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Hazardous Influence of Hydroxyurea on Spermatogenesis in Thalassemia Intermedia Patients: An Egyptian Cohort Study

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Abstract

Background: Hydroxyurea (HU), frequently used in thalassemia intermedia (TI), might have adverse effects on spermatogenesis.

Aim: To assess the effects of HU treatment on sperm parameters and potential reversibility on its discontinuation in TI patients.

Methods: Twenty fully-pubertal male TI patients regularly followed-up at the Ain Shams University Thalassemia Center were classified according to previous HU treatment (1:1); first group had received HU for ≥ one year, while the second had never received HU. All recruited patients were subjected to full clinical assessments. Sperm parameters (number, abnormal forms, motility and forward progression) were assessed at enrollment and reassessed six-months after stopping HU treatment.

Result: Eleven patients on HU therapy had statistically significant lower sperm count in comparison to those who had never received HU. At six months off HU therapy, there was statistically significant improvement of all sperm parameters. Nevertheless, such parameters were still lower than those of patients who had never received HU. Statistically significant relationships were noted between total sperm count and HU dose, compliance and duration of therapy.

Conclusion: HU appears to have a hazardous yet reversible effect on sperm health in pubertal TI patients. Counseling should be offered with close follow-up of its effect on fertility.

Keywords: Thalassemia intermedia; Hydroxyurea; Spermatogenesis; Sperm parameters

Abbreviation: HU: Hydroxyurea; TI: Thalassemia Intermedia; HPLC: High Performance Liquid Chromatography.

Introduction

Thalassemia intermedia (TI) has a wide clinical spectrum extending between the clinically more severe thalassemia major (TM) on the one end and the asymptomatic thalassemia carrier state on the other [1-7].

Hydroxyurea (HU) is an s-phase-specific and non-DNA-hypomethylating chemotherapeutic agent capable of inducing hemoglobin F synthesis [8]. It was first synthesized in 1869, and is on the World Health Organization's List of Essential Medicines; a list of the most important medications needed in any basic healthcare system [9]. It has been used in the management of TI patients since 1994 [10]. HU has relatively mild and transient side effects that are tolerable, yet periodic patient followed-up is advised [11,12].

Sperm abnormalities such as oligozoospermia, azoospermia, decreased motility, and increased morphologic abnormalities occur in males with sickle cell disease receiving HU [13]. Higher degrees of DNA damage in spermatozoa are correlated with reduced sperm motility in both beta-thalassemia and sickle cell disease. Whether these abnormalities are directly related to HU therapy is unclear [13-16]. There appears to be little published data regarding the effects of HU on spermatogenesis in pubertal males with TI. Our aim was to assess such effects, focusing on the duration, dose, compliance and age of start of HU treatment. We also aimed to assess the degree reversibility of the effects of HU upon discontinuation of treatment.

Patients and Methods

This is an open label prospective follow-up study that was conducted, over a three-year period, at Thalassemia Center, Children's Hospital, Ain Shams University at Cairo Governorate, Egypt. Thirty-two fully pubertal male TI patients with Tanner scores of five and older than 16 years of age were recruited. Their diagnosis was confirmed by means of high performance liquid chromatography (HPLC) using the D-10 (BioRad, Marnes La Coquette, France). Patients must be able and willing to provide a sample for semen analysis, not suffering from chronic liver disease or endocrinal disorder. Any history of drug intake known to affect spermatogenesis was sought. Regular compliance to hydroxyurea treatment was assessed. Patients were labeled "regular" if they received HU >5 days/week or >45 weeks/year.

Patients selected randomly from our Thalassemia center, and were classified according to previous HU treatment; first group had received HU for ≥ one year, while the second had never received HU. Hydroxyurea was started before enrollment in our study because it is well established that HU is an antimetabolite inhibitor with cellular

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and hematological effects that increases total Hb, HbF, MCH and MCV [17]. The group of patients who were on HU treatments were evaluated at enrollment where efficacy and side effects were evaluated and the patients were counseling about continuing treatment. Of this group, those were decided to stop HU were enrolled and reevaluated after six months for reconsidering the restart of medication and none were decided to restarted and requesting further semen analysis follow-up test first.

