Cardiac iron and cardiac disease in males and females with transfusion-dependent thalassemia major: a T2* magnetic resonance imaging study

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ABSTRACT

Background

It has been repeatedly reported that female patients with thalassemia major survive longer than males and that the difference is due to a lower rate of cardiac disease in females.

Design and Methods

We compared the cardiac iron load as measured by T2* magnetic resonance imaging in 776 patients (370 males) examined at the National Research Council as part of an Italian cooperative study. We also established normal left ventricular ejection fraction values for our population.

Results

The prevalence of cardiac disease was higher in males than in females (105 males *versus* 69 females; $P<0.0001$). Cardiac T2* was significantly lower in patients with heart dysfunction ($P<0.0001$), but no difference was observed according to sex. Twenty males and five females had a history of cardiac arrhythmias. Their cardiac T2* was not significantly lower than that of patients without arrhythmias (24 ms *versus* 26 ms; $P=0.381$), nor was there a difference between sexes. Liver T2* was significantly lower in males and females with heart dysfunction compared to those without. Ferritin levels were higher in patients of both sexes with heart dysfunction without significant differences between males and females.

Conclusions

Males and females are at the same risk of accumulating iron in their hearts, but females tolerate iron toxicity better, possibly as an effect of reduced sensitivity to chronic oxidative stress.

Key words: thalassemia, cardiac iron, MRI, T2*, sex.

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Introduction

The survival of patients with thalassemia major has significantly improved in recent decades, as a result of regular transfusions and chelation therapy. This favorable trend continues, thanks to the introduction of new oral iron chelators and imaging methods, which allow better management of iron overload. However, complications are still frequent and cardiac disease remains the leading cause of death in these patients. It has been repeatedly reported that female patients have a better prognosis than males. All the studies have been concordant in documenting significantly better results for female than for male patients.[1-3]

In a Greek study, women had a 23% longer life expectancy than men[1] and in a large report from Cyprus, including the entire population born after 1974, a multivariate analysis demonstrated that women had less than half the risk of death compared to men.[4] The difference in survival was mainly due to a lower prevalence of cardiac disease in females. Male patients were more likely to develop heart failure and arrhythmias than were female patients. An Italian cooperative study showed a highly significant association between sex and heart failure. In fact 71% of the patients with heart failure were males.[2] It has been suggested that females have a better compliance than males and, therefore, accumulate less iron in crucial organs such as the heart and the liver. This hypothesis is not, however, confirmed by ferritin levels, which, in our series, were not significantly lower in females.

The aim of our retrospective study was to verify whether the decreased prevalence of cardiac disease in females could be attributed to lesser iron accumulation in their hearts as measured by a multislice multiecho T2* magnetic resonance imaging (MRI) technique.

Design and Methods

The Myocardial Iron Overload in Thalassemia (MIOT) project is a network involving 57 Italian thalassemia centers and six Italian sites where cardiac MRI is performed using homogeneous, standardized and validated procedures.[5] All centers are linked by a web-based network, configured to collect and share the patients' history, clinical and diagnostic data.[6] At all six sites, cardiac MRI examinations were performed using 1.5 T scanners. (GE, Milwaukee, Wisconsin, USA). For the measurement of myocardial iron overload, we used a multislice multiecho T2* approach, as previously described.[7,8] A T2* gradient-echo multiecho sequence was used for the measurement of liver iron overload.[9] A single transverse slice through the liver was obtained at nine echo times in a single end-expiratory breath-hold.

