Does blood transfusion affect pituitary gonadal axis and sperm parameters in young males with sickle cell disease?

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ABSTRACT

Objective: We evaluated the effect of packed red cell transfusion (PCTx) on serum concentrations of gonadotropins luteinizing hormone and follicle-stimulating hormone (LH and FSH) and testosterone (T) levels and measured sperm parameters in young adults with sickle cell disease (SCD) on top-up transfusion (TTx) and those on exchange transfusion (ETx) regimen. **Materials and Methods:** Basal serum concentrations of FSH, LH, and T and semen parameters were evaluated before and 7 days after PCTx in 18 young adults with transfusion-dependent SCD, aged 20.7 ± 2.88 years. They had full pubertal development (Tanner's stage 5), and capacity to ejaculate. They were regularly transfused since early childhood. Chelation therapy was started early during the first 2 years of life using desferrioxamine and was replaced by deferasirox for the last 4-5 years. Ten patients were on TTx and eight were on ETx regimen. **Results:** PCTx significantly increased hemoglobin (Hb) from 8.5 ± 1.17 g/dl to 10.5 ± 0.4 g/dl, T from 12.3 ± 1.24 nmol/L to 14.23 ± 1.22 nmol/L and gonadotropins' concentrations. Sperm parameters improved significantly after PCTx including: total sperm count from 87.4 ± 24.6 million/ml to 146.2 ± 51.25 million/ml, total progressive sperm motility (TPM) from 40.8 ± 11.1 million/ml to 93.4 ± 38.3 million/ml, rapid progressive sperm motility (RPM) progressive motility from 29.26 ± 8.75 million/ml to 67.4 ± 29 million/ml. After PCTx the total sperm count, TPM and RPM were significantly better in the ETx group versus the TTx group. Before and after PCTx, T concentrations were correlated significantly with sperm total count, volume, TPM and RPM (r = 0.53, 0.55, 0.42, and 0.38, respectively, P < 0.01). Hb concentrations were correlated significantly with sperm count, TPM, RPM, and % of sperms with normal morphology (r = 0.60, 0.69, 0.66, and 0.86, respectively, P < 0.001). **Conclusion:** Our study suggests that in males with SCD blood transfusion is associated with significant acute enhancement of sperm parameters and with increased concentrations of serum T, LH, and FSH. Improvement of sperm parameters were significantly better in the ETx group verses the TTx group. These "acute" effects on spermiogenesis are reached with an unknown mechanism/s and suggest a number of pathways that need further human and/or experimental studies.

Key words: Desferrioxamine, gonadotropins, sickle cell disease, spermatogenesis, testosterone

Introduction

Recent progress in the therapy of sickle cell disease (SCD), has considerably improved the prognosis of patients with this disease.[1] Their mean life expectancy is currently about 50 years, their quality of life has improved and many more patients now reach reproductive age.[2]

Few analyses of sperm parameters in patients with SCD have been published till now. All these studies described alterations of spermatozoa concentration, motility and morphology. Some reported a decrease in ejaculate volume and sperm vitality. The majority of the patients with SCD had an impairment of at least one sperm parameter.[3-7]

In addition, patients with SCD often have delayed puberty and moderate to severe hypogonadism, of unknown

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origin.[8-11] Several mechanisms have been suggested including primary hypogonadism, hypogonadism induced by repeated testicular infarction, and zinc deficiency.[11-15]

In SCD, hypoxic conditions promote sickling of erythrocytes containing Hemoglobin–S (HbS), with resultant impairment in blood flow, ischemia, micro-infarct, and organ necrosis. Micro-radio-angiographic studies in patients with SCD show a significantly reduced number of small vessels, abnormal dilation, or obliteration of the remaining capillaries with consequent loss in the function.[16-18] In addition, clinical studies show that chronic anemia has much greater impacts on tissue and organ functions than previously thought.[19,20]

Transfusions have been the traditional modality for treatment of chronic complications of SCD. Blood transfusions packed red cell transfusion (PCTx) improve blood flow by reducing the proportion of red cells capable of forming sickle hemoglobin (Hb) polymer. This limits hemolysis and the endothelial damage that result from high proportions of sickle polymer-containing red cells. Additionally, transfusions are used to increase blood oxygen carrying capacity in sickle cell patients with severe chronic anemia or with severe anemic episodes.