The details of the study design and laboratory investigations were explained to all patients and/or their parents or legal guardians, and an informed consent was obtained from each patient or their legal guardians before enrollment into the study. The study was approved by the Institutional Regulatory Board of the Childrens' Hospital, Faculty of Medicine, Ain Shams University. The study adhered to the principles of the Declaration of Helsinki for working with humans with the code of Ethics of the World Medical Association.

All files of the recruited were sought from the record keeping unit of the Thalassemia Center and reviewed. Self-reported histories from the patients during their follow ups were collected. Additionally, demographic data and transfusion histories were copied. Histories of hydroxyurea therapy including doses, durations and degree of compliance were collected. Thorough clinical assessment was performed with special emphasis on tanner staging [18], fully pubertal male patients were only recruited.

Laboratory investigations included complete blood counts (CBC) and red blood cell (RBC) morphology studies as well as differential white blood cell (WBC) counts, all done using Leishman-stained smears. Mean pre-transfusion hemoglobin levels over the six months immediately prior to the study were calculated. Serum ferritin (SF) concentration studies (chemiluminescence principles (Im-Multie) were performed. Semen analysis, in accordance with the WHO criteria [19], were conducted both at the time of enrollment into the study and after six months of stoppage HU for those patients who were treated with HU. Analysis included the examination of seminal volume, color, sperm count, motility, percentages of abnormal forms and types of abnormality.

Statistical Analysis

Data were collected, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20 (Armonk, NY: IBM Corp 2011). Output of qualitative data entries was in the form of numbers and percentages while that of quantitative data was in the form of means, standard deviations and ranges when parametric, and in the form of medians with interquartile ranges (IQR) (IQR; 75th and 25th percentiles) when non-parametric. The Kolmogrov Smirnov test was used to test the distribution of normality. Comparison of qualitative data of two groups was performed using the Chi-square test and/or the Fisher exact test (the latter when count of any cell type was found less than five). Quantitative data with parametric distribution was compared using the independent t-test, whilst non-parametric data was compared using the Mann-Whitney test. Pearson correlation coefficients were used to assess the association between two normally distributed variables. When a variable was not normally distributed, a Spearman correlation test was performed. The confidence interval was set to 95% and the margin of error accepted was set to 5%. Therefore, a p-value of <0.05 was considered significant.

Result

Out of the thirty-two patients who initially recruited, twelve were excluded; twenty patients that fulfilled inclusion criteria were enrolled, as illustrated in Figure 1.

Their mean age at diagnosis was 4.05 ± 1.85 (3-8) years and their mean age at assessment was 18.80 ± 1.40 (17-21) years. Seven patients (35.0%) had previously received blood transfusion therapy, with a mean frequency of 3.71 ± 1.50 (2-6) per year. The mean pre-transfusion hemoglobin in the 12 months prior to the study was 8.4 ± 0.74 (7.4-10.2) gm/dl. Iron overload assessment was performed using serum ferritin tool with mean level of 367.3 ± 89.48 (240-525) ng/dl. None of the patients had previously received iron chelation therapy.

Eleven patients out of twenty (55%) had received HU therapy, with a mean dose of 21.21 ± 5.42 (16.67-29.17) mg/kg/dose. Only five of these patients (45.45%) used HU regularly. Differences between the demographic and clinical characteristics of patients on HU therapy and those who had never received HU were non-significant, as is shown in Tables 1 and 2. There were statistically significant lower total sperm counts in patients receiving HU compared with those that had never received such treatment.

Data are expressed as Mean ± SD (Range) where independent t test was used for comparison, unless other specified as number (%)* where Chi-square test was used for comparison. NA: Not applicable for comparison.

