Puberty During Sickle Cell Anemia In Cameroonian Children: A Case Control Study

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Abstract

Background Puberty is reported to be impaired in children with Sickle Cell Anemia (SCA). We therefore, aimed to explore clinical and hormonal features of puberty in Cameroonian children with emphasis on the factors associated with delayed puberty during SCA.

Methods During a case-control study, we included 64 children aged 8 to 18 years with SCA matched to healthy controls. We assessed height, weight, body mass index, body composition and Tanner stages. Hormonal measurements included Follicle Stimulating Hormone, Luteinizing Hormone and sex steroids (estrogens/ testosterone) with radio-immunologic assays. We used a non-parametric test (Mann U Whitney Wilcoxon) to compare the median values between cases and controls. We looked into associations between severity criteria of SCA and delayed puberty through multivariate analysis.

Results Delayed puberty was reported in 27.3% of girls and 10% of boys with SCA. Median age of menarche was delayed by 2 years compared to controls. SCA patients had low free fat mass compared to controls ($p = 0.03$). Abnormal levels of Antimullerian hormone were reported in cases. History of severe infection, acute chest syndrome as well as low hemoglobin level were associated with delayed sexual maturation in children with SCA.

Conclusion Our study reveals delayed puberty in children with SCA. Moreover, puberty is affected by severity of the disease. This highlights importance of regular monitoring of puberty during follow-up of these children.

Background

Puberty is reported to be impaired in children with Sickle Cell Anemia (SCA)[1]. Indeed, delayed puberty accounts for 6.8% of chronic complications of this condition in Europe[2]. The gonadotropic axis is affected by hypoxia secondary to chronic anemia and recurrent vaso-occlusive events [3]. Hypogonadotropic hypogonadism is reported in 3–5% of children with sickle cell anemia [3].

Some studies report a delayed onset of puberty correlated with low levels of gonadotropins and sex steroids[4][5][6]. Clinical and biological features related to delayed puberty in SCA include severity of disease, nutritional status and baseline hemoglobin level.

Few studies in sub-Saharan Africa highlight relationship between low hemoglobin levels, clinical

features of SCA and delayed onset of puberty.

A previous study in Cameroon reported delayed puberty in 11, 54% of boys and 29, 7% of girls[7]. But, the relationship between severity of SCA and puberty is not well known in our country. We aimed to explore clinical and hormonal features of puberty in Cameroonian children compared to healthy controls with emphasis on the factors associated with delayed puberty during Sickle Cell Anemia.

Methods

Patients: During a 10 months' period (September 11, 2015 to June 16, 2016), we did a case-control study during which we compared 2 groups of children. We included 64 children aged 8 to 18 years for girls and 9 to 18 years for boys with confirmed diagnosis of SCA in steady state at the time of the study and attending the Mother and Child Center (MCC) in Yaounde for at least three months. Each case was matched for age and sex to a control with an AA hemoglobin electrophoresis. We performed an alkaline pH cellulose acetate electrophoresis in the MCC's laboratory in order to confirm hemoglobin genotype for controls. In the two groups, we excluded those who had another known medical condition which may affect puberty (chronic kidney failure, cardiac disease, HIV, endocrine disease). Controls were recruited among relatives of the SCA patients.

Procedure: We collected data related to SCA from files and parent's interviews. Severity criteria included more than three vaso-occlusive crises, three hospitalizations for infection and/or three blood transfusions during the last year, past history of acute chest syndrome and/or priapism. We also reported lab results for the last three months' visits in steady state for hemolysis (hemoglobin level, Lactate dehydrogenase, free bilirubin level) and bone marrow activity (white blood cells and platelets count, percentage of Hemoglobin F).