The T2* images were analyzed using a custom-written, previously validated software program (HIPPO MIOT®, IFC-CNR).[8] The software was able to map the myocardial T2* distribution into a 16-segment model of the left ventricle according to the American Heart Association/American College of Cardiology standardized myocardial segmentation.[10] A T2* value greater than 20 ms was considered as a conservative cut-off for all 16 segments, for the mid-ventricular septum and for the entire heart.[11,12] The intra-observer, inter-observer and inter-study variabilities of the proposed methodology have been previously assessed.[5,7] The transferability of multislice multiecho T2* within the MIOT network has been previously validated.[5]

Steady-state free procession cine images were acquired to assess ventricular function parameters (left ventricular volumes, mass and ejection fraction) quantitatively in a standard way.[13] In brief, 9 to 14 breath-hold short-axis slices (depending on left ventricular size) from the atrioventricular ring to apex were acquired with an 8-mm slice thickness and a 0-mm gap. The images were analyzed using MASS software (Medis, Leiden, The Netherlands) by each site. Papillary muscles were included when measuring left ventricular mass and excluded when measuring volumes. Patients with thalassemia major undergo T2* MRI to quantify and monitor segmental and global myocardial iron overload and liver iron overload. The data collected in a specially prepared form include age, sex, diagnosis of heart disease (in particular, heart dysfunction and arrhythmias documented by electrocardiography and requiring medications), and mean yearly serum ferritin levels. Risk factors for heart disease (smoking, family history, hypertension, diabetes mellitus type 1 and 2, dyslipidemia, obesity) were also considered, since previous studies showed the presence of coronary artery disease in patients with thalassemia major[2] and, moreover, cardiac risk factors were reported to be significantly correlated with myocardial fibrosis.[14]

We performed a retrospective review of the MRI results and of clinical data in thalassemia major patients who had undergone at least one cardiac MRI examination at the time of the study. For patients who had undergone more than one MRI, the results of the first examination were considered in order to avoid the effect of intensive chelation started in some patients found to have a low T2*.

Thalassemia major patients with a normal myocardial T2* have different normal values for left ventricular ejection fraction (LVEF) compared to healthy individuals, as demonstrated by Westwood *et al.* using the cardiac magnetic resonance tools software (Cardiovascular Imaging Solutions, London, UK).[15] In order to avoid bias due to the use of different software, we defined our limit of normal for LVEF. Among 776 thalassemia major patients present in the MIOT database who had undergone at least one cardiac MRI examination at the time of the study, we selected 93 patients with no history of cardiac disease, a normal electrocardiogram, no known risk factors, and T2* values greater 20 ms in all cardiac segments. Therefore, as far as it was possible to ascertain with conventional, standardized, non-invasive approaches,[13,15] we included all thalassemia subjects with no evidence of cardiac disease. Since left ventricular parameters can differ with age and gender, we divided the population into three age groups (< 20 years, 20-30 years and > 30 years), also differentiating males and females. The lower limit of normal for LVEF within each category was defined as the mean minus two standard deviations (SD) (Table 1). Accordingly, in the present study heart dysfunction was defined as a LVEF lower than the identified thresholds and/or a history of heart failure requiring treatment.

Patients

All the patients were affected by thalassemia major and were on chronic transfusion regimens receiving red cells every 2 to 4 weeks to maintain a pretransfusional hemoglobin level greater than 95 g/L. All patients had been chelated with deferoxamine for the

Table 1. LVEF thresholds calculated as a function of age and sex in patients with no history of cardiac disease, normal electrocardiogram, no known risk factors and T2* values >20 ms in all cardiac segments.

		< 20 years	20-30 years	>30 years
Males	mean±SD	63.3±4.2	62.4±3.1	64.6±4.4
	threshold	55	56	56
Females	mean±SD	64.0±3.3	64.4±4.3	66.7±5.2
	threshold	57	56	56

majority of their lives. At the time of the MRI examination 35% of the patients were on deferoxamine, 18% on deferiprone, 20% on deferasirox, and 22% on combination deferoxamine plus deferiprone and 5% on sequential deferoxamine-deferiprone. The study complied with the Declaration of Helsinki. All patients gave written informed consent to the protocol. The institutional review board approved this study.