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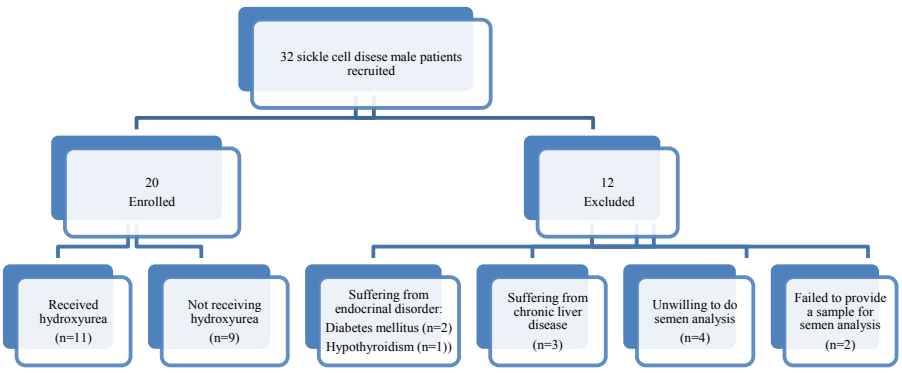

Figure 1: Flowchart for the screened and enrolled subjects.

Variables	Patients not receiving HU	Patients receiving HU	Test value	p-value
Age at diagnosis (years)	4.00 ± 2.24 (1-8)	4.09 ± 1.58 (2-6)	-0.107	0.916
Age at assessment (years)	20.11 ± 1.76 (17-22)	19.55 ± 1.04 (18-21)	0.895	0.383
Receiving transfusion therapy*	5 (55.6)	2 (18.2)	3.039*	0.081
Frequency of transfusion/year	3.80 ± 1.79 (2-6)	3.50 ± 0.71 (3-4)	0.22	0.835
Pre-transfusion hemoglobin in 6 months prior to study (g/dl)	8.13 ± 0.62	8.61 ± 0.78	-1.485	0.155
Serum ferritin (ng/dl)	388.44 ± 105.21	350.00 ± 75.00	0.954	0.353

Table 1: Comparison between patients receiving hydroxyurea (HU) and those not receiving HU regarding clinical and laboratory characteristics.

Comparison between patients receiving HU and those not receiving HU at enrollment of the study regarding semen analysis characteristics				
Variables	**Patients not receiving HU (N=9)**	**Patients receiving HU (N=11)**	***Test value***	***p-value***
Volume (millimeter)	1.59 ± 0.93 (0.2-3)	2.40 ± 1.00 (1.2-4.2)	-1.86	0.079
Yellow color*	9 (100)	11 (100)	NA*	NA
Total sperm count in million (cm^3)	126.14 ± 52.64 (31.36-180)	6.33 ± 3.14 (1-10.2)	7.575	<0.001
Abnormal forms (%)	35.44 ± 6.21 (22-42)	35.45 ± 11.07 (20-60)	-0.002	0.998
Comparison between patients receiving HU and those not receiving HU six months after enrolment of the study regarding semen analysis characteristics				
Variables	**Patients not receiving HU (N=9)**	**Patients receiving HU (N=11)**	***Test value***	***p-value***
Volume (millimeter)	1.23 ± 0.48 (0.5-2)	1.81 ± 1.01 (0.8-4.5)	1.578	0.132
Yellow color*	9 (100.0%)	11 (100.0%)	NA*	NA
Total sperm count in million (cm^3)	57.67 ± 34.06 (25-140)	45.99 ± 28.03 (8.5-88)	0.842	0.411
Abnormal forms (%)	34.89 ± 7.01 (20-42)	38.45 ± 9.63 (25-60)	0.925	0.367
Comparison between semen analysis at enrolment and after 6 months for patients receiving HU				
Patients receiving HU (N=11)	**Semen analysis at enrollment**	**Semen analysis after 6 months off HU**	***Test value***	***p-value***
Volume (millimeter)	2.40 ± 1.00 (1.2-4.2)	1.81 ± 1.01 (0.8-4.5)	1.381	0.197
Yellow color*	11 (100)	11 (100)	NA*	NA
Total sperm count in million (cm^3)	6.33 ± 3.54 (1-10.2)	45.99 ± 28.03 (8.5-88)	-5.252	<0.001
Abnormal forms (%)	35.45 ± 11.07 (20-60)	38.45 ± 9.63 (25-60)	-1.451	0.177
Comparison between semen analysis at enrolment and after 6 months for patients not receiving HU				
Patients not receiving HU N=9	**Semen analysis at enrollment**	**Semen analysis after 6 months off HU**	***Test value***	***p-value***
Volume (millimeter)	1.59 ± 0.93 (0.2-3)	1.23 ± 0.48 (0.5-2)	1.002	0.346
Yellow color*	9 (100)	9 (100.0%)	NA	NA
Total sperm count in million (cm^3)	126.14 ± 52.64 (31.36- 180)	57.67 ± 34.06 (25-140)	4.418	0.102
Abnormal forms (%)	35.44 ± 6.21 (22-42)	34.89 ± 7.01 (20-42)	0.204	0.844

