Pituitary iron and factors predictive of fertility status in transfusion-dependent thalassemia

Long-term survival of patients with transfusion-dependent thalassemia (TDT) raises hopes of attaining a decent quality of life, having a family and maintaining reproductive potential. However, for many these remain significant challenges despite advances in the management of iron overload. While pregnancies are possible, there is inadequate information to guide patients on their reproductive ability and on markers that predict when they will have infertility issues. Preservation of fertility and timely interventions remain pervasive concerns. Although the association of iron toxicity with cardiac and pancreatic dysfunction has been investigated extensively, the progression of iron deposition in the anterior pituitary leading to hypogonadotropic hypogonadism and infertility has not been adequately explored.[1]

To address these issues, we sought to investigate pituitary dysfunction and stratify the risk of infertility utilizing reproductive data and information on systemic iron load. We also evaluated the progression of pituitary dysfunction and systemic iron parameters and their effect on reproductive capacity. Magnetic resonance imaging (MRI) measurements of anterior pituitary iron (R2) and volume have established reference data in normal controls and proved an adequate method to predict clinical hypogonadism in thalassemia.[2-4] Identifying risk thresholds could assist in establishing the timing and effectiveness of intensifying chelation treatment to halt further damage and potentially restore function.

The study was approved by institutional review boards and all patients provided written informed consent to participation.

Fifty-three TDT patients, 10 years and older, and 12 healthy subjects (age 12-40 years) underwent MRI (Philips Achieva®, 1.5 T) to determine cardiac iron (T2*), anterior pituitary iron (R2) and three-dimensional volume.[5] Levels of non-transferrin bound iron, labile plasma iron (LPI), and ferritin were measured in blood samples, and liver iron concentration (LIC) and myocardial iron were quantified by MRI and a superconducting quantum interference device (SQUID), respectively. In order to assess fertility, plasma gonadotropins (luteinizing hormone [LH] and follicular-stimulating hormone [FSH]), estradiol and testosterone levels were assayed. Anti-Müllerian hormone (AMH) levels were determined in women in order to evaluate ovarian reserve and inhibin B levels were measured in men to assess impairment of spermatogenesis.[6,7] In 17 patients these measures were repeated 2-3 years later to assess for changes over time. The patients' pubertal and reproductive history was recorded. Z scores for anterior pituitary iron accumulation, Z(R2), and volume, Z(V), were calculated for each patient.[3] Parametric statistics were applied to characterize patients: mean ± standard deviation, t-test, and Pearson correlation. Associations of Z(R2) and Z(V) with age and LH and FSH levels were demonstrated by boxplots and linear regression. Receiver operating characteristic (ROC) curve analysis was used in patients for categorization into Z(R2) ≤4 and >4 to examine the sensitivity and specificity for systemic iron parameters.

Moderate-severe pituitary iron deposition Z(R2) >2, mean 5.4) was observed in 33 patients (62%, age 26±7 years), a rate comparable to that in the report by Noetzli et al.[4] The rest of the patients (32%, age 23±8 years) had no iron accumulation (mean Z(R2) of 0.03). Thirty-two patients (60%) had a Z(V) < -2 (mean -2.8) indicating sig-

nificant gland shrinkage; the remaining 40% had a Z(V) of -0.7. There was a negative correlation between Z(R2) and Z(V): r=-0.36, P=0.02 (Figure 1A). We found that a Z(R2) value >4.0 predicted pituitary volume loss: Z(V) < -2.0 was present in 17/20 patients with a Z(R2) >4 and in only 16/33 with a Z(R2) <4.0 (P<0.009).

Twenty-three patients (43%, 28±6 years) simultaneously had Z(R2) >4 and Z(V) <-2, of whom ten (43%) were splenectomized. Overall, patients who underwent splenectomy had higher pituitary R2, smaller gland volume and a significantly higher cardiac iron in comparison to the non-splenectomized patients (P<0.004) while LIC was similar (Table 1). This supports the notion that

Table 1. Patients' disease characteristics.