We reported anthropometric parameters using the 2007 WHO charts for children aged 5 to 19 years to calculate height and BMI z- scores. We measured weight using a TANITA® BC–420 class III device to the nearest 0.1kg. We measured height using a Leicester Height Measure ® MK II stadiometer to the nearest 0.1cm. The Body Mass Index (BMI) was calculated from the weight and height measured using the Quetelet index: Weight (in kilograms) / square of the height (in meters). According to WHO norms: Stunting was defined by a height for age z-score (TAZ) less than—2 standard deviations;

Wasting was defined by a BMI z-score for age (BMI-Z) less than—2 standard deviations; Overweight and obesity were defined by a BMI-Z respectively greater than 2 and 3 standard deviations.

We measured body composition parameters with bioelectrical impedance analysis. We used a TANITA® BC 420 device (TANITA BC 420 MA, Tokyo, Japan). After recording the weight and height previously obtained, the children step on the electrodes with bare feet. Then, we recorded lean body mass and free fat mass (in Kg) values.

Pubertal development was evaluated during physical exam according to the Tanner staging. For girls, we recorded age of menarche, then we classified pubic hair (P) and breast development (B). For boys, we classified pubic hair (P) and testicular development (G) through testicular length (cm).

Hormone assays were also performed to assess pubertal development. We measured basal levels of Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), estradiol for girls and testosterone for boys. We collected a 2 ml sample of venous blood on a dry tube for each participant. After centrifugation, the serum was divided into 4 tubes of 0.5 ml and stored at the Mother and Child Centre’s laboratory at –20 ° C. Radioimmunoassays were performed at the Hormonology Laboratory of the Robert-Debré University Hospital in France. During transport, the right temperature was maintained with dry ice in an approved package for organic products. Serum concentration of each hormone was compared with reference values from the Robert Debré University Hospital laboratory adjusted to age, gender and Tanner stage.

Statistical analysis: We performed statistical analysis with SPSS software version 20.0 (IBM Corporation, Chicago, USA). WHO ANTHRO plus version 1.0.4 from WHO was used for the analysis of anthropometric data. Quantitative variables were expressed as median with the interquartile ranges. The Mann U Whitney Wilcoxon non-parametric test was used to compare the median values between cases and controls. We did a univariate analysis with Odd ratio estimate to look for association between SCA characteristics (severity criteria, biological markers of bone marrow activity, hemolysis markers) and pubertal development. A p value less than 5% was considered statistically significant.

Ethics approval: This study received ethical approval from the institutional board review of the Faculty of Medicine and Biomedical Sciences of Yaounde. We obtained parental consent for all children

enrolled during the study period. Also, children above 12 years gave their assent for this study.

Results

I. CHARACTERISTICS OF THE STUDY POPULATION

During the study period, we included 64 children with SCA and 64 controls with a median age of 11.6 and 11.3 years respectively (Range: 8-18 years). Girls were more represented in each group. Weight, height and BMI values were lower in children with SCA compared with controls. This difference was statistically significant for BMI (*p = 0.02*). With regards to body composition, there was no difference for fat mass in the two groups. Whereas, free fat mass (muscle mass) of SCA children was lower than values in controls (p = 0.03). (**Table 1**).

Table 1: Characteristics of the study population

Variables	Cases (n = 64) Median (IQR)	Controls (n = 64) Median (IQR)	P value
Age (years)	11.6 (8.2-17.1)	11.3 (8.1- 17.8)	0.5
Weight (Kg)	32 (27.9 – 36.1)	35.6 (30.7 – 44.7)	**0.05**
Height (cm)	142 (133 – 148)	146 (137 – 153)	0.1
HAZ	-1.17 (-1.95 ; -0.42)	-0.1 (-0.81 ; 0.5)	**< 0.001**
BMI (Kg/m2)	16.1 (15.3 – 17.4)	17 (15.6 – 19.1)	**0.02**
BMI-Z	-0.99 (-1.35 ; -0.39)	-0.29 (-1.08 ; 0.52)	**0.008**
Fat mass (%)	5 (5 – 10.6)	5 (5 – 11.4)	0.2
Free fat mass (Kg)	29.3 (26.5 – 33.4)	33.4 (28.6 – 37.2)	0.03

HAZ= Height for age z-score; BMI = Body Mass Index; BMI-Z= Body Mass Index z-score;

IQR= Interquartile range

Children with SCA presented with a median hemoglobin F of 9.9% and high levels of LDH with a median value of 1 464 IU/l and free bilirubin. In addition, median value of leukocytes and platelets were respectively 12 800 cell/mm3 and 407 500 cells/mm3 (**Table 2**).

