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Impaired pubertal development and testicular hormone function in males with sickle cell anemia

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ABSTRACT

Changes in weight/height ratio, delayed sexual maturation, hypogonadism and impaired fertility have been demonstrated in sickle cell disease (SCD). This study aimed to evaluate the clinical and laboratory views of the Leydig cells function after stimulation with hCG in adults with sickle cell disease. We studied 15 patients with SCD (18 to 40 years; median = 27 years old), fourteen homozygous S, and one with SC disease. The control group, composed by adult males, was divided into two groups: I – 10 relatives (18–39 years, median = 26 years) with the same socioeconomic level of the patients, and II – 9 normal individuals (23–28, median = 31 years) randomly chosen. Clinically it was observed a slight degree of malnutrition, important puberty delay, rarefaction of chest, underarm and pubic hair, and important reduction of the testis and penis size, featuring a mild hypogonadism in patients with SCD. The hormonal level assessment of testosterone at baseline and at 24, 48 and 72 h after hCG stimulation showed no significant differences between the groups studied. We can presume that adult men with SCD showed clinical hypoandrogenism with normal testicular hormonal function, a fact inconsistent with the hypothesis of primary hypogonadism.

Introduction

Variation on growth and sexual development, mainly regarding external sexual characteristics, has been observed in individuals with sickle cell disease (SCD) [1–4]. These manifestations can cause serious psychological problems, difficulties on school and work adaptation, and loss of self-esteem [5].

Prospective studies involving children and adolescents with SCD have demonstrated an overall growth delay and a significant retardation in sexual maturation and consequent puberty [6–9]. Hypogonadism and fertility problems in adult life have been observed in some of these subjects [10,11].

Abbasi et al. [12], studying changes in gonadal function of 14 adult males with SCD, found low levels of androgen, high basal levels of luteotrophic gonadotrophins (LH) and follicle-stimulating hormone (FSH), as well as an exacerbated response of LH to gonadotrophin-releasing hormone (GnRH). The authors attributed these findings to primary hypogonadism such as other investigators [13,14].

On the other hand, a Nigerian study showed that SCD patients presented a significant decline in plasma levels of testosterone (T), LH and FSH, comparing with other individuals [10]. The authors concluded that these patients had secondary hypogonadism due to pituitary dysfunction and suggested that this should be the result of multiple pituitary thrombi, in agreement with other authors [11].

Given the high incidence of SCD in the Brazilian population [15] and the fact that the etiology of the delay in growth and sexual development remains controversial, the aim of the present study was to evaluate testicular function of adult men, in order to contribute to a better understanding of the pathogenesis of this important dysfunction.

Materials and methods

Fifteen adult patients with SCD (PG), 14 with homozygosis for hemoglobin (HbSS) and 1 with double heterozygosis for HbS and HbC, were evaluated. The patients were followed at the Outpatients Clinic of Hereditary Anemias at Escola Paulista de Medicina/UNIFESP, and the chronological age ranges from 18 to 40 years of age (median: 27 years). All of the patients were in steady state, without infectious process or vaso-occlusive crisis. No one of them was on chronic transfusion program or had received blood transfusions in the past 3 months.

The control group (CG) was divided in two: a) CG-I composed of 10 clinically healthy patients' relatives with the same socioeconomic status, aged 18–39 years (median: 26 years), 80% with sickle cell trait; b) CG-II composed of 9 voluntary individuals consisted of hospital workers and medical students, with ages ranging from 23 to 38 years

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Table 1
Nutritional assessment in patients (PG), in relatives of patients (CG-I) and in normal men (CG-II).

Group	Weight (kg)[a]	Wrist (cm)	Height (cm)	BSA (m^2)[a]
	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$
PG ($n = 15$)	56.99 ± 10.46	15.53 ± 0.85	169.15 ± 6.25	1.65 ± 0.16
CG-I ($n = 10$)	73.62 ± 10.09	17.90 ± 0.88	170.15 ± 3.76	1.83 ± 0.11
CG-II ($n = 9$)	77.74 ± 12.37	17.33 ± 1.00	175.72 ± 9.40	1.93 ± 0.18
	p-value			
ANOVA-F	0.0001	<0.0001	0.0700	0.0003
PG × CG-I:	0.0050	0.0001	0.9400	0.0279
PG × CG-II	0.0010	0.0006	0.1083	0.0014
CG-I × CG-II	0.7035	0.3871	0.1957	0.3801

$\overline{X}$: mean; SD: standard deviation; PG: patient group; CG: control group; BSA: body surface area. [a]$p < 0.005$.

(median: 31 years). The ethnic composition of the PG group was 12 African descendants (80%), two Caucasian descendants (13.3%) and one mulatto (mixed Caucasian and African descendants) (6.7%). CG-I group was 5 African descendants (50%), one Caucasian descendant (10%) and four mulattos (40%). Differently of the other groups, the CG-II presented a higher percentage of Caucasian descendant individuals (89%).

This study was approved by the Ethics and Research Committee of the Institution. Written Informed Consent Form was obtained from all of the men involved in the research.

The hematological parameters were obtained by electronic cell counter. The reticulocyte counting was carried out by standardized microscopic methods. The diagnosis of SCD was established by hemoglobin electrophoresis on cellulose acetate at alkali pH and confirmed on agar gel electrophoresis at acid pH, and fetal hemoglobin (HbF) was measured by the alkali denaturation.

