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## Fertility in transfusion-dependent thalassemia men: Effects of iron burden on the reproductive axis

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### To the Editor

Decreased reproductive capacity in transfusion dependent thalassemia (TDT) men is common but addressed infrequently. Previous reports estimate that more than one half of men with TDT are affected by oligospermia and asthenospermia and have abnormal sperm quality due to transfusion-induced iron overload [1,2]. Hypogonadotropic hypogonadism and subfertility due to increased iron in the pituitary gland has been reported [3]. However, additional mechanisms affecting reproduction in TDT men and progression to infertility as it relates to systemic and pituitary iron load are not fully understood, delaying efforts for effective intervention. Oxidative stress, through increased production of reactive oxygen species (ROS), is considered a major contributory factor to male infertility causing damage to the sperm membrane, nucleus, and proteins, thereby impairing sperm quality [4]. Normal

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#### Authorship Contributions

ST Singer, JF Smith, DW Killilela, and J Suh served as investigators on this trial, involved in the study design, data acquisition, interpretation, and reporting as well as drafting and critically reviewing the manuscript. K Ivani and P Evans participated in data acquisition and analysis. J Wang, Q Yuan, and R Fischer performed and interpreted pituitary iron measurements and contributed to the analysis and reporting of the trial data. E Vichinsky participated in interpreting the data and critically reviewing this manuscript.

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seminal plasma contains defense mechanisms against ROS consisting primarily of glutathione (GSH) as well as carnitine, folate, vitamins E, C, and A, zinc, and selenium [5]. In iron-overload thalassemia patients, excess non-transferrin bound iron (NTBI) and labile plasma iron (LPI) can act as pro-oxidants, resulting in increased generation of ROS. This coupled with low antioxidant defense molecules was suggested as a basis of tissue damage and impaired organ function in thalassemia [6,7]. Whether abnormalities involving these biochemical pathways are the basis for abnormal spermatogenesis and subfertility in thalassemia men with a high iron burden is not well studied. Additionally, the extent of iron-induced pituitary damage resulting in low gonadotropins and affecting reproduction capacity is not known. Thus, we investigated the pituitary-gonadal axis in a subset of TDT patients in the context of their total iron burden.

All TDT men, 18 years and older, at our thalassemia center were approached about the study, there was no preselection. Seven TDT men and two normal controls were studied. Anterior pituitary volume and iron accumulation (R2) were measured by pituitary MRI and corresponding Z scores calculated. Sperm count and motility were determined and sperm DNA integrity (DNA fragmentation Index; DFI) was assessed using Sperm Chromatin Structure Assay. Semen plasma elemental content was analyzed by inductively coupled plasma optical emission spectrometry and GSH was quantified using a liquid chromatography linked tandem mass-spectrometry (LC/MS/MS) assay. NTBI/LPI were analyzed (London, England) and liver iron concentration (LIC), cardiac T2\* MRI, ferritin, vitamins C and E, and plasma zinc levels reviewed.

All patients (median 26, range 21–30 years) went through spontaneous puberty at age 13–16. Retrospective analysis showed consistent low iron burden in two patients (1 and 4) and variable iron overload in the others (Table I). Mean pituitary iron (R2) was increased and mean anterior pituitary volume was decreased compare to normal age-matched male controls: R2  $16.2 \pm 2$  vs.  $11.2$  Hz ( $Z = 6.0$ );  $329.6 \pm 83.3$  vs.  $596.1 \pm 94.4$  mm<sup>3</sup> ( $Z = -3.0$ ), respectively. Anterior pituitary volume and LH level correlated with sperm count. Five of the seven patients (70%) had either low sperm count or complete azoospermia and variable sperm motility (Table II), an important measure of fertilization capacity. DFI was normal indicating no increase in sperm DNA fragmentation. Seminal plasma analysis revealed elevated iron concentration in 5/6 patients, highest in the three patients with azoospermia while iron was undetected in the normal controls. Seminal iron was inversely associated with sperm count and motility (Table II). The three patients with azoospermia, also exhibited low seminal plasma zinc levels despite a normal plasma range, as well as the lowest GSH concentration, inversely correlating with systemic iron overload. Plasma vitamins C and E were within the low-normal range (not shown) and zinc levels were in low or low-normal range (62–97; nl 70–120 mcg/dL).