Table 2: Biological features of children with sickle cell anemia

Variables	N	Median (IQR)
Hemoglobin F (%)	48/64	9.9 (6.4 – 14.2)
White blood cells (cells/mm^3)	56/64	12 800 (10 325 – 15 375)
Hemoglobin level (g/dl)	56/64	7.4 (6.5 – 8.1)
Platelets (cells/mm^3)	56/64	407 500 (326 000 – 555 500)
LDH (UI/L)	47/64	1 464 (1 028 – 1 789)
Free Bilirubin (mg/L)	46/64	33.8 (23.1 – 45)

II. PUBERTY

As for puberty, girls with SCA were older than controls at the onset of puberty (B2) **(Table 3).** Only 16.7% of girls with SCA had their menses compared to 22.2% of controls. The median age of menarche was 14.5 years in cases compared with 12 years in controls (*p = 0.002*). We found pubic

hair growth (P) in 69.4% of controls and 27.8% of cases (p = 0.01). We reported a delayed puberty among 27.3% of girls with SCA older than 13 years. At the onset of puberty (S2), median values of FSH, LH and estradiol were in the normal range in the two groups (**Table 4**).

Table 3: Median age at different Tanner stages for girls

Variable	Cases (n = 36)	Controls (n = 36)	P value
Breast development	**Median (IQR)**	**Median (IQR)**	
S1	10 (9.1 – 10.9)	9.5 (8.4 – 10.3)	1
S2	11.6 (10.4 – 13.3)	10.9 (10.1 – 11.3)	0.1
S3	14.1 (13.9 – 15.2)	11.4 (10.9 – 12.5)	**0.02**
S4	16.4 (16.3 – 17.2)	13.2 (12.7 – 14.2)	0.1

Table 4: Hormone levels at each Tanner stage for girls

	FSH (IU/L)		*p*	LH (IU/L)		*P*	Estradiol (pg/ml)		*P*
	Cases Median (IQR)	Controls Median (IQR)		Cases Median (IQR)	Controls Median (IQR)		Cases Median (IQR)	Controls Median (IQR)	
S1	1.9 (1.1 – 2.3)	1.7 (1.4 – 3.4)	**0.706**	0.1 (0.1 – 0.2)	0.1 (0.1 – 0.2)	**0.538**	14 (11 – 20)	11 (11 – 11)	**0.004**
S2	3.9 (2.2 – 5.1)	2.8 (2.1 – 4.2)	**1.000**	0.4 (0.2 – 2.3)	0.3 (0.2 – 0.6)	**0.738**	23 (11 – 29)	16.5 (11 – 40)	**0.442**
S3	4.3 (3.6 – 5.1)	3.8 (3.5 – 6.2)	**0.806**	2.8 (2.5 – 4)	2.3 (0.7 – 3.4)	**0.270**	30 (24 – 34)	30 (24 – 67)	**0.841**
S4	3.8 (2.3 – 7.9)	3.8 (2.7 – 5.7)	**0.732**	4.1 (2.2 – 6.8)	3.4 (2.2 – 6.2)	**0.838**	77 (28 – 97)	38 (31.8 – 96)	**0.838**

Among boys, SCA patients were older than controls at the same stages of testicular development with a gap of 1.7 years. The median age at onset of puberty (G2) was 13 years for cases against 11.3 years for controls (**Table 5**). Puberty (G2) had started in 92.9% of controls and 64.2% of cases. Pubic hair growth was present in 21.4% of boys with SCA compared to 53.6% of controls (p = 0.03). We reported delayed puberty among 10 % of boys older than 14 years. At the onset of puberty (G2), median values of gonadotropins were 0.9 IU/l vs 1.2 IU/l for FSH; 0.1 IU/l vs 0.2 IU/l for LH and 0.1ng/ml vs 0.1 ng/ml for testosterone respectively for cases and controls **(Table 6).** As for cases, median values of AMH were significantly higher than those of controls (45.9 ng/ml vs 17.65 ng/ml; *p= 0.018*).