Characteristics	N (%) or mean ± SD
Study patients	53*
Females:males	27:26
Age (years)	25±8 (range 10-45)
β^0 thalassemia	34
E/β^0 thalassemia	18
Hb H-Constant Spring	1
Years on regular transfusions	18.7±6
$\quad\beta^0$ thalassemia major (mean age 24±6)	19.7±6
$\quad$E/β^0 thalassemia (mean age 28±6)	17±5
Chelation treatment	
$\quad$Mono therapy (DFX)	29 (55%)
$\quad$Combination therapy (DFX & DFO)	24 (45%)
Systemic iron measures	
LIC (mg/g dw)	15±11
Cardiac T2* (ms)	27.2±10
Mean annual ferritin (µg/L)	2620±2332
NTBI (µmol/L)	1.9±1.3
LPI (µmol/L)	0.25±0.5
Anterior pituitary	
R2 (s^{-1})	14.5±3
Z score of R2: Z(R2)	3.4±3.6
$\quad$Pituitary Z(R2) > 2 (n=33; 62%)	5.4
$\quad$Females' Z(R2)	3.2±4.4
$\quad$Males' Z(R2)	3.7±4.5
Volume (mm^3)	377±110
Z score of volume: Z(V)	-2.0±1.2
$\quad$Pituitary Z(V) < -2 (n=32; 60%)	-2.8
$\quad$Females' Z(V)	-2.3±1.1
$\quad$Males' Z(V)	-1.7±1.3
Spleen status	
Group I: splenectomized	17
$\quad$Age (years)	31±6
$\quad$Age at splenectomy (years)	15±8
$\quad$Years since splenectomy	17±6
$\quad$Z(R2) score	5.2±3
$\quad$Z(V) score	-2.7±1
$\quad$Cardiac T2*(ms)	21±11
$\quad$LIC (mg/g dw)	17±11
$\quad$Mean annual ferritin (µg/L)	2982±2800
Group II: non-splenectomized	35
$\quad$Age (years)	21±6
$\quad$Z(R2) score	2.6±3
$\quad$Z(V) score	-1.6±1
$\quad$Cardiac T2* (ms)	30±9
$\quad$LIC (mg/g dw)	14±11
$\quad$Mean annual ferritin (µg/L)	2450±2050

Two individuals died from iron overload complications during the study period. SD: standard deviation; DFX: deferasirox; DFO: deferoxamine; LIC: liver iron concentration; dw: dry weight; NTBI: non-transferrin bound iron; LPI: labile plasma iron;. LIC and cardiac iron (T2) were obtained within 6 months of laboratory studies. Normal NTBI and LPI: not detectable.

splenectomy accelerates iron loading in the pituitary gland as previously reported to occur in the myocardium.[8] To assess for a possible confounding effect of older age (mean 31±6 and 21±6 years in splenectomized *versus* non-splenectomized patients, respectively) a multivariable general linear model predicting Z(V) was constructed. It showed that splenectomy was significant while age was not (P=0.002 and P=0.218, respectively).

Patients in the third decade of age had significantly higher pituitary iron accumulation than those in their second decade (Z(R2) 4.9±3 *vs.* 1.1±2, P<0.002), paralleled by a decrease in pituitary volume, most apparent in the fourth decade of life (*Online Supplementary Figure S1*). These findings appear to be a decade later than in a prior study.[4] It is possible that this difference is the consequence of improved adherence to treatment or greater efficacy of the oral chelation drug, deferasirox, used for extended periods by most of our patients. Indeed, two studies have shown stabilization of pre-existing hypogonadism, and a low rate of new endocrine disorders with long-term (5 and 6.5 years) chelation with deferasirox.[9,10] However, further longitudinal study is needed to assess the effect of chelation regimens on pituitary iron.

Pituitary MRI findings in the 12 control patients were consistent with the published reference data used in this study.

The levels of gonadotropins (LH and FSH) were inversely correlated with pituitary iron load, Z(R2), and directly correlated with anterior pituitary volume, Z(V); LH/FSH levels declined with gland shrinkage (*Online Supplementary Figure S2*). Ten of 24 post-pubertal women reported regular menstrual periods (group I) and 14 had primary or secondary amenorrhea (group II). LH and FSH concentrations were normal in group I and significantly lower in group II (P<0.04 and P<0.005, respectively). Pituitary iron load and pituitary volume also differed significantly between the two groups (P<0.03 and P<0.006, respectively) (Figure 1B). These findings suggest low reproductive potential among women in group II, as also indicated by low AMH levels (Table 2), signifying compromised oocyte count[6] and diminished reproductive potential. AMH, a key marker of ovarian reserve, has been reported to be positively associated with pregnancy and live birth rates after assisted reproduction. Its validity in TDT women was demonstrated and it serves as a prognostic factor for the probability of pregnancy when gonadotropin secretion is compromised.[11,12]

