Study of Seminal Fluid Parameters and Fertility of Male Sickle Cell Disease Patients and Potential Impact of Hydroxyurea Treatment

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Abstract

Introduction: Male Sickle cell disease (SCD) patients often have moderate to severe hypogonadism resulting in abnormal seminal fluid parameters due to testicular dysfunction. Hydroxyurea (HU), the only drug found to be effective in preventing morbidity and mortality in sickle cell disease patients has been found to further aggravate the testicular dysfunction.

Material and Methods: This was a prospective study done at a tertiary care hospital over 26 months between September 2011 to October 2013. 100 male sickle cell disease patients of age group 15 to 45 years were recruited in the study. We evaluated seminal fluid indices in all patients and the effect of hydroxyurea on seminal fluid parameters. Hydroxyurea was given at low dose of 10mg/kg/day orally to patients with frequent vaso-occlusive crisis and frequent need of blood transfusion. Seminal fluid analysis was done according to WHO criteria before starting hydroxyurea and every 3 months after initiation of hydroxyurea. Patients with abnormal seminal parameters before hydroxyurea therapy were not given hydroxyurea therapy. Patients with abnormal sperm parameters were subjected for FNAC of testis. In sickle cell disease patients with hydroxyurea therapy, who developed abnormal seminal fluid parameters, hydroxyurea was stopped for 3 months and seminal fluid parameters were re-evaluated. Patients who had recovery of seminal indices after hydroxyurea cessation were restarted with hydroxyurea therapy at low dose.

Results: Among Sickle cell disease patients without hydroxyurea therapy, 18% of patients developed oligospermia and 4% developed azoospermia. Among sickle cell disease patients with hydroxyurea therapy, 20% of patients developed oligospermia and 10% developed azoospermia. Seminal fluid parameters reverted back to normal after stoppage of hydroxyurea for 3 months in 73% of patients.

Conclusion: Alteration of sperm parameters is seen in a significant number of sickle cell disease patients. Also, alterations of seminal fluid parameters are exacerbated by hydroxyurea treatment even with low dose. Therefore, treatment with hydroxyurea in adolescent and adult male sickle cell disease patients should be preceded by routine assessment of seminal fluid parameters and followed up regularly every 3 months for any change in seminal fluid parameters for evidence of hydroxyurea toxicity.

Editorial Viewpoint

• SCD patients have hypogonadism due to testicular dysfunction which may be further aggravated by hydroxyurea.
• This study confirms above even with low doses of hydroxyurea.
• There is a need to monitor seminal fluid parameters regularly in young adults with SCD receiving hydroxyurea.

Introduction

Sickle cell anemia is one of the most common human autosomal recessive disorder caused by a mutational substitution of thymine for adenine in the sixth codon (GAG to GTG) of the globin gene on chromosome 11p. Sickle cell anemia patients often have moderate to severe hypogonadism of unknown origin, although several mechanisms have been suggested like: primary
hypogonadism, hypogonadism induced by repeated testicular infarction, zinc deficiency, and puberty delay due to span height retardation.

Hydroxyurea remains the only approved disease modifying therapy for sickle cell anemia. Hydroxyurea increases the fetal hemoglobin which has higher oxygen carrying capacity and does not undergo sickling under low oxygen tension. Low dose hydroxyurea therapy (10 mg/kg/day) is found to be effective in improving clinical and hematological parameters in sickle cell anemia with improved quality of life. Hydroxyurea being an antimitotic agent has been reported to impair human spermatogenesis. Hydroxyurea is also associated with testicular atrophy, a reversible decrease in sperm count and abnormal sperm morphology and motility. So, hydroxyurea treatment can aggravate the testicular dysfunction in sickle cell disease patients.

Sickle cell anemia is a major public health problem in the state of Odisha, India. The sickle gene frequency is 10–30% in general population in Odisha (Patel DK). The use of hydroxyurea is increasing in sickle cell anemia patients due to its beneficial effect in reducing painful crises and blood transfusion requirement but there are limitations to hydroxyurea because of its toxicities. Sickle cell disease itself causes abnormalities in seminal fluid parameters, which also may be aggravated by hydroxyurea therapy. On the basis of the above observations, we conducted this study to evaluate seminal fluid parameters and fertility of men suffering from sickle cell disease and to analyze the potential impact of hydroxyurea.