Clinical assessment consisted of anamnesis and complementary interview to obtain information about the installation of the pubertal phase, libido, fertility, gynecomastia, and priapism, in both patients and control groups. They were classified in relation to pubertal staging according to Tanner criteria [16]. The measurement of the width and length testicular with a pachymeter, the testicular volume [17], and the length and diameter of the penis were also determined. The bone age was assessed by hand and wrist radiographies, calculated by Greenwich's and Pyle's criteria [18]. The nutritional assessment of the patients and control individuals was carried out according to WHO's criteria [19].

All groups were submitted to the human chorionic gonadotrophin (hCG) stimulation test. For this purpose, three peripheral blood samples were collected and pooled for the measurement of basal testosterone at −30, −15 and 0 min (between 7 and 10 am). hCG (5000 IU) was administered intramuscularly at time zero and new blood samples were collected 24, 48 and 72 h after injection [20]. The serum obtained from the six samples was stored at −20 °C until the time of the testosterone assay. Plasma testosterone was determined by radioimmunoassay according to the technique of Lox and Mallory [21]. Standardization of the assays provided coefficients of intra-assay and interassay variation of 7.3% and 10%, respectively, for values within the normal range.

The data were subjected to a descriptive analysis from measures of centrality and dispersion. The variables were tested with the Shapiro–Wilk normality and homogeneity of variance Bartlett. For comparison of variables between groups was performed initially an ANOVA F-one-way followed by Tukey's multiple comparisons test. The trend in the testosterone level at baseline within 72 h was performed from the linear regression model by least squares method of Pearson. For all statistical tests the significance level of 5% was used.

Table 2
Age in years, the onset of puberty, the appearance of hair, first ejaculation and voice disorders in patients (PG), relatives of patients (CG-I) and normal men (CG-II).

	Onset of puberty	Appearance of hair	First ejaculation	Change of voice
	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$
PG ($n = 15$)	15.80 ± 1.61	16.00 ± 1.81	16.80 ± 1.81	16.80 ± 1.78
CG-I ($n = 10$)	12.80 ± 0.92	13.20 ± 1.55	13.20 ± 1.40	14.30 ± 1.83
CG-II ($n = 9$)	12.67 ± 0.87	13.11 ± 1.45	13.67 ± 1.22	14.32 ± 0.83
	p-value			
ANOVA-F	<0.0001	0.0001	0.0002	0.0003
PG × CG-I:	0.0001	0.0019	0.0011	0.0043
PG × CG-II	0.0002	0.0024	0.0094	0.0052
CG-I × CG-II	0.9731	0.9929	0.8028	0.9943

$\overline{X}$: mean; SD: standard deviation; PG: patient group; CG: control group.

Table 3
Volume, length and width in patients with testicular (PG), in relatives of patients (CG-I) and in normal men (CG-II).

	Testicular volume (mL)	Testicular length (cm)	Testicular width (cm)
Group	$\overline{X} \pm SD$	E	D
PG ($n = 15$)	17.93 ± 3.58	4.00 ± 0.57	1.69 ± 0.23
CG-I ($n = 10$)	25.50 ± 1.58	4.51 ± 0.31	2.18 ± 0.17
CG-II ($n = 9$)	23.33 ± 2.29	4.46 ± 0.37	2.17 ± 0.14
	p-value		
ANOVA-F	<0.0001	0.0169	<0.0001
PG × CG-I:	0.0002	0.0484	0.0001
PG × CG-II	0.0009	0.1066	0.0002
CG-I × CG-II	0.6549	0.9661	0.9884

$\overline{X}$: mean; SD: standard deviation; PG: patient group; CG: control group.

Results

Fetal hemoglobin measurement in PG ranged from 1.0% to 5.6%. In relation to clinical severity, all patients were out of crisis and had not been transfused in the past 3 months.

Data on the nutritional assessment of patients and controls are shown in Table 1. Excluding height, mean values observed were significantly lower in patients-PG compared to controls (CG-I and CG-II) (see Table 1).

As can be noticed in Table 2, there were significant differences between patients and controls regarding age at onset of puberty, at onset of hair development, at first ejaculation and at time of voice change, being all them higher in patients. No significant differences were observed between CG-I and CG-II. In addition, 93.3% of the patients presented scant chest, axillary, and pubic hair.

Width and volume testicular were significantly lower in PG compared with CG. Significant differences were also observed regarding testicular length between patients and CG-I (Table 3).

The mean penis length was 9.9, 11.0 and 10.5 cm in PG, CG-I and CG-II, respectively. Significant differences were only observed between patients and CG-I. The Tanner criteria of pubertal stage classified one patient as G3P3 and two patients as G5P4, whereas all controls and the rest of the patients were G5P5.

Standardization of the hCG stimulation test: Table 4 shows the mean testosterone levels obtained in patients and CG-I and -II at different

Table 4
Levels of testosterone ng/dL at baseline and after 5000 IU of hCG in patients (PG), in relatives of patients (CG-I) and in normal men (CG-II).

Group	Basal	24 (h)	48 (h)	72 (h)
PG ($n = 15$)	434 ± 154	554 ± 115	596 ± 193	671 ± 155
CG-I ($n = 10$)	551 ± 161	644 ± 175	698 ± 175	781 ± 135
CG-II ($n = 9$)	505 ± 103	634 ± 177	618 ± 120	757 ± 109

Variance (DF × CI × CII) FcR = 3.32, Fc basal = 2.05, Fc 24 h = 1.35, Fc 48 h = 1.07 and Fc 72 h 2.18. PG: patient group; CG: control group.