Our results elucidate causes of infertility in TDT men; we found high seminal iron in most patients, associated with azoospermia or oligospermia that to the best of our knowledge was not demonstrated before. High seminal plasma iron can lessen sperm motility to 10% of normal, thus significantly affecting reproductive potential [8]. Further, the elevated systemic NTBI and LPI and low seminal GSH in our patients, correlating with sperm motility, imply that iron-induced oxidative stress may have a major role in the subfertility often detected in

TDT men, beyond the hypogonadism caused by iron in the pituitary gland. Indirect evidence for seminal oxidative stress in TDT men was suggested [9]. In nonthalassemia subfertile men low seminal GSH levels were present, while normal GSH levels were associated with better sperm qualities [10]. Our results of normal sperm DNA fragmentation, an important measure for fertilization rate, contradict previous findings of increased sperm DNA fragmentation in TDT men [1,11]. It is possible that the relative low iron burden and normal seminal GSH levels found in our three patients were protective against increase DNA fragmentation. If confirmed in a larger study, it could suggest better preserved sperm quality in patients with consistent good iron chelation habits. Alternatively, diminished sperm DNA integrity was attributed to the use of the iron chelator desferrioxamine [11,12], while our three patients were not using this chelation agent. Additionally, low seminal plasma zinc levels detected in most our patients also affects fertility, as zinc is an important micronutrient for DNA repair during spermatogenesis. In males, low zinc levels adversely affected fertilization ability that improved with increase in dietary zinc intake [5,13]. Since zinc deficiency is common in TDT [14] examining the effect of increase zinc intake in TDT men, even with apparent normal plasma levels may be warranted.

This study suggests that individuals with high iron burden have decreased pituitary volume and low gonadotropin levels; additionally their seminal environment has a detrimental effect on sperm vitality and quality. In patients with consistent adherence to iron chelation the pituitary size and fertility measures are better preserved. The value of serial measures of pituitary volume and iron concentration needs further study but may prove to have a better predictive value for fertility potential than gonadotropin levels. Aggressive iron reduction along with antioxidant and zinc supplementation could assist with fertility preservation. The use of antioxidants supplementation was shown to improve sperm qualities, though the overall effectiveness remains controversial [4,15]. Clarifying the risk thresholds of these causative factors in larger studies can assist in treatment interventions and fertility preservation.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE I

## Patients' Iron Overload and Fertility Profiles

Subject	1	2	3	4	5	6	7	C1	C2	nl range
Age (years)	26	21	26	27	27	30	23	26	24	–
<b>Iron burden</b>										
LIC <sup>a</sup> (mg iron/gm dry liver)	6	25	23	6.9	8.2	6	19.6	–	–	<3
Range previous 5 years	5.4–8.2	16–25	12–24	4.4–10.2	4.8–13.8	12–30	na	–	–	>30
Cardiac T2* MRI (ms)	30.5	29.1	6.5	29	22	15	13.15	–	–	>30
Range previous 5 years	13–30	20–29	5.8–7.6	30–32	9–15	9–10	–	–	–	11.4–11.7 <sup>c</sup>
Pituitary R2* (SE) (s <sup>-1</sup> )	13.5	16.4	17.0	14.5	–	16.7	19.5	–	–	–
R2* Z score	2.2	5.7	6.0	3.3	–	5.7	9.1	–	–	–
Pituitary ant. vol. (mm <sup>3</sup> )	447	279	176	227	–	359	336	–	–	550–561 <sup>c</sup>
Vol Z score	-1.1	-3.0	-4.0	-3.5	–	-2.0	-2.4	–	–	–
Ferritin (µg/L)	634	3700	4290	1050	1965	2500	2325	–	–	12–300
Range previous 5 years	668–1100	900–2900	3000–4900	611–1340	1524–4000	2000–3880	–	–	–	–
NTBI/LPI (µM)	2.09/0.01	1.52/1.09	1.86/1.67	1.61/0.01	2.93/0.52	–	3.1/0.2	–	–	0
<b>Reproductive hormones</b>										
LH/FSH (mIU/mL)	4.9/7.9	0.3/0.9	0.0/0.0	1.6/2.7	2.85/3.42	2.8/0.3	0.07/0.3	–	–	1.5–9.3/1.6–8
Testosterone (ng/ml)	373.8	144	142	131	335	668	378	–	–	300–1200
<b>Sperm and seminal plasma<sup>b</sup></b>										
Volume (ml)	3.5	2.5	1.4	2.3	1.5	4.5	1.8	5	2	>2
Sperm concentration (Million/ml)	178	0	0	14	11	94	0	25	93	>20
Sperm motility (%)	75	0	0	65	37	75	0	45	65	>50
DFI (%)	13	–	–	9.7	na	8.6	na	na	na	<15% <sup>d</sup>
Zn (mg/L)	141.4	54.4	6.7	163	na	120.4	80.2	82	81	144 ±42 <sup>e</sup> 156 ±50 <sup>f</sup>
Fe (mg/L)	1.2	2.7	2.5	2.2	na	<dl	2.4	<dl	<dl	0.29 ±0.12 <sup>f</sup>
Total GSH (µmol/L)	9.5	2.2	0.3	8.6	na	7.5	1	na	4.8	3.2 ±0.38 <sup>g</sup> 7.7 ±2.4 <sup>h</sup>

<sup>a</sup> LIC determined by superconducting quantum interference device (SQUID).