Material and Methods

This was a hospital based prospective study undertaken on sickle cell anemia patients in age range of 18 to 45 years enrolled in Sickle cell clinic and molecular biology lab of Veer Surendra Sai Medical College and Hospital, Burla, Odisha, India during the period from September 2011 and October 2013.

Screening, Diagnosis and Clinical Evaluation

Screening of Sickle Cell Anemia was initially done by sickling slide test. Those found positive were subjected to agarose gel Hb electrophoresis in an alkaline medium (pH 8.6). Quantification of various hemoglobin including Hbf, HbS was done by High Performance Liquid Chromatography (HPLC) using VARIANT™ Hemoglobin Tasting System (Bio-Rad Lab, Hercules, CA, USA) on the principle of cation exchange HPLC according to manufacturer's guidelines. Confirmation of Sickle Cell Disease (codon 6=GAG to GTG mutation) was done by amplification refractory mutation system-Polymerase Chain Reaction (ARMS-PCR) using established protocols.

Total 100 patients were divided into 2 groups. Group I included 50 patients without hydroxyurea therapy and Group II included 50 patients who needed hydroxyurea therapy and had normal sperm parameters prior to hydroxyurea therapy. Group II patients were given low dose hydroxyurea therapy 10 mg/kg/day. Sickle cell anemia with 1 or more of the following complications were included in Group II and were given low dose hydroxyurea therapy: (a) painful crises ≥3 episodes in previous 1 year.; (b) ≥2 blood transfusions in last 1 year. Painful crisis was defined as an acute painful event that required oral/injectable analgesics and that lasted for at least 4 hrs when no other cause could explain the symptom.

A detailed history was taken in all cases with reference to age, marital status, fertility in form of number of issues, family history, history of painful crisis, blood transfusion. Married male sickle cell disease patients with regular sexual activity in form of regular unprotected sexual intercourse for 12 months or more were asked whether he had caused a pregnancy or not. Routine hematological evaluation like complete blood count, liver function tests, serum creatinine were done in all patients and repeated every 3 months following hydroxyurea therapy.

Group I patients were evaluated for seminal fluid analysis and those who had azoospermia were subjected for FNAC of testis. In Group II, seminal fluid analysis and routine hematological evaluation were done prior to hydroxyurea therapy and during hydroxyurea therapy every 3 months. Hydroxyurea was temporarily stopped in those who developed oligo or azoospermia during hydroxyurea treatment and again tested for seminal quality after 3 months. Those who developed azoospermia were subjected to FNAC of testis. Hydroxyurea was restarted after normalization of sperm parameters.

Exclusion Criteria

Patients with following criteria were excluded from the study: -(a) patients with other sickle cell syndrome such as HbS/ß-thal, HbS/HbE, HbS/HbC, HbS/HbD Punjab and others; (b) male patients <18yr and >45 yr; (c) patients who refused to give consent; (d) patients who had taken hydroxyurea for less than 80% of days.

Evaluation of Seminal Fluid Parameters

Seminal fluid sample was collected in a sterile container by masturbation after minimum 3 days of sexual abstinence and analyzed after liquefaction according to WHO criteria. The parameters assessed included volume of ejaculate, sperm concentration, motility, sperm morphology and viability. The normal semen volume is 2-6 ml, normal sperm concentration ≥15 million/ml, normal motility ≥40% motile [progressive (PR)+ nonprogressive(NP)] or ≥32% with
In our study, we analyzed the seminal fluid parameters of 50 sickle cell disease patients without hydroxyurea therapy and 50 sickle cell disease patients with hydroxyurea therapy. In the sickle cell disease patients without hydroxyurea therapy, the mean sperm concentration during hydroxyurea therapy was 39.26 ± 29.32 million/ml with normal morphology 73.3 ± 30.96% with oligospermia seen in 4 patients (20%) and azoospermia in 5 patients (10%). Comparison of sperm parameters before and during hydroxyurea therapy showed significant reduction in sperm concentration (p value <0.0001) with reduction in both normal morphology and motility (Table 2).