The aim of simple additive or top-up transfusion (TTx) in SCD is to restore Hb to normal steady state. The Hb should is raised acutely to 10 g/dl or hematocrit to = 30%. The aim of exchange transfusion (ETx) using the COBE Spectra is to reduce the percentage of HbS to < 30% whilst keeping the Hb below 11 g/dl. ETx is used in patients with previous neurological complications. However, the major and unavoidable complication of transfusions in SCD is iron overload. Therefore, in the transfusion-dependent type of SCD, iron toxicity may also add to different organ damage including the pituitary gland and gonads.[21-23]

The purpose of this study was to evaluate the acute effect/s of blood transfusion on the pituitary testicular axis and sperm parameters in 18 males with SCD.

Materials and Methods

Eighteen young adults with SCD on packed cell transfusion (10 on TTx, and eight on ETx after previous stroke during their childhood period. Patients were on iron chelation therapy using oral deferasirox in the last 3-4 years (30-40 mg/kg body weight once a day).

All patients were examined with special emphasis on anthropometric measurements including weight, height, and body mass index (BMI). Sexual maturation was assessed according to Tanner *et al*. maturity rating[24] and testicular volume was measured by Prader's orchidometer. One patient from the ETx group was on hydroxyurea therapy. Patients with hepatitis or abnormal liver functions tests were excluded.

The following investigations were performed before and 7 days after blood transfusion:

1. Semen analysis (count, morphology, and motility) after an abstinence interval of 3-4 days.
2. Measurement at 8 a.m. of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T) serum levels.

Conventional semen analysis was carried out using manual procedures and light microscopy, in the central hospital laboratory, according to the last WHO guidelines.[25] The semen analyzer was blinded to patients' names and diagnosis.

The following nomenclature was used to define the semen quality:

1. Normozoospermia: A total number of spermatozoa, and a percentages of progressively motile and morphologically normal spermatozoa, equal to or above the lower reference limit
2. Oligospermia: A total number of spermatozoa below the lower reference limit (5th percentile: 12 million/ml)
3. Asthenozoospermia: A percentages of progressively motile spermatozoa below the lower reference limits (5th percentile: 31%)
4. Oligoasthenozoospermia was defined as a total number of spermatozoa, and percentage of progressively motile spermatozoa, below the lower reference limits
5. Teratozoospermia: A percentage of morphologically normal spermatozoa below the lower reference limit (5th percentile: 3%)
6. Asthenoteratozoospermia: A percentage of both progressively motile and morphologically normal spermatozoa below the lower reference limits.

Normal serum levels for hormones in our central lab for age (18-30 years) are: T =21.4 ± 5.9 nmol/L, LH = 4.5 ± 1.2 IU/L, FSH = 3.2 ± 0.9 IU/L).

Hypogonadism was diagnosed when serum T values are below 7.6 nmol/L (220 ng/dL).

The ethical committee of Hamad Medical Center (HMC) approved the study and informed consents were obtained from all the patients.

All hormone levels were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, California 90045, U.S.A.)

The results are presented as mean ± standard deviation (SD), and the paired *t*-test was applied to analyze the data when normally distributed and Wilcoxon test was applied when the data were not normally distributed. Linear regression equation was used to study the relations between different variables ($P < 0.05$ was chosen as the limit of significance).

Results

All patients with SCD (n = 18, age 20.7 ± 2.9 year) had a normal sexual development (Tanner's stage 5), with normal secondary sex characteristics, normal testicular volume (17.4 ± 3.1 ml) and were able to ejaculate. None of the 18 patients had oligospermia, asthenozoospermia, teratospermia, or asthenoteratozoospermia.