Statistical Analysis

All data were analyzed using the SPSS version 13.0 statistical package. All continuous variables are described as mean ± SD. Categorical variables are expressed as frequencies and percentages. The coefficient of variation was calculated as the ratio of the standard deviation of the half mean square of the differences between the repeated values, to the general mean, and is expressed as a percentage. Comparisons between groups were made by independent-samples t-tests for continuous values with normal distribution. The Wilcoxon rank sum test was applied for continuous values with non-normal distribution (i.e. T2* data). For non-continuous variables, χ^2 testing was performed. In all tests, a two-tailed probability value of 0.05 or less was considered statistically significant.

Results

At the time of the study, information on 776 thalassemia major patients who had undergone at least one cardiac MRI examination was present in the MIOT database. Three hundred and seventy (48%) were males and 406 (52%) females. Of the 776 patients studied, 174 (22%) had a diagnosis of one or more cardiac problems, including heart dysfunction (66.6%), arrhythmias (14.4%), and both heart dysfunction and arrhythmias (19%).

The prevalence of cardiac disease (heart dysfunction and/or arrhythmias) was significantly higher in males than in females (P<0.0001). In fact, 105 males (28.4%) had active or prior and resolved cardiac disease as compared to 69 females (17%).

Heart dysfunction

The coefficient of variability among centers for the quantification of LVEF in patients without cardiac iron or dysfunction was 6.3%, confirming that the MIOT network is a reliable system in which cardiac function parameters were analyzed using reproducible procedures. We identified two groups of patients with heart dysfunction: patients with a history of clinically symptomatic heart failure requiring therapy with a normal LVEF at the time of the MRI (group 1) and patients with LVEF at the time of MRI below the thresholds identified in our study population with or without a history of symptomatic heart failure (group 2). When the two groups were considered together, there was a significantly higher percentage of males with heart dysfunction (23%) than females (16%) (P=0.014). The difference between sexes was statistically significant in the third decade of life (Figure 1).

Serum ferritin levels were higher in patients of both sexes with heart dysfunction but the difference was not statistically significant (1931±1861 ng/mL *versus* 1588±1456 ng/mL; P=0.099) and no significant differences between males and females were found (Table 2). The correlation between cardiac R2* and ferritin was statistically significant (R= -0.359, P<0.0001).

Global heart T2* values were significantly lower in both males and females with heart dysfunction (Table 2) than in those without dysfunction, but no difference was observed according to sex (Figure 2). The correlation between LVEF and cardiac R2* was statistically significant (R= -0.327, P<0.0001). The percentage of patients with LVEF lower than the normal limits and a global heart T2* greater than 20 ms was significantly higher in males (9%) than in females (3%) (P<0.0001) (Figure 3). Within this subgroup of patients, 38% showed heterogeneous myocardial iron over-

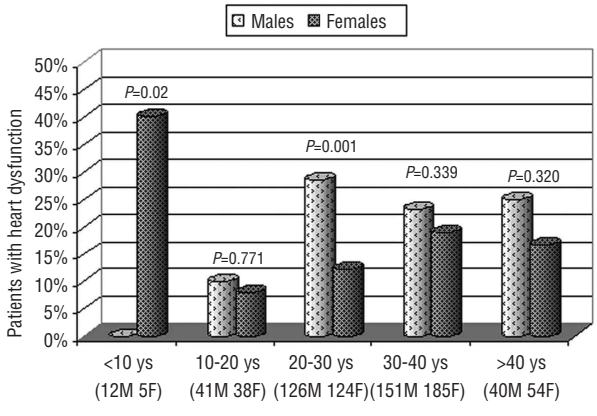

Figure 1. Percentage of heart dysfunction in males and females according to age. The difference between sexes was statistically significant in the third decade. (M, male; F, females). Two female patients <10 years of age had a LVEF below normal (see text).

Table 2. Clinical characteristics of the population studied.

