Ultrasonographic Assessment of Spleen size and Pattern of Change Among Sickle Cell Disease Patients and Healthy Controls in North-Eastern Nigeria

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Abstract

Background

Ultrasonography is an established and reliable method for assessing the spleen. Because of variation due to genetic and other environmental factors including malaria endemicity, interpretation of spleen sizes requires a knowledge of the normal reference range for a given population. This study aimed to identify spleen size reference ranges across age groups of healthy controls to serve as a baseline to assess changes in spleen size in patients with SCD.

Methods

Using a cross-sectional study design, spleen size was measured in healthy people of different age groups, and steady-state SCD patients (children and adults) using abdominal ultrasonography. Using the age-group specific reference values obtained from the controls, spleens were classified into small, normal size, or enlarged among the SCD patients.

Results

The study consisted of 109 (34.8%) healthy controls and 204 (65.2%) steady-state SCD patients. The spleen was visualised in all the controls (n=109) and in 107 (52.4%) SCD patients. Using cut-off values for spleen length among the controls across age groups [< 5 years (5.0cm -7.0cm); 5-9 years (5.5cm - 8.5cm); 10-14 years (6.0cm – 11.0cm); and ≥ 15 years (7.0 - 12.5cm)], spleen size was classified as small (n=18/204; 8.87%), normal (n=68/204; 33.3%) and enlarged (n=21/204; 10.3%) among the SCD patients.

Conclusion

Model-based age-group reference ranges and percentile curves for splenic dimensions based on ultrasonography among normal controls in North-Eastern Nigeria were established and may be of value in assessing spleen sizes among SCD patients living in malaria-endemic regions of Africa.

Introduction

Sickle cell disease (SCD) is an inherited condition of red blood cells widely prevalent across Sub-Saharan Africa, affecting up to 3% of births in some parts of the continent [1]. The spleen is the largest organ in the reticulo-endothelial system with an active role in immune defence against infection and is one of the earliest organs to be affected in patients with SCD. In SCD, the spleen initially enlarges, followed by progressive atrophy due to repeated episodes of vaso-occlusion and infarctions [2]. Part of the routine clinical evaluation of patients with SCD involves examining for the spleen [3]. However, clinical examination by palpation has a low sensitivity for the detection of splenic enlargement because the organ would have to enlarge two to three times its normal size before becoming palpable on abdominal examination [4]. Ultrasonography provides a non-invasive method of assessing the spleen size; it is the test of choice in most climes because of its accuracy, low cost, flexibility, and safety profile [5]. Despite the widespread use of ultrasonography in the clinical evaluation of splenic enlargement, there is no consensus on how to define splenomegaly among SCD patients; some authors have used spleen length to define splenomegaly [6, 7], while others have used the spleen volume or index [8, 9]. In addition, various cut-off values for spleen length including > 11cm [10], > 12cm [11] and ≥ 13cm have been used to indicate enlarged spleens [12-15].

Furthermore, knowledge of normal spleen size in a healthy population is required to interpret changes in spleen size, as changes in spleen size occur due to population-specific variations in genetics and environmental factors (e.g. infections such as malaria). Establishing a reference range for populations living in the same area as the patients' population is needed to aid interpretation of ultrasonography assessments. However, few studies are available on normal spleen dimensions in children and adults in Africa, particularly those living in malaria-endemic regions [16-18]. The aim of this study was to identify spleen length reference ranges for healthy controls to serve as a baseline to assess changes in spleen size in patients with SCD

Materials and methods

Study design and participants

This hospital-based, cross-sectional study was conducted from October 2020 to November 2021 at the University of Maiduguri Teaching Hospital (UMTH), North-Eastern Nigeria. Steady-state SCD patients (children and adults) [19] on follow-up at the outpatient paediatric and haematology clinics during the study period were invited to take part. Healthy individuals consisting of medical students, children of hospital personnel, and post-op paediatric patients (without any acute or chronic illness likely to influence splenic size) on follow-up in the surgical clinic served as controls. The study participants were divided into four age groups: 1: less than 5 years; 2: 5–9 years; 3: 10–14 years and group 4: 15 years and above.

Data collection

Clinical examination

Clinical data of the study participants were collected using a structured questionnaire. The spleen size was assessed by palpating the anterior axillary and mid clavicular line by a single examiner. Palpable splenomegaly was reported as the distance in cm the spleen extends under the left coastal margin in the mid clavicular line.