Seminal fluid parameters after stoppage of hydroxyurea therapy: Among the total 30% of hypogonadism patients (oligospermia + azoospermia), 11 patients (73%) reverted back to normal following stoppage of hydroxyurea therapy for 3 months but 4 patients (27%) did not revert back (Table 2).

FNAC of testis: FNAC of testis was done in 2 non-hydroxyurea patients with azoospermia and in 4 hydroxyurea receiving patients with azoospermia. In non-hydroxyurea (Group I), one patient showed absent spermatogenesis with other being normal. In hydroxyurea receiving group (Group II), three out of four patients showed absent spermatogenesis (Table 3).


discussion

Because of the longer life expectancy of sickle cell disease patients due to advent of hydroxyurea therapy, the evaluation of possible side effects of hydroxyurea on male fertility has therefore become a question of public health. Any deleterious impact of hydroxyurea on spermatogenesis and sperm parameters would represent a major concern necessitating advice on sperm cryopreservation as a preventive measure in order to preserve future male fertility.

In our study, we analyzed the seminal fluid parameters of 50 sickle cell disease patients without hydroxyurea therapy and 50 sickle cell disease patients with hydroxyurea therapy. In the sickle cell disease patients without hydroxyurea therapy, the mean sperm concentration
was 48.60±27.73 million/ml with oligospermia seen in 18% patients and azoospermia in 4% of patients. Overall we detected 22% of oligo-azoospermia in patients without hydroxyurea therapy. In the study by Berthaut I. et al, 40% of individual values of concentration of spermatozoa was below normal and at least one sperm parameter was abnormal in 91% of patients before hydroxyurea treatment.

A decrease in semen volume in patients with sickle cell disease was previously described, suggesting associated abnormalities like disease of seminal vesicle, prostate. In our study, the volume of ejaculate was in normal range, in agreement with Osegbe et al study, which did not find any significant difference in ejaculate volume between sickle cell disease patients and fertile male controls.

In the present series of 50 patients who received low dose hydroxyurea therapy, there is significant reduction in mean sperm concentration when comparing semen before and during treatment (54.28 ± 16.2 vs 39.26 ± 29.32 million/ml). During hydroxyurea treatment, 20% of patients developed oligospermia and 10% developed azoospermia. In the study by Berthaut Let al, where high dose hydroxyurea (20-30 mg/kg/day) was used, showed affection of all sperm parameters in semen samples.

After stopping hydroxyurea treatment for 3 months in patients who developed oligo or azoospermia, 73% of patients reverted back to normal, indicating reversibility of hydroxyurea induced gonadal dysfunction in most cases.

In our study, FNAC of testis of two azoospermic patients without hydroxyurea therapy showed absent spermatogenesis in one patient. FNAC of testis in 4 azoospermic patients on hydroxyurea therapy showed absent spermatogenesis in 3 patients. Jones et al had found that, testes from hydroxyurea treated rats showed significant atrophic degeneration in seminiferous tubules compared to control.

**Conclusion**

The study has several limitations including a small sample size, lack of testing for gonadal hormones like FSH, LH and testosterone for confirming central or peripheral cause of hypogonadism and lack of testicular biopsy. Despite these limitations, the study indicates that alteration of sperm parameters is seen in a significant number of sickle cell disease patients. Also, alterations of sperm parameters are exacerbated by hydroxyurea treatment even with low dose. Therefore, treatment with hydroxyurea in adolescent and adult male sickle cell disease patients should be preceded by routine assessment seminal fluid parameters and followed up regularly every 3 months for any change in sperm parameters for evidence of hydroxyurea toxicity. Hydroxyurea treatment should be stopped temporarily in patients who develop alteration of sperm parameters and again restarted after normalization of sperm parameters.

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