After PCTx significant increase of Hb from 8.5 ± 1.2 g/dl to 10.5 ± 0.4 g/dl was associated with increased concentrations of T (from 12.3 ± 1.2 nmol/L to 14.2 ± 1.2 nmol/L), LH (from 4.4 ± 0.9 U/L to 5.7 ± 0.85 U/L), and FSH (from 5.4 ± 1.46 U/L to 6.6 ± 1.8 U/L). Total sperm count increased significantly from 87.4 ± 24.6 million/ml to 146 ± 51 million/ml and rapid progressive sperm motility (RPM) increased from 29.3 ± 8.75 million/ml to 67.4 ± 29.1 million/ml [Table 1].

Comparison between the two study groups (ETx vs. TTx) revealed that after PCTx patients on ETx had significantly better semen parameters including total sperm count, total progressive sperm motility (TPM), RPM, and percentage of sperms with normal morphology [Table 1]. Gonadotrophins and T concentrations did not differ among the two groups.

Before and after PCTx, T concentrations were correlated significantly with sperm total count, volume, TPM, and RPM (r = 0.53, 0.55, 0.42, and 0.38 respectively, P: 0.01). Before and after PCTx, Hb concentrations were correlated significantly with Sperm count, TPM, RPM, and percentage of sperms with normal morphology (r = 0.60, 0.69, 0.66, and 0.86, respectively, $P < 0.001$) [Figures 1 and 2].

Table 1: Hormonal and sperm parameters in patients with sickle cell disease before and after transfusion

	SCD-exchange *n*=8	SCD-top-up *n*=10	SCD-total *n*=18
Age (year)	20.33±2.16	21±3.67	20.7±2.88
LH-1 U/L	3.83±0.75	4.8±0.97	4.4±0.94
LH-2 U/L	5.5±0.55#	5.9±1.17#	5.7±0.85#
FSH-1 U/L	5.5±1.05	5.8±0.97	5.4±1.46
FSH-2 U/L	6.67±1.75#	7.11±0.93#	6.6±1.8#
Testost-1 (nmol/L)	12.1±1.45	12.48±1.42	12.3±1.24
Testost-2 (nmol/L)	14.2±1.17#	14.26±1.32#	14.23±1.22#
Hb-1 g/dL	7.9±1.5	8.1±1.9	8.5±1.17
Hb-2 g/dL	10±1.2#	10.9±0.2#	10.5±0.4#
Ferritin	2085±375*	1189±225	1488±557
Sperm count-1 (M/ml)	85.3±15.5	90.56±22	87.4±24.6
Sperm count-2 (M/ml)	197±38.4#*	122.7±23.5#	146.2±51.25#
Volume-1 (ml)	2.5±0.32*	122.7±23.5#	146.2±51.25#
Volume-2 (ml)	2.83±0.4#*	2.22±05	2.35±0.7#
Total PM (M/ml)-1	44.7±7.3*	41.8±7.7	40.8±11.1
Total PM (M/ml)-2	129.2±37#*	75.3±19.16#	93.4±38.3#
RPM (M/ml)-1	32±10.1*	27.44±7.7	29.26±8.75
RPM (M/ml)-2	88.2±31#*	53.56±18.5#	67.4±29.1#
NPM (M/ml)-1	17.8±9.15	25.3±10.8	21.6±10.25
NPM (M/ml)-2	27.8±12.2#*	29.7±9.15#	28.5±12.7#
Immotile-1 (M/ml)	22.8±6.7	23.44±9.2	23.2±8
Immotile-2 (M/ml)	40±15.9#*	24.67±7.26	30.8±13.45#
Normal morphology-1%	32.7±5.43	31.44±5.43	31.9±5.27
Normal morphology-2%	57.2±3.3#*	51.56±1.13#	53.8±3.57#