Sonographic evaluation

A board-certified radiologist with more than 15 years of experience in abdominal ultrasonography performed all the examinations using Logiq P5 Premium BT11 ultrasound scanner (GE Medical Systems, USA) equipped with a low frequency (3-5MHz) curvilinear transducer. The measurements were taken on images through the splenic hilum using electronic callipers. Splenic length, defined as the maximum distance between the dome and the tip of the spleen, was measured in a longitudinal view. Splenic width, defined as the maximum distance between the medial and lateral borders, was measured in a transverse view. Splenic depth,

defined as the maximum anteroposterior dimension, was also measured on a transverse view. Spleen volume was determined using the formula - length x width x depth x 0.523 [20]. Autosplenectomy was defined as the non-visualisation of the spleen in the splenic bed [5]. The spleen sizes of the SCD patients were compared with control values stratified by age. The spleen size was considered normal if the value fell within the 2.5th and 97.5th centile of the expected range for the age-specific group. Spleen sizes below and above the cut-off ranges were considered small-sized spleens or enlarged respectively [16, 21].

Statistical analysis

The data was analysed using Statistical Package for the Social Sciences (SPSS) (version 25; SPSS, Chicago, IL, USA). Categorical data were summarised using frequency and proportions while continuous data reported as mean and standard deviation (±) if normally distributed, or median and interquartile range if the data was skewed. Spearman's correlation was used to determine the relationship between the various spleen dimensions Model-based age-specific reference ranges for the various spleen dimensions were computed with age modelled as fractional polynomials using MedCalc® v.20.114 (MedCalc Statistical Software Ltd, Ostend, Belgium) [22]. Log-transformation of variables was applied before model fitting as needed. The model-based 2.5th, 10th, 50th, 90^{th,} and 97.5th percentiles of the (log-transformed) variables were then plotted against age. Comparison of spleen dimensions between controls and SCD patients was performed using non-parametric analysis. Scatter plots were drawn to assess the relationship between age and spleen length among the study participants. The level of significance was set at two-tailed P-value <0.05.

Results

Ultrasonography data were available for 109 of the controls (median age 14.0 years; range 1-34 years) and 204 of the SCD patients (median age 12.5 years; range 1- 45 years). The

haemoglobin (Hb) phenotypes of the SCD patients consisted of homozygous sickle cell disease (Hb SS) (n = 196), sickle-haemoglobin C disease (Hb SC) (n = 5), and sickle cell β-thalassaemia (Hb Sβ) (n=3). The spleen was clinically palpable in five of the SCD patients (2.4%) (range, 2 - 10 cm), but in none of the controls. Three of the HbSS patients (aged 1, 6 and 24 years) had past history of acute splenic sequestration.

Sonographic evaluation of the spleen among the control participants

The spleen was visualised on ultrasonography in all the control participants. Spleen volume correlated highly with spleen width (rho=0.891, $P < 0.001$), depth (rho=0.920, $P < 0.001$), and length (rho=0.858, $P < 0.001$). The various spleen dimensions by age groups and non-parametric reference limits are shown in Table 1. Specific (log-transformed) reference limits for spleen dimensions in relation to age are shown as curves in Figs. 1A-D.

Sonographic evaluation of the spleen among the SCD patients

Of the 204 SCD patients, the spleen was visualised in 107 (52.3%) and not visualised in 97 (47.5%) by ultrasonography. Based on the reference ranges generated from the control participants, spleen sizes were classified as small [18/204; 8.87%], normal [68/204; 33.3%] and enlarged [21/204;10.3%] among the SCD patients (Figs. 2 A-C). Further classification of spleen size across age groups among the SCD patients is shown in Table 2. Enlarged spleens were more frequently found in children less than five years old by both spleen length (31%) and volume (35.7%), and among patients 15 years above (25% and 20% by length and volume respectively). Small-sized spleens were more frequently encountered in the age group 10-14 years (21.1% and 31.6% by length and volume respectively) (Table 2).

Relationship between spleen size and age among the SCD patients and the controls

The mean spleen length ($P = 0.0001$) and volume ($P = 0.002$) were significantly higher in the control group compared to SCD patients; this difference was particularly striking in those aged

10 - 14 years (Table 3). Of note however, the spleen length was almost three-fold higher among the SCD patients compared to the controls in the first two years of life, followed by a rapid decline in spleen size around the third year (Fig. 3A). Also, the fitted line between spleen size and age had a positive slope among different age groups of the control group (Fig. 3B), while the slope was negative across all age groups among the SCD patients (Fig. 3C).