Table 2: Comparison between patients receiving hydroxyurea (HU) and those not receiving HU regarding semen analysis characteristics.

There was a statistically significant improvement in total sperm counts after six months stoppage of HU, as is depicted in Table 2. Patients who were receiving hydroxyurea regularly; those receiving doses higher than 25 mg/kg/day, as well as those that had been receiving HU therapy for more than ten years had statistically significantly lower sperm counts comparted with patients receiving HU at smaller doses and those who had been on HU treatment for shorter durations, as summarized in Table 3. Moreover, these differences continued to be significant even after six months of stoppage of HU therapy, as shown in Table 3.

Data are expressed as mean ± SD (Range) where independent t test was used for comparison

*Patients were labeled "regular" if they received HU > 5 days/week or > 45 weeks/year.

Attempts to correlate total sperm counts of patients on HU treatment at enrollment to this study and at six months off HU with other parameters yielded no statistically significant findings. This included hemoglobin at enrollment (r= -0.009, p=0.979); and at 6 months off therapy (r= -0.185, p=0.586), as well as serum ferritin levels (r=0.018, p=0.957) and (r=0.048, p=0.889).

Discussion

Hydroxyurea therapy in TI patients has significant positive effects, increasing hemoglobin levels and thus potentially reducing blood transfusion dependency, as well as decreasing skeletal deformities and splenomegaly and increasing the energy state [20]. Potential adverse events with associated with HU therapy include gastrointestinal disturbances, rashes, alopecia, headache, and myelotoxicity [21]. Of all potential adverse effects, HU's impact on rapidly dividing cells remains a major concern. Young children who receive HU appear to have normal growth, but the effect on pubertal development is less clear. The two potential reproductive concerns with the use of HU in both the adolescent and adult populations are the resultant abnormal spermatogenesis and potential teratogenic effects. [13].

Published literature on the effect of HU on spermatogenesis in TI is limited. This makes evidence-based counseling on the risk of developing sperm abnormalities on HU therapy quite challenging. In this study, patients on HU therapy showed a statistically significant lower total sperm count in comparison with those that had never received HU. Abnormalities such as oligozoospermia, azoospermia, have also been reported in males with SCD receiving HU [13]. De Santicis and his colleagues [22] studied eleven patients, and found that (68.7%) had

Variable		TSC in million in patients on HU at enrollment	Test value	p-value	TSC in million in patients on HU after six months off therapy	Test value	p-value
Hydroxyurea dose; mg/kg/dose	>25 mg/kg/dose	9.05 ± 0.90 (8-10)	2.307	0.046	67.75 ± 26.43 (31-88)	2.342	0.044
	≤ 25 mg/kg/dose	4.77 ± 3.56 (1-10)			32.25 ± 21.55 (8.5-66)		
hydroxyurea compliance*	Regular n=5	3.90 ± 2.98 (1-8)	3.835	0.004	28.15 ± 17.65 (8.5-53)	3.216	0.011
	Irregular n=6	9.24 ± 0.89 (8-10.2)			67.4 ± 22.91 (31-88)		
Duration in years	> 10	3.90 ± 2.98 (1-8)	3.835	0.004	28.15 ± 17.65 (8.5-53)	3.216	0.011
	< 10	9.24 ± 0.89 (8-10.2)			67.4 ± 22.91 (31-88)		

Table 3: Hydroxyurea (HU) variables effect on total sperm count (TSC) in TI patients receiving HU at enrollment and six months off therapy.

