). The ossification stage of the proximal humeral epiphysis was scored using a combination of the staging systems published by Schmeling et al. [18] and by Kellinghaus et al. [19]. Stages describe the ossification process of the proximal humeral epiphysis, as seen below, and applying the classification schemes, as shown in detail in Figure 1 and 2.

All MRI slices were evaluated in all cases for stage detection. The decision for stages 1,2a-2b-2c and 3a-3b-3c was based on the most-developed epiphysis in the slices. For stage 4, at least one epiphyseal scar should be observed in all sections. For stage 5, epiphyseal closure and no epiphyseal scar should be observed.

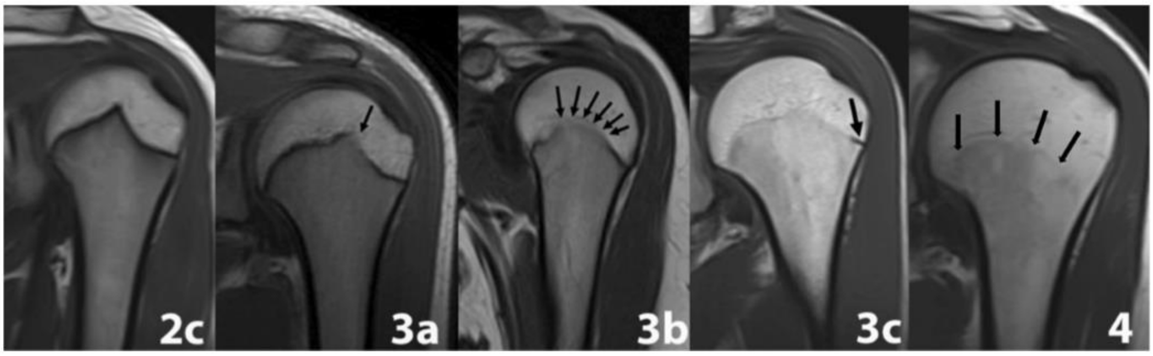

Figure 1.Ossification stages of the proximal humeral epiphysis; T1weighted turbo spin echo (T1 TSE) sequences in coronal oblique orientation. The length of the ossified epiphysis is over two thirds compared to the width of the metaphyseal ending (2c). Epiphysealmetaphyseal fusion completes one third or less of the former gap between epiphysis and metaphysis. Arrow shows fused epiphysealmetaphyseal part (3a). Epiphyseal-metaphyseal fusion completesbetween one third and two thirds of the former gap between epiphysis and metaphysis. Arrows show fused epiphyseal-metaphyseal part (3b). Epiphyseal-metaphyseal fusion completes over two thirds of the former gap between epiphysis and metaphysis. Arrow shows unfused epiphyseal-metaphyseal part (3c). The epiphyseal cartilage is fully ossified, and the epiphyseal scar is visible. Arrows show epiphyseal scar (4).

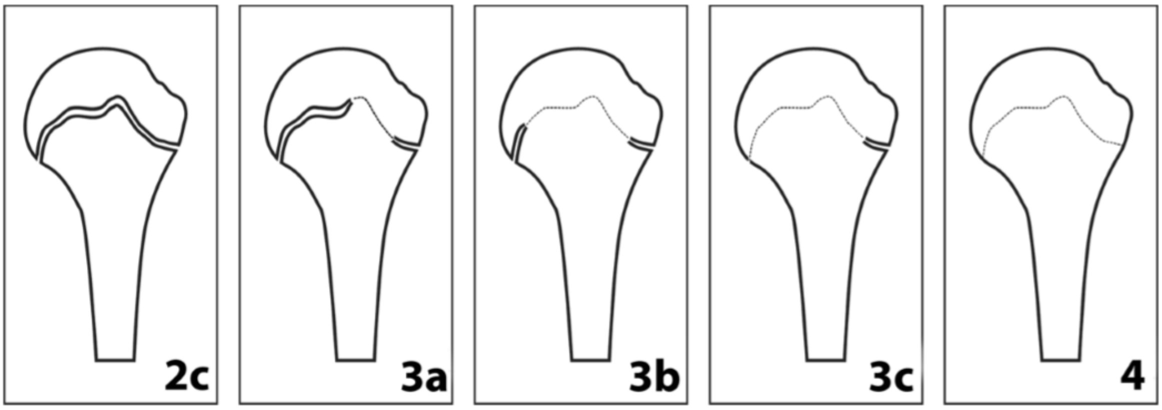

Figure 2. Schematic drawings of the stages of the proximal humeral epiphysis

Statistical Analysis

SPSS ver. 17 software (SPSS, Chicago, IL, USA) was used for the statistical analysis. Data were expressed as the mean or median with standard deviation (SDs), 95% confidence intervals, and ranges, as appropriate. Associations between age and ossification stage were evaluated via Spearman's correlation analysis. Between-sex comparisons were performed using the Mann–Whitney U-test. A p-value < 0.05 was considered to be significant.