Discussion

Ultrasonography is non-invasive, not associated with radiation exposure and provides real-time information. It is widely available in resource limited settings, and routinely used to evaluate the size of internal organs such as the spleen [21]; however, there are no standards for defining spleen size among SCD patients. We documented spleen size and volume in SCD patients and age-matched healthy individuals from the same geographical area in North-Eastern Nigeria. We documented spleen length and volume among apparently healthy participants from the same geographical area and used this as a reference for assessing spleen size among our SCD patients. We generated non-parametric (2.5^{th} - 97.5^{th} percentiles) reference ranges according to four age groups and percentile curves to provide specific reference values for spleen sizes for individuals aged 1-36 years.

Our data on spleen length among the controls were similar to previous findings among paediatric [16-18] and adult population in Africa [5, 23, 24]. In contrast, the upper limit of spleen length in our controls aged <5 years (median, 6.1 cm), 5-9 years (median 6.9 cm) and 10-14 years (median 7.7 cm) were smaller when compared among a normal population in the USA aged 2-4 years (median 7.4 cm), 6-8 years (median 8.2 cm) and 10-12 years (median 9.9 cm) [25]. Our data for the upper limit of spleen length were also smaller than those recorded among comparable age groups in Europe [21]. In a study involving 631 American athletes, the spleen was larger in white Americans compared to the African-American athletes [26]. These findings

suggests that the spleen may be inherently smaller in the African population compared to the White population; contrary to the general notion of the spleen being bigger in areas where infections like malaria are endemic [27]. Other studies from Nigeria have made similar observations of smaller spleen sizes among their population compared to published data among the White population [5, 23, 24]. This underscores the importance of using population-specific reference values in classifying spleen sizes among individuals with SCD and other disease conditions affecting the spleen.

Spleen size assessment using length or volume

While most studies have used spleen length to define splenomegaly in SCD [6, 7, 10, 12-15], others used spleen volume [8, 9, 28, 29] or both length and volume [30, 31]. Given the strong positive correlation between spleen length and volume in our normal controls (rho = 86.4; P <0.001), consistent with earlier reports [32, 33], both parameters were used to classify spleen sizes in the current study. The ease of acquiring spleen length when compared to the cumbersome nature of assessing volume favours the use of spleen length to determine spleen size in routine clinical practice and future studies. Another drawback of using spleen volume lies with the use of the prolate ellipsoid volume method in its determination formula; because the spleen can become irregularly shaped in SCD patients due to the recurrent vaso-occlusion and infarctions, estimating volume using this formula may not be accurate [26]. Despite similarities in the range of values obtained for the different categories of spleen sizes using spleen length and volume, we noted a tendency for higher frequencies of enlargement when using spleen volume.

Comparison of spleen size between the SCD patients and controls

We noted a difference in the pattern of age-related increase in spleen size between the controls and SCD patients. Among the controls, the spleen length showed a progressive increase in children less than five years to the adult mean size of 9.0 cm before levelling off, consistent

with reports from the USA [25], Europe [21], and India [34]. The SCD patients in our cohort had larger spleens early in life, but the rate of increase in length was slow thereafter. Compared to the progressive increase in length observed in the control group, the spleen length remained steady in SCD patients aged 5 - 9 years group, with a slight increase in the 10-14 years group, before increasing to the adult (>15 years) mean length of 9.9 cm. The slow increase in spleen length observed during childhood and adolescence among the SCD patients is indicative of progressive splenic injury, which counteracts the normal age-related physiological increase in spleen size observed in the control group.

Clinical implication

Although the spleen is usually relatively larger in infants and toddlers than in adults [21], we observed a more than 3-fold increase in size of the spleen among the SCD patients compared to the normal controls in the first two years of life. This is not unexpected as obstruction of the inter-endothelial slits in the basement membrane by the relatively rigid sickled red blood cells could result in passive splenic enlargement [35]. Extra medullary haemopoiesis and infections may also account for enlarged spleens in early childhood among SCD patients [36, 37]. Also, the spleens among a quarter of the older SCD patients (n=5/20) with visible spleens appeared to be markedly enlarged. It is not clear if the presence of compound heterozygosity for the Hb S gene may be associated with this finding (they consisted of 3 HbSS, 1 HbSC and 1 HbS thal). Patients with enlarged spleens may be prone to complications related to splenomegaly including sub-clinical red cells sequestration and hypersplenism resulting in worsening anaemia, splenic infarction and therefore may need close monitoring [38]. Furthermore, the finding of enlarged spleens in 31% (n = 13/42) of our SCD patients less than five years of age using ultrasonography compared to 4.8% (n = 2/42) by clinical examination aptly demonstrates the low sensitivity of the latter technique and the fact that the spleen may be enlarged 2-3-fold before it becomes palpably enlarged. It is possible that red cell sequestration and hypersplenism