*P<0.05 exchange transfusion group (ETx) versus top-up group (TTx), #P<0.05, after versus before PCTx, 1: Before, 2: After PCTx, SCD: Sickle cell disease, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, Hb: Hemoglobin, RPM: Rapid progressive sperm motility, NPM: Normal progressive motility

Discussion

Infertility is a major problem in SCD patients, especially in males. In addition to low serum T, other abnormalities involving the accessory sex organs, such as the seminal vesicles and the prostate gland, as well as marked decrease in ejaculate volume and sperm parameters may be observed in male patients.[3-15,26] Recently, some data showed that blood transfusion produce significant acute changes in the hormonal milieu and sperm parameters of patients with chronic hemolytic anemia.[27-29] Therefore, we studied the acute effects of PCTx on spontaneous spermatogenesis and pituitary testicular axis in young eugonadal males with transfusion-requiring SCD (10 were on TTx and eight were on ETx).

Significant improvement of sperm count and sperm morphology and motility [Table 1] occurred after 7 days of PCTx and was associated with a significant increase in serum levels of T, LH, and FSH. Both T and Hb levels were significantly correlated with all the sperm parameters.

Hormones act on all phases of spermatogenesis. FSH and LH act directly on the testes to stimulate somatic cell function in support of spermatogenesis. Three phases of spermatogenesis that are regulated by gonadotropins in men are: (i) The maturation of type A spermatogonia to type B spermatogonia, (ii) meiosis, and (iii) spermiation, both acutely and chronically. Both FSH and T actions are important for the progression of meiosis, perhaps by the regulation of the survival via the intrinsic and the extrinsic apoptotic machineries, and also spermiation in both rodents and humans. Data from the literature have shown that FSH predominantly regulates spermatogonial development, while T regulates the latter phase of spermiogenesis, while both FSH and T seem to be equally important in supporting spermatocyte development.[30-32] T is important for the conversion of step 7 round spermatids into step 8