<sup>b</sup> Semen analysis was performed in random times during transfusion cycle, mean 10 ±4 days after previous transfusion. The effect of this timing on the results presented is unknown.

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Testosterone: All patients except subject 1 were receiving testosterone replacement therapy.

NTBI and LPI were determined at a standardized research laboratory (University College London, London UK).

DFI: DNA fragmentation index; dl: detection limit.

Minimum detection limit for Fe =0.208 mg/L.

CI,C2: 2 normal control subjects.

TABLE II

Pearson Correlation Between Selected Iron and Fertility Variables

	R2	Ant pit vol	LJC	Ferritin	LH	FSH	LPI	GSH	Sperm count	Sperm motility	Zinc	Iron
R2		-0.201	0.572	0.554	-0.726	-0.789 <sup>a</sup>	0.291	-0.780 <sup>a</sup>	-0.609	-0.688	-0.555	0.241
Ant pit vol	-0.201		-0.519	-0.603	0.760 <sup>a</sup>	0.625	-0.642	0.521	0.824 <sup>b</sup>	0.513	0.514	-0.609
LJC	0.572	-0.519		0.810 <sup>b</sup>	-0.838 <sup>b</sup>	-0.564	0.763	-0.938 <sup>c</sup>	-0.625	-0.950 <sup>c</sup>	-0.889 <sup>b</sup>	0.782 <sup>a</sup>
Ferritin	0.554	-0.603	0.810 <sup>b</sup>		-0.739 <sup>b</sup>	-0.769 <sup>b</sup>	0.944 <sup>c</sup>	-0.839 <sup>b</sup>	-0.580	-0.762 <sup>b</sup>	-0.933 <sup>b</sup>	0.374
LH	-0.726	0.760 <sup>a</sup>	-0.838 <sup>b</sup>	-0.739 <sup>b</sup>		0.838 <sup>b</sup>	-0.571	0.877 <sup>b</sup>	0.869 <sup>c</sup>	0.849 <sup>b</sup>	0.702	-0.726
FSH	-0.789 <sup>a</sup>	0.625	-0.564	-0.769 <sup>b</sup>	0.838 <sup>b</sup>		-0.601	0.702	0.732 <sup>a</sup>	0.587	0.590	-0.228
LPI	0.291	-0.642	0.763	0.944 <sup>c</sup>	-0.571	-0.601		-0.731	-0.466	-0.712	-0.922 <sup>b</sup>	0.612
GSH	-0.780 <sup>a</sup>	0.521	-0.938 <sup>c</sup>	-0.839 <sup>b</sup>	0.877 <sup>b</sup>	0.702	-0.731		0.743	0.972 <sup>c</sup>	0.915 <sup>c</sup>	-0.649
Sperm count	-0.609	0.824 <sup>b</sup>	-0.625	-0.580	0.869 <sup>c</sup>	0.732 <sup>a</sup>	-0.466	0.743		0.744 <sup>b</sup>	0.548	-0.731 <sup>b</sup>
Motility	-0.688	0.513	-0.950 <sup>c</sup>	-0.762 <sup>b</sup>	0.849 <sup>b</sup>	0.587	-0.712	0.972 <sup>c</sup>	0.744 <sup>b</sup>		0.863 <sup>b</sup>	-0.786 <sup>a</sup>
Zinc	-0.555	0.514	-0.889 <sup>b</sup>	-0.933 <sup>c</sup>	0.702	0.590	-0.922 <sup>b</sup>	0.915 <sup>c</sup>	0.548	0.863 <sup>b</sup>		-0.504
Iron	0.241	-0.609	0.782 <sup>a</sup>	0.374	-0.726	-0.228	0.612	-0.649	-0.731 <sup>b</sup>	-0.786 <sup>a</sup>	-0.504	

<sup>a</sup>Trend of association (*P* value 0.05–0.08).<sup>b</sup>*P* 0.05.<sup>c</sup>*P* 0.01.