occurs within a spleen that is not palpable and so splenic ultrasonography may be a useful adjunct to clinical management.

Limitations

Being a hospital-based and single centre study with small sample size for the controls used to generate the reference ranges may limit generalisability of the findings. Secondly, assessment of inter-observer variability for the measurements obtained by ultrasound was not done; however, having a single board-certified radiologist to obtain all images in healthy participants and SCD patients brought about consistency and improved accuracy of comparison between the groups.

Conclusion

This study has provided reference ranges for spleen length across age groups [< 5 years (5cm -7cm); 5-9 years (5.5cm - 8.5cm); 10-14 years (6cm - 11cm); and 15 years (7.0 - 12.5cm)] and percentile curves for splenic dimensions for different ages based on ultrasonography among normal controls in North-Eastern Nigeria; this may be of value in assessing spleen sizes among SCD patients living in malaria-endemic regions. Regular spleen scans can help identify patients with enlarged spleens, who may require close monitoring for development of complications such as subclinical acute sequestration (especially in vulnerable age groups) and hypersplenism.

Table 1

Title: Splenic dimensions according to age group and non-parametric reference ranges among the normal controls (n=109)

Parameter	**Number (n)**	**Mean (SD)**	**Non-parametric measures**		
			2.5^{th} centile	0.5 median	97.5^{th} centile
Spleen length (cm)					
< 5 years	21	6.3 (0.7)	5.2	6.1	7.0
5 - 9 years	22	6.8 (0.9)	5.3	6.9	8.3
10 - 14 years	21	8.0 (1.2)	6.0	7.7	11.1
≥15 years	45	9.0 (1.4)	7.2	8.8	12.5
Spleen width (cm)					
< 5 years	21	6.3 (0.7)	5.1	6.2	8.1
5 - 9 years	22	7.1 (0.9)	5.4	7.1	8.5
10 - 14 years	21	8.1(1.3)	6.2	7.7	10.7
≥15 years	45	9.2 (1.5)	6.9	8.9	12.4
Spleen depth (cm)					
< 5 years	21	2.6 (0.3)	2.1	2.6	3.1
5 - 9 years	22	3.0 (0.5)	2.2	3.0	3.8
10 - 14 years	21	3.3 (0.4)	2.6	3.2	4.0
≥15 years	45	4.0 (0.8)	2.9	3.9	5.8
Spleen vol (cm^3)					
< 5 years	21	54.0 (14.9)	32.0	53.5	86.4
5 - 9 years	22	76.9 (27.3)	33.0	78.6	133.3
10 - 14 years	21	117 (48.7)	56.2	102.8	226.0
≥15 years	45	180.8 (87.0)	80.0	152.7	414.7

Table 2

Title: Frequency of different spleen sizes among SCD patients (n=107) by age group

Parameter	Reference range		Category of spleen size, n, (%)			Total
	Lower limit (2.5^{th} centile)	**Upper limit (97.5^{th} centile)**	**Small-sized**	**Normal**	**Enlarged**	
Spleen length (cm)						
< 5 years	5.0	7.0	7 (17.7)	22 (52.4)	13 (31.0)	42
5 – 9 years	5.5	8.5	2 (7.7)	21 (80.8)	3 (11.5)	26
10 -14 years	6.0	11.0	4 (21.1)	14 (73.7)	1 (5.3)	19
>15 years	7.0	12.5	3 (15.0)	12 (60.0)	5 (25.0)	20
Spleen volume (cm^3)						
< 5 years	30.0	85.0	4 (9.5)	23 (54.8)	15 (35.7)	42
5 – 9 years	35.0	135.0	2 (7.7)	17 (65.4)	7 (26.9)	26
10 -14 years	55.0	225.0	6 (31.6)	12 (63.1)	1 (5.3)	19
>15 years	80.0	415.0	5 (25.0)	11 (55.0)	4 (20.0)	20

Footnote: Age-group based reference ranges were generated from the corresponding spleen length and volume of the controls. The values were rounded up to the nearest integer. Spleen size below the 2.5^{th} centile were considered small size; normal-sized spleen are dimensions between the 2.5^{th} and 97.5^{th} centiles; enlarged spleens are values above the 97.5^{th} centiles.