Table 5: Median age at different Tanner stages for boys

Variable	Cases (n = 28)	Controls (n = 28)	P value
Testicular length	**Median (IQR)**	**Median (IQR)**	
G1	11.3 (10.7 – 11.6)	10.9 (10.2 – 11.7)	1
G2	13 (11.5 – 14.8)	11.3 (10.5 – 12.2)	0.4
G3	13.2 (12.9 – 15.6)	11.4 (10.8 – 12.8)	0.1
G4	15.9 (15.3 – 17.8)	12.8 (11 – 14)	**0.03**
G5	18.2 (17.8 – 18.6)	15.6 (15.6 – 15.6)	1

III. CLINICAL AND BIOLOGICAL FACTORS ASSOCIATED WITH RISK OF DELAYED PUBERTY IN SICKLE CELL ANEMIA

Boys with SCA were more likely to experience delayed puberty if they had a past history of infection and blood transfusion (**p = 0.03**). Whereas, in girls, the risk of delayed puberty was associated with past history of acute chest syndrome**.**

With respect to biological characteristics of SCA, percentage of hemoglobin F of less than 10% (OR = 2.2) and hemoglobin level less than 7 g / dl (OR = 1.4) were more likely to expose to breast development delay in girls. The same factors were relevant for delayed puberty in boys with SCA.

Discussion

Delayed puberty is commonly reported among children with SCA. We aimed to have an overview of pubertal development of children with SCA in our setting compared with healthy controls. In addition, we searched for clinical and biological factors which may expose to delayed puberty for this specific population. This study highlights delayed onset of puberty in children with SCA compared to controls. Some severity criteria such as recurrent infections and low hemoglobin levels increase the risk for delayed puberty.

We included SCA patients followed up in a reference center, but they were not representative of all children with SCA in Cameroon. However, our results give relevant data about pubertal development in patients with this condition.

In our study, 27.3% of girls and 10% of boys with SCA presented a delayed onset of puberty. A previous study in Cameroon in 2014 reported similar results[7]. But this result is lower than proportions reported in previous studies around the world. It varies from 37% to 50% for girls and

28.57–73 % for boys[8][6]. These differences could be explained by the smaller size of their study populations. On the other hand, their study groups included adolescents and young adults who were older than our subjects. This fact may have increased the probability to have a greater proportion of delayed puberty with respect to age groups.

According to literature, many mechanisms are described for delayed puberty during SCA. Chronic hypoxia related to recurrent vaso-occlusive events and chronic anemia lead to hypoplasia of pituitary gland and gonads[3]. On the other hand, iron overload secondary to hemolysis and multiple transfusions also affects the gonadotropic axis[9][10]. Micronutrient deficiency is also mentioned by some authors as part of pathogenesis of delayed puberty. Zemel et al. in 2002 found an improvement in weight and height in prepubertal children after zinc supplementation[11]. Since weight is a determining factor in the onset of puberty, zinc intake may be a protective factor in delayed puberty. Many factors could explain delayed puberty in our study population. Firstly, SCA children had median hemoglobin level of 7.4g/dl which suggest a state of chronic hypoxia. Moreover, their values of leukocytes and thrombocytes revealed a state of blood hyperviscosity which increase the risk of vaso-occlusive crises. Then, they had high levels of bilirubin and LDH which are markers of hemolysis.