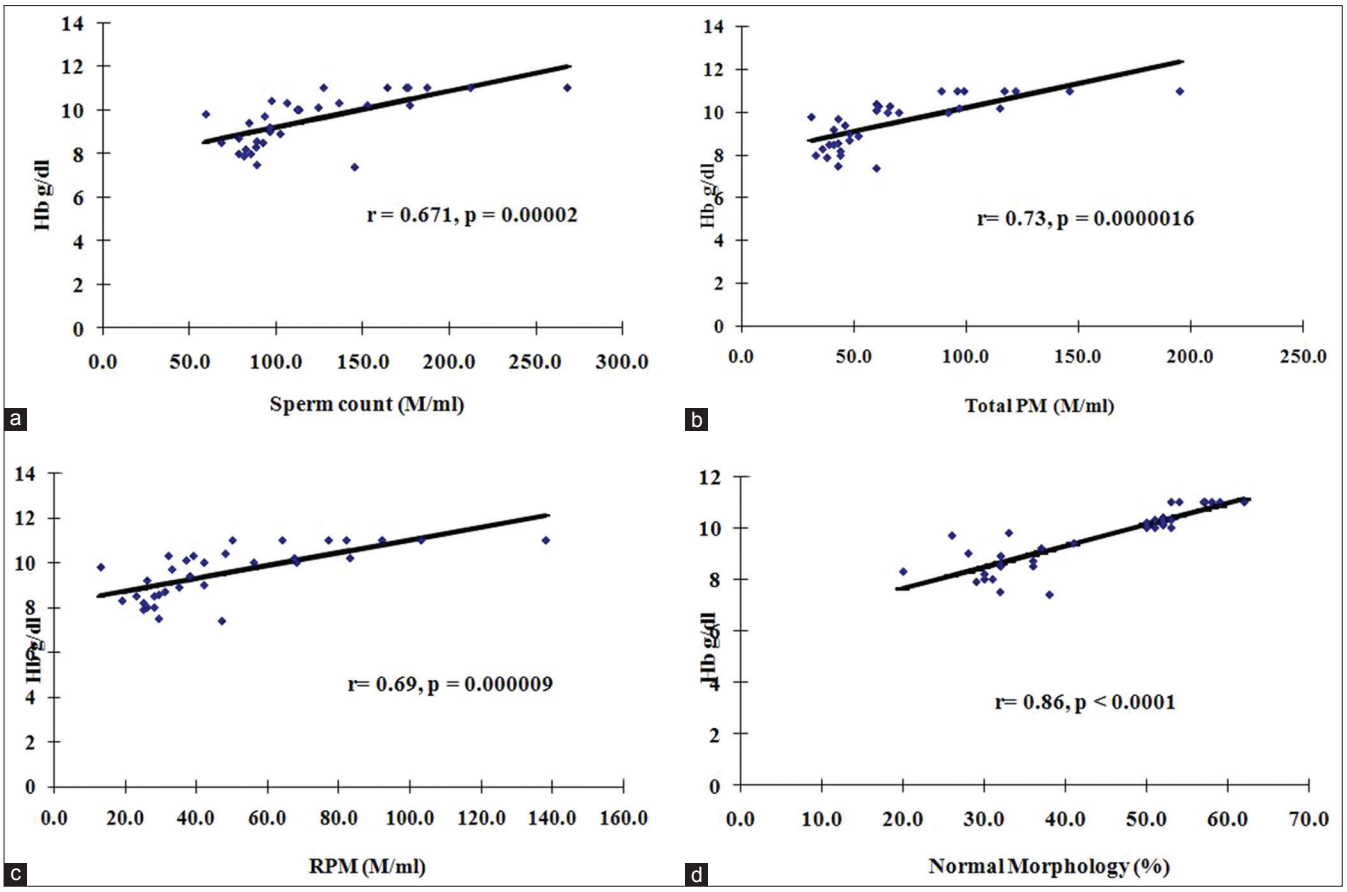

Figure 1: Regression of sperm parameters on hemoglobin (Hb) levels: (a) Regression of total sperm count on Hb levels. (b) Regression of total progressive sperm motility on Hb levels. (c) Regression of rapid progressive sperm motility on Hb levels. (d) Regression of percentage of sperms with normal morphology on Hb levels

spermatids by regulating the adhesion between Sertoli cells-and round/elongating/elongated spermatids at the apical ectoplasmic specialization (ES) adherent junctions.[32] The normal time duration for spermiogenesis (process of differentiation of spermatids to spermatozoa) is 21 days.[33]

In our patients with SCD, many possible mechanisms can contribute in the improvement of sperm parameters after PCTx. These include: Improving oxygenation (by increasing Hb-A level), increasing blood flow in the microcirculation (by decreasing Hb-S), and decreasing iron toxicity (by suppressing excessive ineffective erythropoiesis). In addition, increasing LH and T secretion can improve the viability of epididymal sperms and possibly rapid progression of the late stages of spermatogenesis. In an animal study by Schoff *et al*. Bovine sperms were subjected to extended anoxia (2.5 h) then diluted into oxygenated medium. In response to anoxia sperm adenosine triphosphate (ATP) titers dropped from 15 to 20 < mu > moles/108 cells to 1-2 < mu > moles/108 cells in the first 5 min then remained extremely low until re-oxygenation. Cyclic adenosine monophosphate cAMP titers declined slowly over the anoxic period, but did not show the same scale of depression as ATP. After re-oxygenation ATP recovered to pre-anoxia levels within 1 min, and cAMP rose to about the pre-anoxia levels. Motility was substantially depressed after extended anoxia.[34]

Moderate hypoxia has been shown to decrease gonadotropins' secretion within 2 days of arrival at moderate altitude.[35] Previous studies indicated that hypoxia reduced the fertility of men from lowland by decreasing sperm count and sperm motility in semen.[36] In rats and mice, hypobaric hypoxia markedly inhibits spermatogenesis as well as epididymal sperm parameters.[37] Testicular damage is related overproduction of reactive oxygen species (ROS) by the hypoxic stress, with increased sperm lipo-peroxidation.[38]