Men who received testosterone supplementation (group II) had lower LH and FSH levels compared to men who did not (group I); P<0.03 and P<0.001, respectively. Although testosterone treatment could contribute to the low levels, our findings indicate that the main causes are pituitary iron toxicity and volume loss since Z(R2) and Z(V) differed significantly between the two groups (P<0.02 and P<0.0002, respectively). Such low FSH levels, as observed in group II (1.3 mIU), are associated with low spermatogenesis as also shown in thalassemic men with severe iron overload.[13] Furthermore, the levels of inhibin B, a sensitive marker for male infertility, differed significantly between these two cohorts (P<0.03) and were lower than the reported mean level in men with normal fertility parameters. The concentration of inhibin B was below the 10th percentile in 8/26 men (31%), in a range that considerably decreases the likelihood of retrieving testicular spermatozoa.[14]

Cardiac iron, in addition to strongly correlating with pituitary iron, had an optimal cutoff for predicting a pituitary iron threshold associated with reduced volume and loss of function; area under the curve (AUC) = 0.96, P=0.009 (Figure 1C). The close association between cardiac and pituitary iron might be caused by the additional cellular iron uptake through L-type Ca^{2+} channels.[15] Fifteen of 16 patients with a T2* ≤20 ms had a Z(R2) >4.0 while only 5/35 with a T2* >20 ms had a Z(R2) >4.0 (P<0.006). Therefore, adult patients with a T2* <20 ms are at risk of a decline in fertility potential, but assessing fertility markers even before this cardiac T2* threshold is recommended. LIC and ferritin concentration were less predictive of pituitary iron load; the AUC for these were 0.76 and 0.80, respectively, possibly because of the greater variation in liver and plasma iron. We found no correlation of pituitary iron with non-transferrin bound iron or labile plasma iron.

The effect of chelation over time has not been examined before. We conducted a second measurement (n=17) after an interval of 2.6±1 years which demonstrated decreases in systemic iron: LIC by 46±20%, ferritin by 44±21% and cardiac iron by 34±30% in 11, 10 and 12

Table 2. Patients' fertility and hormone data.

Characteristics	N (%) or mean (± SD)
Females^	24
Group I: regular menses	10 (42%)
Age (years)	20±5
LH/FSH (mIU/mL)	8±7;5±2
Estradiol (pg/mL)	84±75
AMH (ng/mL)	4±3.5
Pituitary Z(R2) score	1.3
Pituitary Z(V) score	-1.7
Cardiac T2* (ms)	28±7
Group II: amenorrhea	14 (58%)
Age (years)	31±5
LH/FSH (mIU/mL)	2±2/2.9±3
Estradiol (pg/mL)	40±47
AMH (ng/mL)	1.6± 1
Pituitary Z(R2) score	5.1
Pituitary Z(V) score	-2.9
Cardiac T2* (ms)	20±9
Pregnancies/live children	5/4
With assistant reproductive therapy	2
Males^	26
Group I: no testosterone treatment	16 (62%)
Age (years)	22±7
LH/FSH (mIU/mL)	3.7±2.7/4.5±2.4
Inhibin B (pg/mL)	140±69
Testosterone (ng/dL)	385±240
Pituitary Z(R2) score	2.3
Pituitary Z(V) score	-1. 0
Cardiac T2* (ms)	30±7
Father of a child	3
With assistant reproductive therapy	3
Group II: on testosterone replacement treatment	10 (38%)
Age (years)	28 ± 6
LH/FSH (mIU/mL)	1.5±1.8/1.3±1.6
Inhibin B (pg/mL)	80±55
Pituitary Z(R2) score	5.7
Pituitary Z(V) score	-2.7
Cardiac T2* (ms)	23±8