	Without heart dysfunction				With heart dysfunction		
	Males (N=285)	P	Females (N=342)	P	Males (N=85)	P	Females (N=64)
Age (years)	29.1±9.1	0.062	31.0±8.5	0.081	31.1±7.7	0.243	32.6±8.2
Hemoglobin pre-transfusion (g/dL)	9.7±0.9	0.782	9.6±0.8	0.747	9.5±1.4	0.270	9.7±0.9
Ferritin (ng/L)	1561±1401	0.745	1611±1504	0.099	1814±1717	0.546	2085± 2040
Alanine transaminase (μ/L)	50.2±38.4	<0.0001	37.3±25.7	<0.0001	62.6±43.3	0.169	62.6±62.7
Aspartate transaminase (μ/L)	43.6± 32.6	<0.0001	34.6±24.1	0.001	52.7± 41.1	0.473	55.7±54.2
Global heart T2* (ms)	29.0±11.7	0.120	27.3±12.6	<0.0001	20.4±14.7	0.759	17.9±11.9
Liver T2* (ms)	8.0±7.5	0.417	8.3±7.6	0.001	6.5±6.6	0.619	6.3±6.3
Left ventricular ejection fraction (%)	62.4±4.5	<0.0001	64.6±5.1	<0.0001	49.9±6.9	0.012	52.9±6.9
Right ventricular ejection fraction (%)	60.6±6.2	<0.0001	63.5±6.7	<0.0001	51.4±9.9	0.012	55.4±8.2

load, 33% had myocardial fibrosis, and 19% suffered from arrhythmias, but no differences were observed according to sex.

Global heart T2* in group 1 was higher (23±13 ms) than in group 2 (18±14 ms), although the difference did not reach statistical significance (*P*=0.069). In neither group was there a significant difference between sexes for global heart T2* values.

Cardiac arrhythmias

Twenty males and five females had a history of cardiac arrhythmias (*P*=0.001). Global heart T2* was not significantly lower in patients with arrhythmias than in those without arrhythmias (24±14 ms *versus* 26±13 ms; *P*=0.381), nor was there a significant difference between sexes (Figure 4).

Cardiovascular risk factors

Data on risk factors for heart disease were available for 726 (345 males, 381 females) of the 776 patients studied. It was found that 154 males and 183 females had at least one risk factor. There was no significant difference between sexes (*P*=0.360). Smoking was significantly more frequent among males than among females (68 *versus* 40; *P*=0.001), but there were no significant differences in LVEF in either males or females. In addition, the prevalence of diabetes was significantly higher in patients with heart dysfunction (type 1: *P*=0.029 and type 2: *P*=0.023), but the difference between sexes was not statistically significant (*P*=0.263 and *P*=0.164).

Chelation treatment

The analysis of different chelation treatments did not demonstrate a significant difference between patients with heart dysfunction and without heart dysfunction (*P*=0.604), nor between sexes (*P*=0.46). In addition, there was no difference in the reported compliance to chelation therapy between males and females (*P*=0.518).

Liver iron

Liver iron was not correlated with heart iron. Liver T2* values were significantly lower in both males and females with heart dysfunction than in those without dysfunction but no difference was observed according to sex.

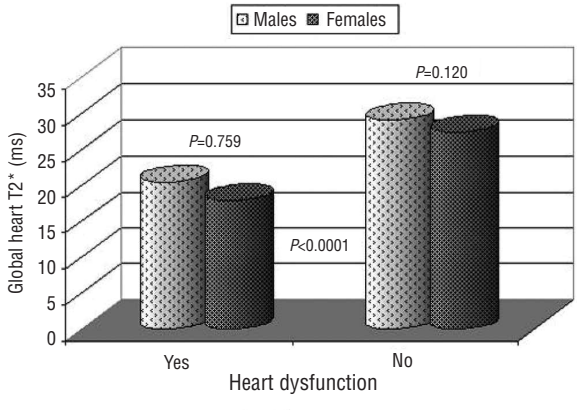

Figure 2. Global heart T2* values in patients with and without heart dysfunction.