normal seminal parameters; one (1.6%) had oligospermia; three (18.7%) had asthenospermia and one (1.6%) had oligoasthenospermia. We found that there was no statistically significant difference in the percentage of abnormal sperms between patients with TI on HU therapy and those who were not. Our data support the theoretical risk of HU affecting sperm development, which is based on the fact that it is an antimetabolite, ribonucleotide reductase inhibitor primarily acting as an S-phase-specific cytotoxic agent that impairs DNA synthesis [23]. Although mean sperm count decrease in those who did not receive HU from the time of enrollment to time of follow-up, this decrease was not clinically significant, this this may be explain by intra-individual variability. Auger et al. [24], found that there were quit important difference in intra-individual variation among participant and from on sample to another, and the average coefficient of variation for the studied 12 participant were 15.8%.

The effect of HU on the patients in this study was reversible and there was statistically significant improvement in total sperm counts six months after stoppage of HU therapy. This finding supports the idea that the effects of HU are relatively short lived and dissipate once the drug is stopped. It appears that a once-daily administration of HU has only brief, intermittent cytotoxic effects on dividing cells [23]. Our study data suggest that dosage, duration of therapy, and degree of patient compliance to HU therapy in TI might impact the degree of adverse effects of HU on male fertility. Statistically significantly lower total sperm counts were detected in the semen of patients receiving hydroxyurea regularly; those receiving doses more than 25 mg/kg/dose, and those on therapy for more than ten years compared with patients receiving hydroxyurea in lower dose and/or for shorter durations. Consequently, it is recommended to keep dose of HU below 25 mg/kg/dose and for shorter duration than ten years and close follow-up with semen analysis if otherwise dose and duration is needed. Moreover, these differences continued to be significant even after six months of stoppage of HU therapy. These findings agree with those of other investigators who also suggested that the duration of HU therapy may correlate with the degree of sperm abnormalities, and who emphasized that, of the patients who had started HU in childhood, those who had received HU for longer 12 years or more became azoospermic [25,26].

A retrospective study that reviewed semen analyses of four adult men on HU therapy found a reduction in sperm counts motility and as well as abnormal morphologies. Cessation of HU therapy in three cases resulted in recovery of spermatogenesis; in two of the three, however, sperm morphology and mobility remained impaired [25]. Most of the aforementioned studies provide only limited data because they involved small, retrospective populations. The studies report varied average ages at initiation of HU therapy, varied lengths of therapy, and varied lengths of follow-up studies once HU is discontinued. The exact timing of recovery after discontinuation of HU is unclear and data are inconsistent as to whether this reduction in sperm count is partially or fully reversible [13].

Limitations

This was a cohort study involving a relatively small group of patients with a short follow-up period and assessment that was done at one point in time (at 6 months). Additional follow-up were should be conducted at 3, 9, 12 months after discontinuation of HU therapy to determine the best time to discontinue such therapy. A future prospective study could involve a parent/guardian/patient consented re-challenge of HU therapy, not addressed in our cohort, for those who improve after a period of stoppage, with a look at the balance between the value of HU therapy and the drastic effect on semen parameters over the long term.

Other potential proposal for future studies can address the timing of start HU with focus on effect of earlier initiation of HU therapy, especially initiation in peri-adolescence period, specifically; it could attempt to correlate the degree of severity of the adverse effects with initiation of therapy at specific Tanner stages. As well as the effects of the length of therapy on the degree of adverse effects of HU. The effect of short durations of stoppages of HU therapy compared with longer stoppages with the degree of recovery of semen parameters has not been studied.

Conflict of Interest

The authors declare no conflict of interest; they have neither a commercial nor other association that might pose a conflict of interest (e.g., pharmaceutical stock ownership, consultancy, advisory board membership, relevant patents, or research funding).

Conclusion

In this study, HU was found to adversely affect all sperm parameters in young adults suffering from B-thalassemia intermedia. This was especially evident with higher doses and longer durations of therapy. Oligospermia was reversible in most cases. Consequently, it becomes essential that pubertal male patients be counseled with regards to the potential hazardous effects of HU on fertility, with regular follow up of these effects once HU therapy is initiated.

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