In rats exposed to hypoxia, epididymal sperm count was significantly reduced at day 7 of exposure to high altitude and maintained low levels with respect to sea level up to 42 days. In addition there was significant reduction of the time for many stages (at day 3, stages I, IV-V, VI, VII, and IX-XI were shorter, at day 7, stages VIII, IX-XI, XII, and XIII-XIV were reduced and at day 14, stages VII, VIII,

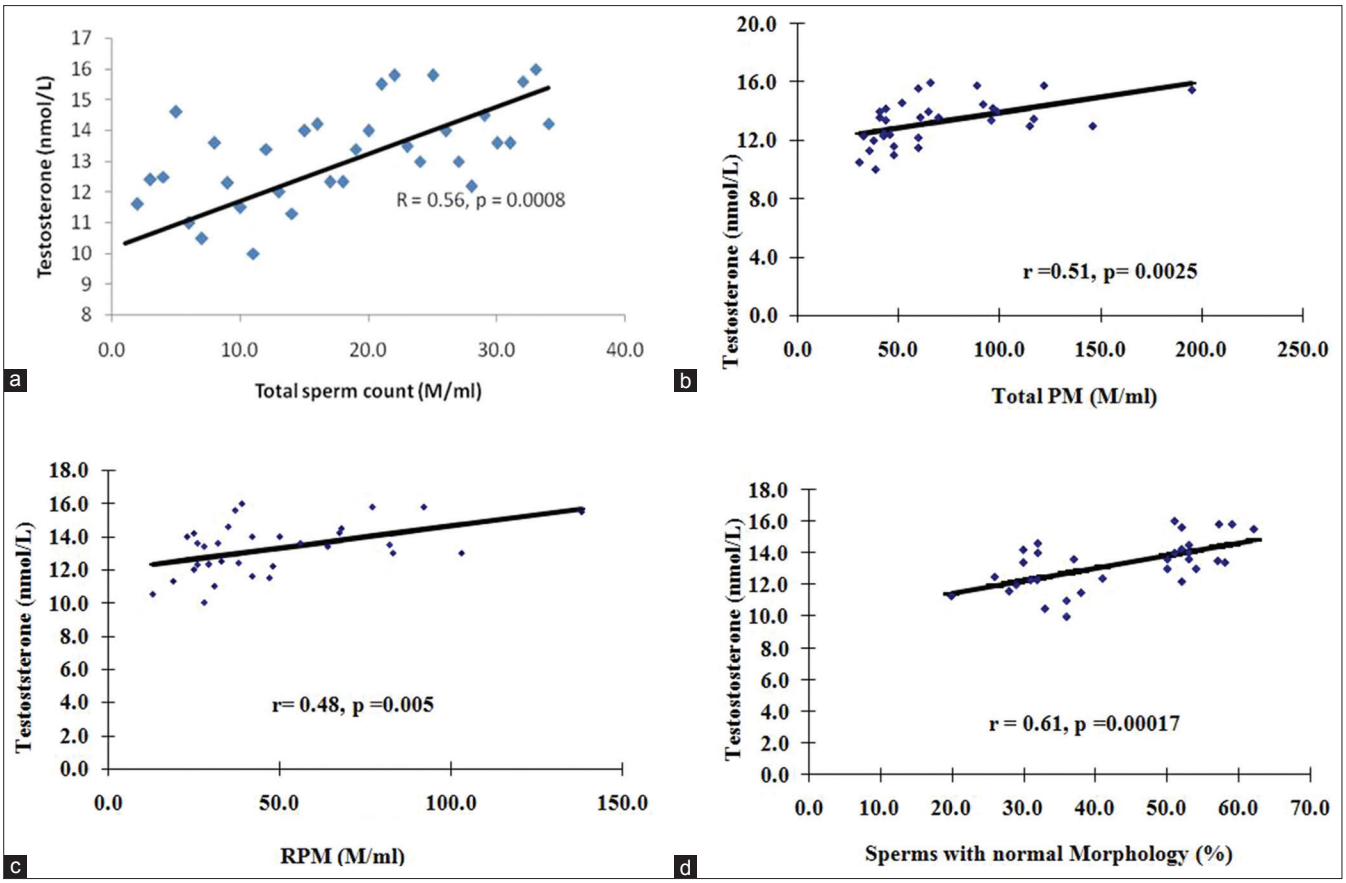

Figure 2: Regression of sperm parameters on testosterone (t) levels: (a) Regression of total sperm count on T levels. (b) Regression of total progressive sperm motility on T levels. (c) Regression of rapid progressive sperm motility on T levels. (d) Regression of percentage of sperms with normal morphology on T levels

and IX-XI were reduced). Hypoxia due to high altitude affects spermatogenesis and in turn affects epididymal sperm count.[39]

The microvasculature is a major target of SCD and endothelial cell activation is a critical component of the microvascular responses accompanying this disease. Vaso-occlusive crises (VOC), which result from blood flow cessation and reduced delivery of oxygen and nutrients to involved tissues, affect multiple organ systems in SCD patients including the testis and the anterior pituitary. Repeated clinical and subclinical sickling can reduce perfusion and oxygenation of the organs including the testes. In support of this effect, testicular and pituitary gland volumes of patients with SCD are reported to be smaller with many infarcts in the testicles and pituitary gland.[22,23,40]

In addition to its effect on microvasculature, HbS has been implicated in the increased levels of the injurious (toxic) superoxide and hydroxyl radical detected in SCD patients.[41,42] The demonstration of significantly better sperm parameters (volume, total count, TPM, RPM, and % of sperms with normal morphology) after transfusion in the group on ETx (with significantly lower Hb-S level) versus the group on TTx, although they have equivalent Hb or T levels, supports the favorable effect of lowering HbS on sperm parameters apart from rising Hb or hormonal control. Moreover, an oxidative stress (secondary to iron toxicity) is suggested to accelerate apoptosis and may contribute to shortened life span of erythrocytes as well as sperms.