^Levels of estradiol and testosterone from patients receiving hormone replacement were excluded from analysis. LH: luteinizing hormone; FSH: follicular-stimulating hormone; AMH: anti-Müllerian hormone. Normal ranges: AMH in fertile women, 1.0-4.0 ng/ml; Inhibin B in adult men, 125-215 pg/mL (both change with age)
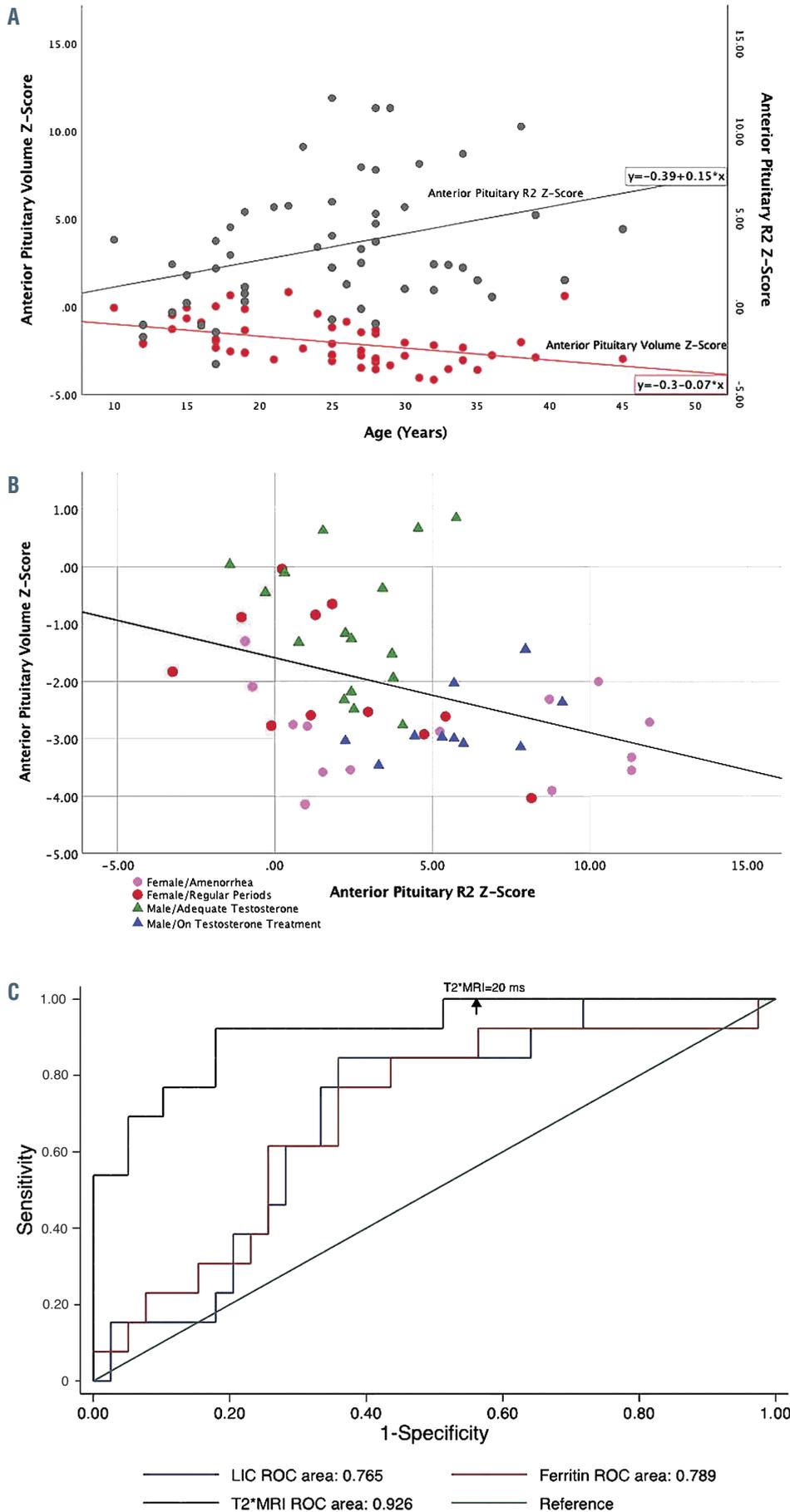
Figure 1. Pituitary iron and volume, relation to hormonal status and to systemic iron. (A) Z scores of pituitary R2 and volume (V) as a function of age in the 53 chronically transfused patients. Pituitary iron and volume are inversely correlated (r= -0.36; *P*<0.02). (B) Distribution by gender and hormonal/reproductive status (based on groups I and II shown in the Tables). The majority of group I patients (men with adequate endogenous testosterone and women with regular periods, represented as green triangles and red circles, respectively) have only moderately elevated pituitary iron accumulation and small changes in pituitary volume; Z scores 0 to 5.0 and 0 to -3.0, respectively. Men requiring testosterone treatment (blue triangles) and women with amenorrhea (pink circles) (group II) had more severe pituitary iron loading and more notable gland shrinkage. (C) Receiver-operating characteristic (ROC) curves for the prediction of pituitary iron show that cardiac T2* correlates strongly with pituitary iron. Liver iron and ferritin concentrations are weakly predictive, the cardiac T2* is superior to both of these measures. The point of cardiac T2* = 20 ms is shown. This value was the optimal cutoff for predicting abnormal pituitary iron; ROC area = 0.93.
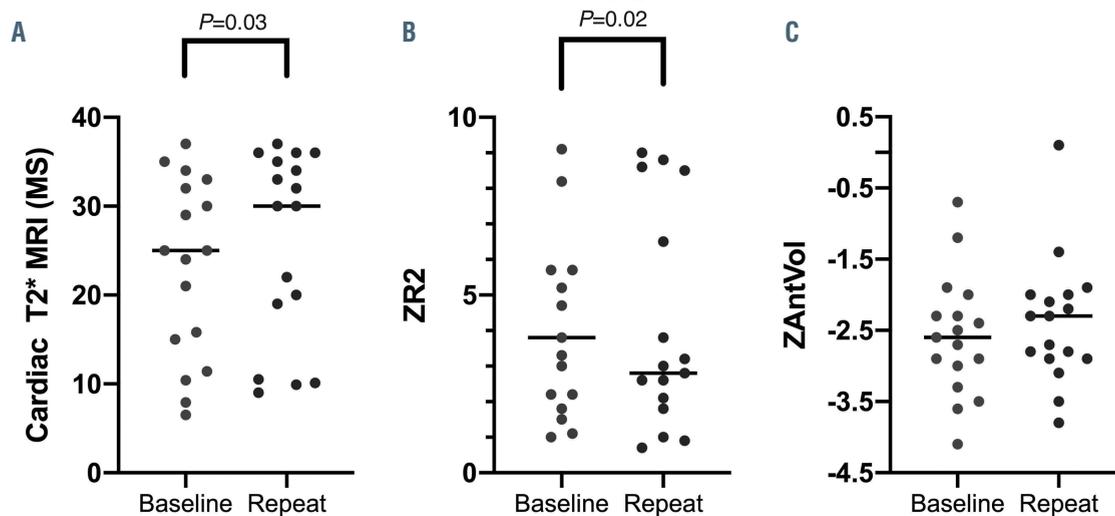
Figure 2. Effect of chelation on pituitary iron and volume. A longitudinal study (mean 2.6 years) showed a significant decrease in pituitary iron ($P<0.02$) correlating with an improvement in cardiac iron ($P<0.03$). The change in pituitary volume was minimal, as expected to be irreversible, and not statistically significant.