Two radiologists (R1 and R2) evaluated the MR images. R1 has 20 years of professional experience, while R2 has 6 years of experience. In addition, R1 has experience in forensic age estimation of living individuals via CT and MRI by measuring the ossification stage of the different epiphyseal areas. Cohen's kappa nonparametric test was used to evaluate intra- and inter-observer variabilities, and the κ statistic was calculated. The Altman [42] system was used to interpret the κ values: κ < 0.20, poor agreement; κ = 0.21–0.40, fair agreement; κ = 0.41–0.60, moderate agreement; κ = 0.61–0.80, good agreement; and κ = 0.81–1.00, very good agreement.

Results

We examined the left proximal humeral epiphysis of 428 patients aged 12–30 years (mean male age, 22.07 ± 4.37 years; mean female age, 22.10 ± 4.75 years; 240 males; 188 females; Table 1). Determination of the ossification stage of the proximal humeral epiphysis was possible in our sample. Figure 2 shows the MRI findings for ossification stages 2c, 3a, 3b, 3c and 4 that were observed. The remaining ossification stages were not found within the study population. Spearman's rank correlation analysis revealed a significant positive relationship between age and ossification stage of the proximal humeral epiphysis (all subjects: rho=0.664, p < 0.001; males: 0.631, p <0.001; females: rho=0.651, p < 0.001).

The intra- and inter-observer reliability for the proximal humeral epiphysis was κ=0.898 and κ=0.828, respectively. These values indicate good agreement.

Table 2 shows the minimum and maximum ages, mean±standard deviation and lower and upper quartiles of all parameters. No significant sex-related differences were found for all stages (P>0.5). The mean age at which stage 2c was seen was 13.44±0.94 years in male and 12.95±0.82 years in female patients. Stage 2c was first seen at 12.1 years of age among male patients and at 12.2 years among female patients. In male patients, the latest appearance of stages 2c was 15.3. In female patients, the latest appearance of stages 2c was 14.4 years. The mean age at which stage 4 was seen was 24.76±3.47 years in male and 24.50±3.22 years in female patients. Stage 4 was first seen at 18.6 years of age among male patients and at 17.2 years among female patients. In males, the latest appearance of stage 4 was 30.6, while in females, the latest appearance of stage 4 was 30.7 years.

Discussion

In recently published research, studies evaluating the development of the proximal humerus epiphysis are mostly based on direct (visual) or x-ray examination of skeletal collections (Table 3). In an anthropological study of proximal humeral epiphyses in the Lisbon skeletal collection, Cardoso [43] reported a complete union at the age of 17 years in both sexes. Another study on a Portuguese sample from Coimbra [44] noted that the minimum age at which the epiphyseal union completes is two years later (at the age of 19) in males. In addition, compared to the Lisbon sample, the oldest individuals with open epiphyses were observed in the Coimbra sample and were 3 years older in both sexes. Both aforementioned studies were conducted in skeletal material using direct examination, the same staging system (3 stages), and similar size samples, which both derive from individuals who lived around the same period in Portugal (Table 3). Nonetheless, compared to the Lisbon study, the Coimbra study reports delayed development of approximately 3 years, which may reflect nutritional stress in the Coimbra sample.

Two more studies of the humeral epiphysis were conducted using direct analysis on skeletal remains from Bosnian and American soldiers [45-46]. For the two Bosnian samples, the minimum age of epiphyseal closure is 18, and the maximum age for which open epiphysis is described is 20 and 21 years, respectively. This finding is more consistent with the Cardoso study results [43]. Conversely, the maximum age for which open epiphysis in the McKern skeletal collection (derived from US soldiers) was 23 years, which indicates a two-year delay in skeletal maturation. These differences can be attributed to any number factors such as population differences or, possibly, different levels and durations of nutritional stress for the US soldiers compared to the Bosnians.

The most important study of the humeral epiphysis using x-rays was done on a large sample of US living patients [47]. This study reported complete union at the ages of 14 and 16 for males and females, respectively. This result is in agreement with two studies on Indian patients which, however, were conducted using undefined protocols [48] or were limited in female patients [49]. The maximum age for which open epiphysis is noted is 21 for males and 20 for females. Interestingly, compared to the anthropological studies, all x-ray studies detected earlier ages of complete fusion (Table 3).

MRI studies of the proximal humerus are limited in the work of Kwong et al. [39] in a sample of 76 US patients. The authors attempted an overview of the humeral growth from 3 months to 17 years and focused on the detailed description of the MRI characteristics from the appearance of the humeral head secondary ossification centers to growth plate transformation, metaphyseal stripe and proximal metaphyseal marrow signal intensity. MRI examinations were performed on different MRI weighting and cross-sections. According to the results, the growth plate remained open until 14 years of age, showed partial closure in the 14-16 years age group (19 cases), and complete closure by 17 years (8 cases). The study had a limited sample with an upper limit at the age of 17 years old and no specifically defined staging system, and sex differences were not investigated. In addition, as stated by the authors, only 9/76 cases were healthy subjects, which could have seriously biased the results.