Discussion

Data from a large cohort of thalassemia major patients supports an association between gender-specific differences in survival with a lower prevalence of cardiac disease in females.[2]

Magnetic resonance imaging results

We compared the cardiac and hepatic iron overload in males and females by means of T2* MRI. Our method measures the global heart iron instead of the more widely used T2* value in the ventricular septum.[11] This can be advantageous in that the distribution of iron in the heart is heterogeneous.[7,8]

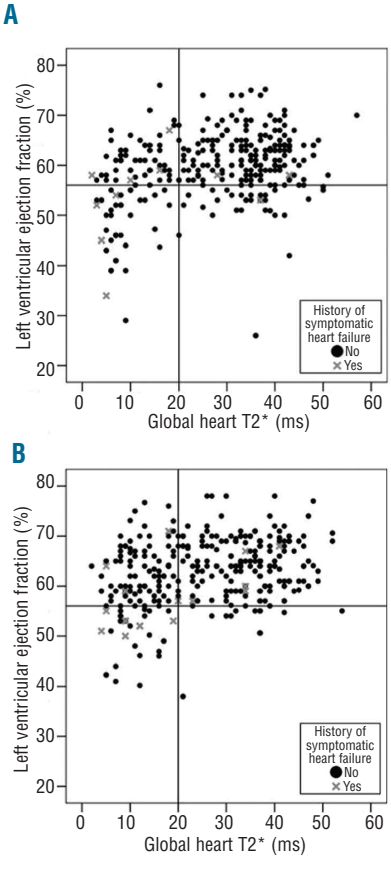

Figure 3. Relationships between global heart T2* values and left ventricular ejection fraction (LVEF) in males (A) and in females (B). The broken lines represent the normal reference ranges for global heart T2* and LVEF.

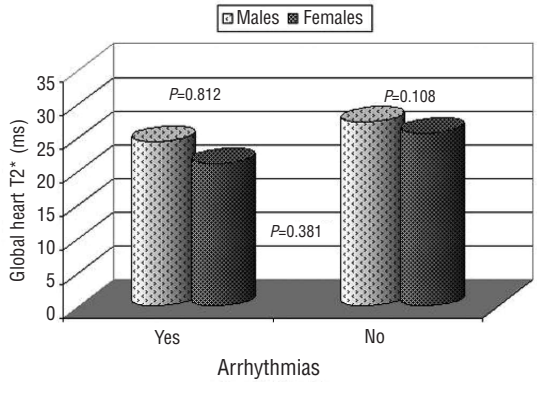

Figure 4. Global heart T2* in patients with and without arrhythmias.

Comparison between sexes

Although the heart T2* in patients with heart dysfunction, both in groups 1 and 2, was significantly lower than in patients without this complication, there was no difference between males and females, indicating that males and females are at the same risk of accumulating iron in their hearts. The LVEF results can be subdivided into three groups: greater than 56%, considered normal; 50-56% considered mildly abnormal, but not necessarily associated with iron, especially in men; and less than 50%, considered grossly abnormal and strongly associated with iron, having a T2* generally less than 10 ms. A few women had values in the mildly abnormal range not associated with iron, while the gender difference was largest in the intermediate group. In contrast, men and women showed fairly similar behaviors in the presence of severe iron overload.

The values of T2* that we observed in our patients with heart dysfunction were higher than those reported in the literature,[11] in which values below 20 ms were considered pathological and associated with heart failure and arrhythmias.[16] However, it can be hypothesized that our patients in group 1 had been chelated intensively after the acute episode of heart failure, therefore increasing their T2*, although we did not calculate the time relationship between the past heart failure episode and the current T2* value, so no firm conclusions can be drawn. In patients in group 2 the T2* values were lower, but still higher than expected. This finding needs to be studied further.