patients, respectively. Results were stable or worse in the remaining ones. A parallel improvement in pituitary Z(R2) was noted in 9/17 (53%) individuals, whose mean baseline Z(R2) was 6.4 (Figure 2). The change was modest in those with a baseline Z(R2) >8, suggesting chelation failure in severe pituitary iron load. Eight of the nine patients with improved Z(R2) also had a decrease in cardiac iron, again showing a good correlation of these two measures. Pituitary volume remained abnormally low in subsequent testing. The mean Z(V) was -2.6 at baseline and -2.4 upon repeat imaging, confirming the irreversible cell destruction in patients with high iron burden. LH/FSH concentrations decreased by a mean of 28% at repeat testing in 7/8 women. AMH levels remained stable overall, supporting reports on preserved gonadal function and successful ovulation induction despite low LH and FSH levels. Longitudinal studies on a larger scale are needed to further explore these findings.

In summary, our data confirm previous pituitary MRI findings of iron and volume changes and risk thresholds for hypogonadism. Our study further highlights the high prevalence and progressive nature of pituitary iron loading, despite advances in chelation therapy, causing irreversible volume loss and deleterious effects on reproductive status. Splenectomy and older age are risk factors for these effects.

Our findings also validate cardiac T2*MRI as a proxy for pituitary R2, which can be used where pituitary MRI is not available. The findings also have implications for modifying chelation treatment, which is effective in mild-moderate cases in preventing or delaying infertility.[16] Discussion of reproductive status, utilizing these hormonal and MRI findings, and informing patients of higher risk of infertility are prudent for proper planning of fertility preservation.

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