The current study used MRI, which is the imaging method of choice for children, to explore the developmental changes of the proximal epiphysis of the humerus in a large sample (N=428) balanced for both sexes, ranging in age from 12 to 30 years old. The study focused on the subtle differences in the epiphyseal line during development in an effort to capture the majority of developmental variation in the manifestation of skeletal age. We applied a combination of the

Schmeling [18] and Kellinghaus [19] staging system, and we found that the minimum age for closure of the epiphyseal union of the proximal humerus is 17.2 for females and 18.6 for males. These results agree with the standards presented for Bosnian males [46-47] but largely disagree with the standards for Americans [47]. These discrepancies can be attributed to differences in ethnicity, nutritional stress, dietary habits, environmental influences, secular changes and the modality used for data acquisition. The intra- and inter-observer reliability was κ=0.898 and κ=0.828, respectively, which is in agreement with other MRI studies in different anatomical regions (e.g., 33). Thus, discrepancies with other studies in the literature are unlikely to be due to human error.

This unequal distribution should be kept in mind as a substantive limitation in the evaluation of our work data. The fact that 345 out of 428 (ca. 80.6%) of our cases were above 18 years of age, and these cases only represented 2/3 (66%) of the total number of case, indicates potential selection bias. This study limitation is especially relevant, since the chance of documenting the lower extremes of a stage, i.e., the minimum age, is lowered. The unequal age distribution is a result of the availability of clinical images, as in any retrospective study of age estimation. In this respect, the results obtained with a balanced age distribution in future prospective studies will be important for demonstrating the applicability of the method.

It is important to define the sex differences for researching age-estimation studies. In our study, no significant difference was detected between the two sexes. At stage 4, however, the difference between the minimum ages (1.4 years) is remarkable. Unfortunately, there is no other proximal humerus MRI study to compare sex differences. Conversely, there are many different estimations of CT and MRI in different populations. In these studies, the differences between the sexes are variable, reaching 2 years for the stages in which the ossification is completed [15-17, 28, 30]. Thus, it is imperative to investigate other factors, such as the effect of socioeconomic status in the maturation of the humerus. Future studies may also reveal new data in discussing this difference.

The fact that stage 5 was not observed in our sample suggests that the minimum age for that stage is over the 30th year of life. Thus, stage 4 represents the final ossification stage for part of the population. There is a need for future studies in older age groups to determine the age at which the epiphyseal scar has completely disappeared, but this research would exceed the purposes of the current work, the focus of which is on the age estimation of living subjects to legal age thresholds.

Although the study was performed on one sectional plane and one MRI weighting, it was of paramount importance for recording the development of the proximal humeral epiphysis. The most prominent example of such limitation was observed in studies with different sectional planes and MRI weighting for the proximal tibial epiphysis, and the distal femoral epiphysis, creating important debates about the development of the methods [17, 24, 28, 30]. Assessing the ossification of the epiphysis at different sectional planes may be important for the reliability of the results. In their original study, Dedouit et al. [17] reported that in addition to the coronal sections, sagittal sections were also evaluated in stages 2-3 and stages 3-4, but this practice was rarely encouraged. On the other hand, the axial and coronal sections of the clavicle CT study were used together for the same purpose in the staging, and Scharte et al. [50] reported a 35.6% difference between axial and coronal planes with clavicle CT. In our study, only coronal sections were used instead of a combination of coronal and sagittal sections. A follow-up study comparing the scores taken on both sectional planes for the proximal humeral epiphysis would enable us to establish a solid methodology for observation of the epiphyseal union using MRI modalities.

Another factor to take under consideration is that the sample derives from a single geographical area. Although this sample is the largest sample of MRI data from the proximal humeral epiphysis, one should stress that skeletal development is subject to many diverse influencing factors, which cannot be controlled in the current study and may have introduced bias to the estimates. For instance, the effects of socioeconomic status on the results should be acknowledged [45-46, 51, 52]. In fact, Schmeling and colleagues [50] suggested that socioeconomic status is a decisive factor for delays in skeletal maturation in contrast to ethnicity, which seems to have no effect. This issue is particularly relevant in all cases that require the assessment of skeletal age, such as for establishing legal responsibility. Franklin et al., [53] correctly stressed that "*the populations most in need of reliable age assessment standards (*e.g.*, comprising* *individuals from localized geographic regions that are more likely to enter the criminal justice system – such as people smugglers) are disproportionately underrepresented in the published literature..."* This property may be true for the present study, as well; thus, there is scope for further expanding the sample to include more socioeconomic and geographical groups.