Table 3: Comparison of spleen sizes between SCD patients and normal controls

Parameters	**SCD patients (n=107)**			**Controls (n=109)**			**P value**
	N	**Mean(SD)**	**Median**	**N**	**Mean (SD)**	**Median**	
Spleen length(cm)							
< 5 years	42	6.4 (1.2)	6.5	21	6.2 (0.6)	6.1	0.423
5 - 9 years	26	6.6 (1.3)	6.5	22	6.8 (0.9)	6.9	0.096
10 - 14 years	19	7.0 (1.8)	7.0	21	8.0 (1.2)	7.7	0.004*
≥15 years	20	9.9 (3.6)	8.8	45	9.0 (1.3)	8.8	0.921
Total	107	7.2 (2.3)	6.7	109	7.8 (1.6)	7.7	0.0001*
Spleen width (cm)							
< 5 years	42	6.7(1.0)	6.4	21	6.3(0.7)	6.2	0.137
5 - 9 years	26	7.0(1.3)	7.0	22	7.1(0.9)	7.1	0.852
10 - 14 years	19	7.2(1.3)	7.0	21	8.1(1.3)	7.7	0.012*
≥15 years	20	9.1(3.0)	8.3	45	9.2(1.5)	8.9	0.286
Total	107	7.3 (1.8)	6.9	109	8.0 (1.7)	7.7	0.000*
Spleen depth (cm)							
<5 years	42	3.1(0.7)	3.1	21	2.6 (0.3)	2.6	0.002*
5 - 9 years	26	3.1(1.0)	2.8	22	3.0 (0.5)	2.9	0.923
10 -14 years	19	3.1(0.9)	3.0	21	3.3 (0.4)	3.2	0.153
≥15 years	20	4.1(1.5)	3.7	45	4.0 (0.8)	3.9	0.659
Total	107	3.3 (1.0)	3.1	109	3.3 (1.0)	3.1	0.166
Spleen vol (cm^3)							
< 5 years	42	73.8(33.7)	66.4	21	53.9 (14.6)	53.5	0.020*
5 - 9 years	26	85.7(55.0)	66.5	22	76.9 (28.0)	78.6	0.979
10 - 14 years	19	90.0(60.0)	70.4	21	117.0(48.3)	102.0	0.013*
≥15 years	20	258.0(270)	145.7	45	183.6(84.7)	154.4	0.599
Total	107	114 (140)	70.4	109	124 (80.8)	99.5	0.002*

Footnote: *Significant P value by Mann Whitney U test for within age group analysis.

Fig. 1

Title: Curves of the model-based reference limits (2.5^{th} to 97.5^{th}) for the spleen dimensions

Legend: Percentile curves of the age-based reference limits for splenic (A) Length (B) Depth (C) Width (D) Volume