Puberty among girls

At the onset of puberty, girls with SCA were older than controls with a gap of 0.7 years. Soliman et al in Egypt and M’Pemba Loufoua in Congo also found a gap of respectively 1.8 and 2 years at stage B2 between SCA subjects and controls[8][12]. The median age of female SCA cases at the onset of puberty was 11.6 years in our study. In contrast, Komba et al in 2015 found a median age of 8.89 years in Cameroonian girls[13]. This delayed onset of puberty in girls with SCA may be due to the deleterious effects of hypoxia and chronic hemolysis on the hypothalamo-pituitary-gonadal axis[3]. The median age of menarche was 14.5 years for girls with SCA compared with 12 years for controls. Whereas the median age of menarche reported by Pasquet et al was 13.18 years in Cameroon for healthy girls[14]. Soliman et al also reported that menarche was delayed by 2.2 years in their cohort of SCA subjects[12]. Our results show a delayed onset of menses in cases compared to controls. This could be explained by low weight and BMI identified in SCA patients compared with controls in our

study. The notion of target weight is described in literature as the determining factor for menarche in girls[15].Since girls with SCA are more likely to have delayed growth, they will reach the target weight needed for menarche later than healthy children. Serjeant et al in 2001 found that weight status was a predictive factor for the age of menarche in their cohort [4].

In our study group, hormone levels were within the normal range at each Tanner stage. This result was different from hypogonadism usually reported in adolescents with SCA.

Puberty among boys

Boys with SCA were older than controls at the onset of puberty with a gap of 1.7 years. Our findings are close to the 2.2-year gap reported by M'Pemba Loufoua et al in 2001. The median age of boys with SCA at the onset of puberty (stage G2) was 13 years in our study. Whereas, Komba et al found a median age of 9.63 years in healthy boys in the urban population of Cameroon [13]. Our results reflect delayed onset of puberty among boys with SCA compared to controls and urban population in Cameroon. This delay can be explained by recurrent vaso-occlusive crises which lead to hemolysis and chronic anemia. Moreover, multiple blood transfusions and chronic hemolysis may induce iron overload which is toxic for the pituitary gland and gonads[10]. Low hemoglobin levels, high bilirubin levels and LDH levels in our study population suggest a high tendency for hemolysis. At the onset of puberty, gonadotropins levels of cases were lower than values of controls. Moreover, AMH levels were higher in cases at G3 Tanner staging. AMH levels usually decrease when testosterone production increases during puberty. This increase of AMH level in SCA may be explained by hypogonadism which is mostly found among boys with SCA [3][12]. This suggests importance of long-term follow-up of these boys to look after fertility features.

Clinical and biological factors associated with pubertal development in Sickle Cell Anemia

Boys with SCA more often had delayed testicular development associated with a past history of blood transfusion (OR = 4) and severe infection (OR = 13). This association was statistically significant in case of severe infection (p = 0.03). Ozen et al in 2012 didn't find an association between the occurrence of an endocrine complication and the severity criteria of sickle cell disease [4].

Biological parameters such as a hemoglobin F of less than 10%, a hemoglobin level of less than 7 g / dl (OR = 3.7) and leukocytes greater than 10,000 cells per mm3 (OR = 9) were associated with delayed testicular development. This association was statistically significant for hemoglobin F (p = 0.02) and leukocytes (p = 0.03). M'Pemba Loufoua et al found association between hemoglobin levels below 7 g / dl in SCA boys and delayed puberty. This may suggest that anemia is a deleterious factor for testicular development. Silva et al found an association between leukocytosis and the occurrence of chronic complications such as delayed puberty [2].

Girls with SCA and history of acute chest syndrome were 2.8 times more likely to have delayed breast development. This association was not statistically significant. This could be explained by the fact that acute chest syndrome induces hypoxia by hypoventilation [14]. This could disrupt tissue oxygen uptake in the pituitary gland and gonads. This would lead to hypogonadism with a slowing down of pubertal maturation[3].