In conclusion, this study focused on proximal humeral epiphysis using a non-ionizing MRI method in living individuals. We encountered difficulties comparing our data with previous research, as notably few studies have been conducted and different methods and populations have been used. Our data show that the MRI method can be very useful, as a supportive method for estimating skeletal age, as it provides a better assessment of bone and cartilage tissue due to its technical advantages and eliminates radiation exposure for living individuals. MRI of the proximal humeral epiphysis can be considered advantageous for skeletal age assessment of normal living subjects in a variety of situations ranging from illegal immigrants/asylum seekers, minor criminals to illegal participants in competitive sports, although it must be stressed that the age thresholds provided in this study are specific to a certain geographic region and should not be applied in a different population without further testing. Future directions of this work will include a prospective study on subjects of known socioeconomic status, nutritional habits and shoulder-related activities to fully exploit the investigative potential of MRI in the study of skeletal development and maturation.

Compliance with ethical standards:

Disclosure of potential conflicts of interest: The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals: This article does not contain any studies with animals performed by any of the authors.

Informed consent : For this type of study formal consent is not required

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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List of Tables

Table 1. Age distribution of male and female subjects.

Age (years)	Male (N)	Female (N)
12	2	6
13	5	-
14	6	12
15	10	2
16	12	13
17	8	7
18	12	11
19	16	8
20	25	13
21	25	24
22	18	11
23	24	14
24	16	9
25	17	16
26	9	9
27	4	7
28	10	8
29	13	9
30	8	9
Total	240	188

Table 2. Minimum and maximum ages, with means ± SDs, lower and upper quartiles and medians, at all stages of proximal humeral epiphysis.

Stage	Sex	N	Mean ±SD	Min-Max	LQ; UQ;Median
2c	Female	8	12.95±0.82	12.2-14.4	12.37; 13.75; 12.65
	Male	10	13.44±0.94	12.1-15.3	12.92; 14.22; 13.25
3a	Female	12	14.87±0.78	14.3-16.7	14.30; 14.90; 14.65
	Male	10	14.97±0.60	14.2-15.9	14.30; 15.37; 15.20
3b	Female	8	16.58±1.15	15.2-18.4	15.72; 17.82; 16.25
	Male	12	16.56±1.02	15.3-18.2	15.80; 17.75; 16.35
3c	Female	43	19.62±2.03	16.2-23.2	18.20; 21.30; 20.30
	Male	61	19.84±2.09	16.2-23.6	17.90; 21.30; 20.10
4	Female	117	24.76±3.47	17.2-30.7	22.05; 27.75; 25.10
	Male	147	24.50±3.22	18.6-30.6	21.80; 26.90; 24.90

Table 3. Comparative table of studies on the closure of the proximal humeral epiphysis.

Population	Life span	Male	Female	Age range	Study protocol	Youngest fused	Oldest with open epiphysis	Reference
Lisbon skeletal collection	1887-1975	65	56	9-29 years	Direct analysis. Staging: 3 stages	Male 17 y, Female: 17 y	Male: 21 y Female:19 y	[43]
Coimbra skeletal collection	1826-1938	69	68	7-29 years	Direct analysis. Staging: 3 stages	Male 19 y, Female: 17y	Male:24 y Female: 22y	[44]
Bosnian skeletal collection	1962-1995	232	0	14-30 years	Direct analysis. Staging: 5 stages	Male: 18 y	Male: 21 y	[45]
Bosnian skeletal collection	1965-1995	114	0	17-30 years	Direct analysis. Staging: 4 stages	Male:18 y	Male: 20 y	[46]
Mckern skeletal collection (US soldiers died in the Korean War)	1924-1954	325	0	17-30 years	Direct analysis. Staging: 4 stages	Male: 17 y	Male: 23 y	[46]
USA-Living individuals/students	Examinations before 2015	258	179	10-21 years	X-ray staging: 4 stages	Male: 16 y, Female:14 y	Male:21 y Female:20 y	[47]
Indian-Living individuals	1951-1971	?	?	14-19 years	X-Ray, staging undefined	Male:16 y, Female:15 y	Male 18 y, Female:17 y	[48]
Indian-Living individuals	Examinations before 2014	0	80	13-21 yeas	X-ray staging: 3 stages	Female: 15-16 y	Female:17-18 y	[49]
USA-Living individuals	Examinations between 2003-2012	44	32	3months -17 years	MRI: staging undefined	15.5 y (sex not defined)	17 y (sex not defined)	[39]
Turkish-Living individuals	Examinations from Jan 2014 to Jan 2016	240	188	12-31 years	MRI Staging: 5 stages	Male: 18.6 y, Female:17.2 y	Male:23.6 y, Female:23.2y	Present study