In our large cohort of thalassemia major patients we confirmed the presence of patients with abnormal heart function and global heart T2* greater than 20 ms, a finding previously reported in a small number of patients.[17] There are several possible explanations for this finding, in contrast to previous reports stressing the absence of left ventricular dysfunction in the presence of a normal T2* value in the mid-ventricular septum.[11] First of all, our well-treated study population with good compliance showed significantly lower body iron overload and myocardial iron overload than the study population reported by Anderson *et al.* (serum ferritin 1652±1543 ng/mL *versus* 2095±1559, respectively; $P<0.0001$). Second, although iron could be removed by chelation treatment, the induced heart damage could be progressive and not totally reversible. Moreover, heart damage in thalassemia does not result only from iron overload (a consistent percentage of our chronically anemic patients showed myocardial fibrosis or arrhythmias). Nutritional deficiencies, including selenium, thiamine, vitamin D and carnitine, and thyroid disease have been reported to cause heart dysfunction in thalassemia. Finally, genetically determined variables could affect susceptibility to heart dysfunction in the presence of iron overload[18] and a consistent percentage of our patients showed a heterogeneous myocardial iron burden.

Global heart T2* was not significantly lower in patients suffering from arrhythmias. The lack of correlation between heart T2* and arrhythmias confirms that, as already suggested in the past, cardiac iron overload contributes less to the development of arrhythmias than to cardiac failure.[16] Two children (both female) below the age of 10 years had an LVEF of 56%, just below the limit of normal (57%), without cardiac hemosiderosis. One of the two had recently immigrated from a South American country in which transfusion therapy was erratic and no chelation was available. Also worth commenting are three patients with severely reduced ejection fraction, but not excessive iron overload (Figure 3). It is likely that they had had severe iron deposition that had subsequently been cleared by intensive chelation.

All the patients had been chelated with deferoxamine for the majority of their lives, and some had later been switched to oral chelators alone or in combination, without significant difference in compliance between males and females. The evaluation of compliance over several years is, however, always approximate, as it is based on the relationship of personal trust between physician and patient, supported by a few objective data, such as the number of vials or pills distributed by the pharmacy or checking for sites of injection. Only in the course of short-term studies is it possible to measure the real adherence to therapy with some accuracy.

Proposed explanations

A better life expectancy in females has also been observed in sickle cell anemia and is the rule in the majority of the world populations. In the most recent update of life expectancy and mortality in 2002-2004 in the modern European Union, life expectancy was 75.1 years for men and 81.3 years for women.[19]

It has been proposed that female longevity is more essential, from a Darwinian perspective, than the prolonged survival of males. But what are the physiological mechanisms set in place to obtain this result?

The common explanation that testosterone increases deaths at a young age because it increases aggression and competitiveness does not apply, in general, to the thalassemic population in which the gender gap in life expectancy is strictly correlated with the presence of heart disease. Similarly, the opposing effects of testosterone and estrogens in regulating blood levels of LDL and HDL cholesterol are not a good explanation for the mortality difference in our patients who, overall, tend to have very low levels of total cholesterol.[20]

However, the gender difference in survival was already present in a survey performed in 1983, including patients born before 1970, when only a small proportion of patients had spontaneous puberty and very few were receiving hormone replacement therapy. In addition, it has been suggested that women survive longer because they have a slower metabolic rate almost from the moment of conception, when male embryos divide faster than female ones. The faster metabolic rate could make male cells more vulnerable to breakdown and, therefore, to disease and death. Several other hypotheses have been proposed, including more active immune function in females, compensatory effects of the second X chromosome, and reduction in the activity of growth hormone and the insulin-like growth factor 1 signaling cascade.[21]

More interesting and to the point could be the more efficient antioxidant defenses available to females. Hydroxyl radicals notoriously implicated in vascular damage, especially in the presence of iron overload, are very damaging and ubiquitous. Their effects can be quenched by antioxidant enzymes in the mitochondria, such as superoxide dismutase and glutathione peroxidase. A study comparing oxidative damage to DNA in males and females demonstrated significantly higher levels of modified DNA bases in males.[22]

Conclusions

Our retrospective study showed that males and females with transfusion-dependent thalassemia major are at the

same risk of accumulating iron in their hearts, but females tolerate iron toxicity better, possibly as an effect of reduced sensitivity to chronic oxidative stress.

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