Biological parameters such as a percentage of hemoglobin F less than 10% (OR = 2.2) and hemoglobin level less than 7 g / dl (OR = 1.4) exposed to delayed breast development among girls. Despite the fact that this finding was not statistically significant, hemoglobin F greater than 10% is a protective factor for vaso-occlusive crises[3]. Thus, a value lower than 10% can promote episodes of hypoxia, deleterious for gonadotropic axis. The use of hydroxyurea in eligible patients improves the concentration of hemoglobin F. Furthermore, a low hemoglobin level exposes the patient to high transfusion requirements. This can lead to an iron overload, which is toxic for gonadal cells[10]. The practice of exchange transfusions increases basic hemoglobin level and therefore improves tissue perfusion[17].

Conclusion

Delayed puberty is present in our setting. Puberty is affected by severity of the disease. Caregivers should check for sexual maturation in the follow-up of children with SCA. Some interventions may improve their puberty.

List Of Abbreviations

AMH: Anti-mullerian Hormone*; BMI:* Body Mass Index*; FSH:* Follicle Stimulating Hormone; *LH:*

Luteinizing Hormone*; MCC:* Mother and Child Center; *SCA:* Sickle cell anemia*;*

Declarations

Ethics approval and consent to participate: The study received ethical approval of the institutional board review of the Faculty of Medicine and Biomedical Sciences of Yaounde 1 University. Parents or guardians provided written informed consent for adolescents enrolled during the study period.

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Competing interests: The authors declare that they have no competing interests

Authors' contributions:

Study concept and design: SNU, MBR, AYA, DS
Literature review: MBR, SNU, KNP
Data collection: MBR, TNJ, DC, DS
Statistical analysis MBR, SNU
Writing: MBR, SNU, TNJ
Review: SNU, CD, DS, DC, AYA
Study supervision: KNP

All authors read and approved the final manuscript.

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Tables

Table 6: Hormones levels at each Tanner stage for boys

	FSH (IU/L)		*p*	LH (IU/L)		*P*	Testosterone (ng/ml)		*p*	AMH (ng/ml)		P
	Cases Median (IQR)	Controls Median (IQR)		Cases Median (IQR)	Controls Median (IQR)		Cases Median (IQR)	Controls Median (IQR)		Cases Median (IQR)	Controls Median (IQR)	
G1	0.6 (0.4 – 2.7)	0.7 (0.7 – 0.7)	0.827	0.1 (0.1 – 0.11)	0.1 (0.1 – 0.1)	0.4311	0.1 (0.1 – 0.5)		/	17.5 (15.9 – 27.6)	23.8 (23.8 – 23.8)	0.8622
G2	0.9 (0.3 – 1.4)	1.2 (0.5 – 1.5)	0.444	0.1 (0.1 – 0.4)	0.2 (0.1 – 0.4)	0.2788	0.1 (0.1 – 0.13)	0.1 (0.1 – 0.11)	0.212	34 (17.7 – 52.7)	46.5 (44.6 – 102.3)	0.3335
G3	2.6 (2.2 – 2.9)	1.9 (1.1 – 2.3)	0.242	1.2 (0.8 – 1.6)	0.8 (0.2 – 1.5)	0.3800	0.13 (0.1 – 0.15)	0.18 (0.1 – 0.4)	0.421	41.6 (41.6 – 41.6)	33.4 (18.2 – 52.7)	1.000
G4	4.3 (2.7 – 9.4)	4.1 (3.5 – 4.7)	1.000	1.9 (1.3 – 3.2)	1.5 (0.9 – 1.9)	0.2488	0.86 (0.3 – 4.9)	1.4 (1.4 – 1.4)	0.655	4.9 (4 – 12.3)	8.4 (8.4 – 8.4)	0.655
G5	9.5 (9.1 – 9.8)	3.4 (3.4 – 3.4)	0.221	9.4 (8.7 – 10.2)	3.7 (3.7 – 3.7)	0.2211	6.3 (5.9 – 6.6)	1.9 (1.9 – 1.9)	0.221	3.5 (3.5 – 3.5)	4.4 (4.4 – 4.4)	0.317