[43] It is possible that in our patients an additional favorable effect of PCTx on epididymal sperm and/or late stages of spermatogenesis and the pituitary gonadal axis is represented by correction of anemia that inhibits the ineffective erythropoiesis, decreases iron absorption, stimulates macrophages to retain iron and attenuates the potential toxic effect of iron on epididymal sperms.[44,45] However, there was no significant correlation between ferritin level and sperm parameters and ferritin levels were higher in the ETx group (with better sperm parameters) versus the TTx group.

Collectively, in our patients with SCD on blood transfusion, increasing oxygen supply through increasing Hb-A, decreasing HBS level (dilution of HbS in TTx and active

reduction of HbS in ETx) and increasing T level appear to be important mechanisms for the favorable effects on epididymal sperm viability and progression of late spermatogenesis. The significant correlations between Hb and T levels with the sperm parameters in these patients supported our view.

Many acute effects have been reported on animal and human sperms quality and quantity in response to drugs. The exact molecular events involved are not understood but it was hypothesized that this acute effect could be due to changes in ionic channel or adenylyl and guanylyl cyclase, which catalyze the formation of cAMP and cyclic-guanosine monophosphate (cGMP), and regulate the levels of these second messengers in cells of reservoir spermatozoa in epididymis and vas deferens.[46-48]

In conclusion, our study suggests that the PCTx, in patients with SCD is associated with significant acute enhancement of sperm parameters and with an increased concentrations of serum T, LH, and FSH. The "acute" effects on spermiogenesis are reached with an unknown mechanism/s and suggest a number of pathways that need further human and/or experimental studies. In this pilot study, rapid improvement of sperm parameters in young adults with SCD after improvement of their Hb level highlights the need to investigate the acute effects of correcting hypoxemia in other hypoxic disorders.

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