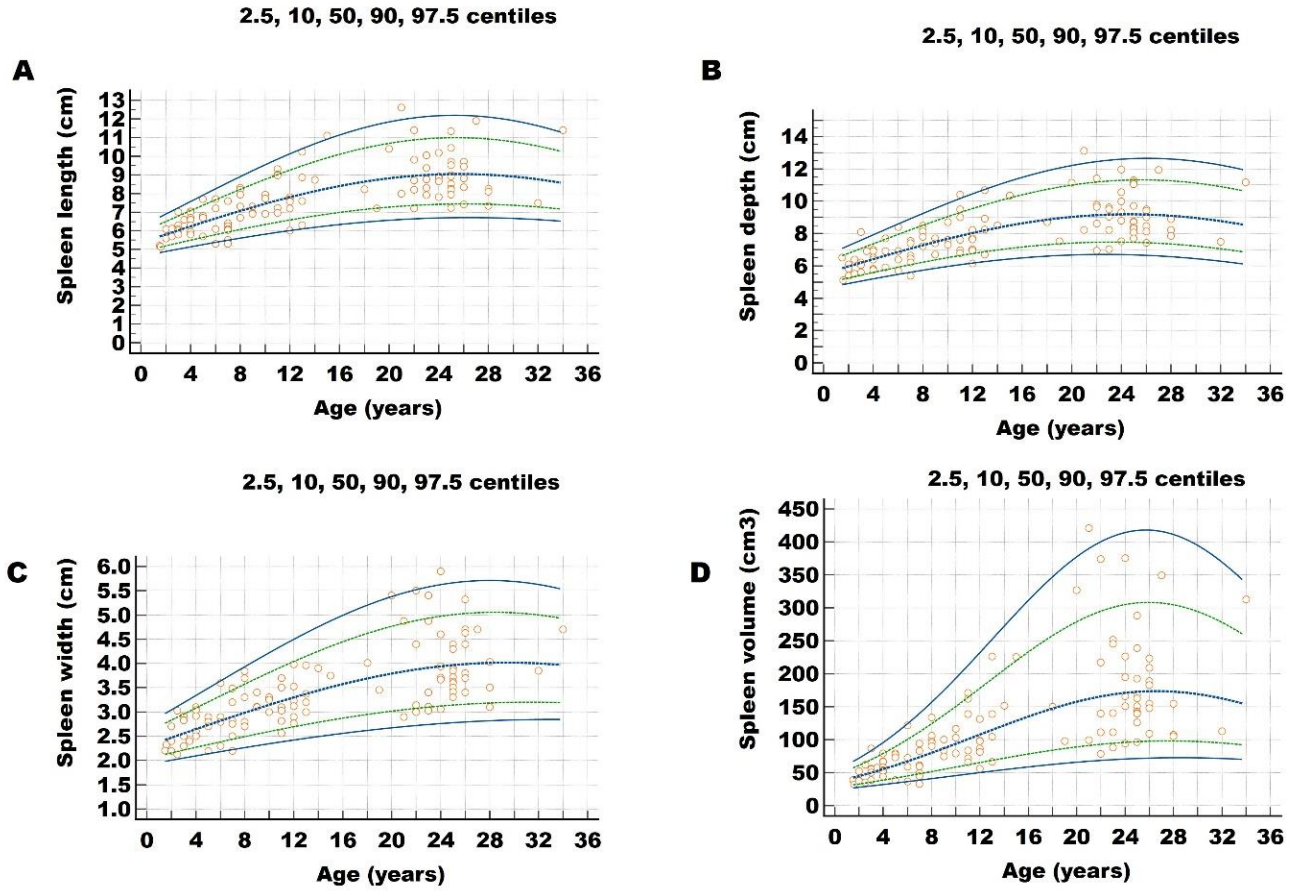


Fig. 2

Title: Abdominal ultrasonography showing various spleen sizes among the SCD patients

Legend: (A) Small-sized spleen (spleen length 4.3 cm) in a seven-year-old Hb SS female (B) Normal sized spleen (spleen length 8.4 cm) in a seven-year-old Hb SS female child (C) Longitudinal view of an enlarged spleen in a six-year-old Hb SS male (spleen length 9.9 cm) (Spleen sizes are compared to controls values as shown in Table 1).

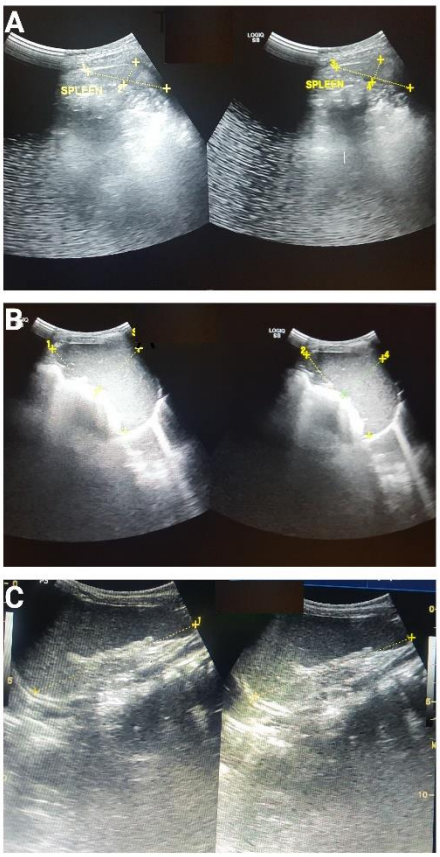
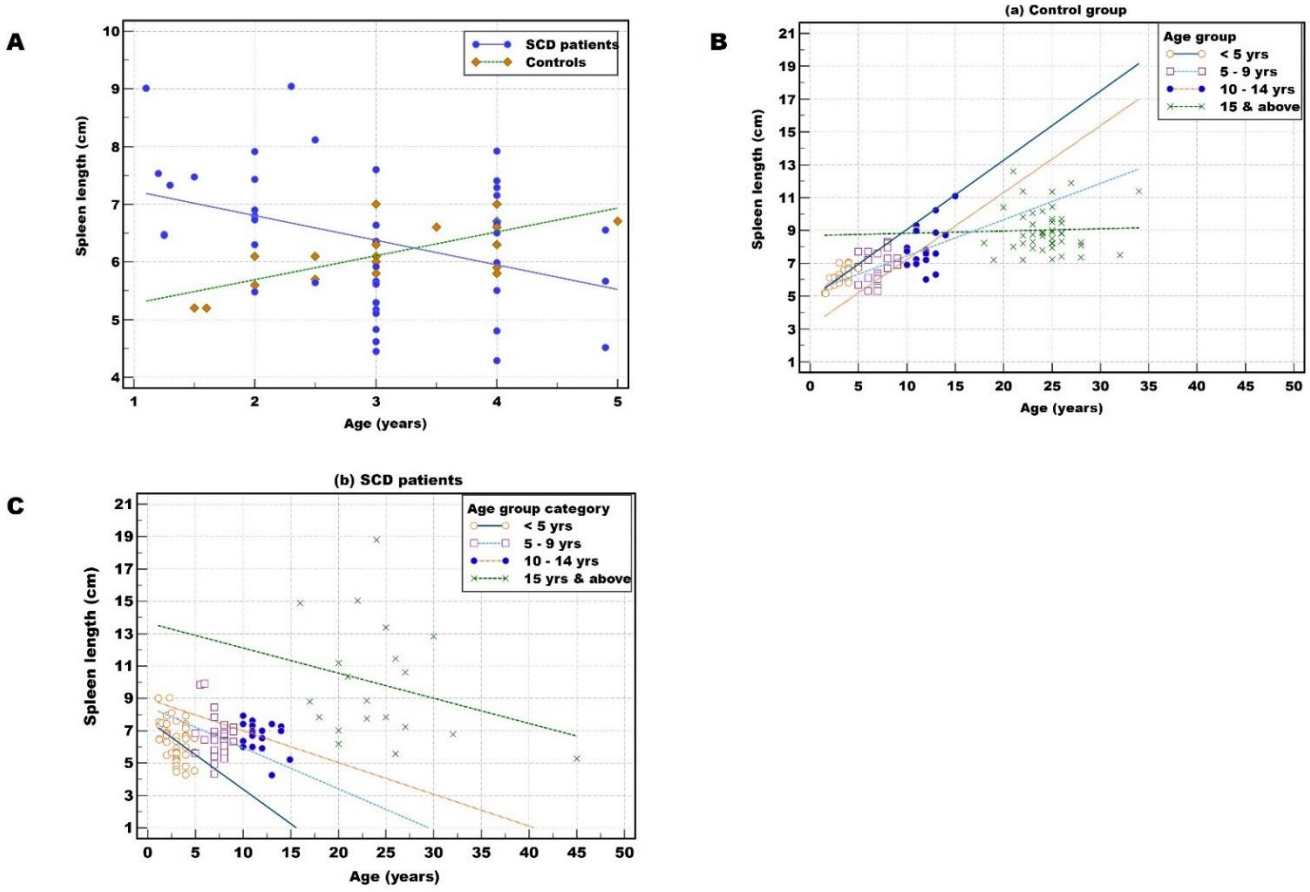


Fig. 3: Scatter plots showing the relationship between spleen length (Y-axis) and age (X-axis among the SCD and controls.



Legend: (A) Among the less than five years old, the spleen length was almost three-fold larger in the SCD group (n=42) in the first two years of life compared to the controls (n=22), before decreasing abruptly around the third year. (B) In the control group (n=109), the spleen length shows a progressive rise with increasing age up to the third decade before levelling off. (C) Among the SCD patients (n=107), the spleen length was similar in the less than five years old and those 5 to 9 years. The spleen length becomes static in those aged 10 to 14 years; after 15 years the spleen length becomes variable with normal and enlarged spleens present. Fitted lines superimposed for all age groups.

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