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Is there a difference in phenotype between males and females with non-transfusion-dependent thalassemia? A cross-sectional evaluation

Maria Marsella^a, Massimiliano Ammirabile^{b,c}, Tiziana Di Matola^d, Alessia Pepe^e, Silvia Costantini^c, Aldo Filosa^c and Paolo Ricchi ^c

^aPediatric Unit, G. Moscati Hospital, Avellino, Italy; ^bLaboratory of Clinical Chemistry and Microbiology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; ^cUOSD Malattie Rare del Globulo Rosso, AORN A. Cardarelli, Naples, Italy; ^dUOC Biochimica Clinica, AORN Ospedale dei Colli, P.O. Monaldi, Naples, Italy; ^eMagnetic Resonance Imaging Unit, Fondazione G. Monasterio, CNR-Regione Toscana, Pisa, Italy

ABSTRACT

Objectives: Non-transfusion-dependent thalassemia includes a variety of phenotypes and genotypes that rarely require regular transfusions. However, these patients can experience a wide range of complications. The objective of this retrospective study was to verify whether there is a significant difference in non-transfusion-dependent thalassemia-related complications and treatment among males and females.

Methods: We performed a re-analysis of samples evaluated in a previously published cross-sectional study, regarding 96 non-transfusion-dependent thalassemia patients followed at the 'UOSD Malattie Rare del Globulo Rosso' Centre of the Cardarelli Hospital in Naples, Italy.

Results: We found that females were more anemic than males, but there was no significant difference in prevalence of common complications among genders, except for hypogonadism. Furthermore, the transitory regular transfusions regimen in women who had been pregnant does not seem to have a significant impact on overall prognosis.

Discussion: In non-transfusion-dependent thalassemia patients, the lower levels of hemoglobin found in females do not seem to indicate a higher prevalence of complications.

Conclusion: This data should be considered in studies with experimental treatments aiming to correct anemia in patients with non-transfusion-dependent thalassemia. It should probably also be taken into account in order to set up different transfusion regimens among genders in transfusion-dependent patients.

KEYWORDS

Thalassemia; gender medicine; severity; pregnancy; iron overload; genotype; phenotype

Introduction

Non-transfusion-dependent thalassemia (NTDT) encompasses a phenotypical and genotypical heterogeneous group of hemoglobinopathies, rarely requiring regular transfusion therapy. Despite relative independence from transfusions these patients experience a wide range of comorbidities and complications, which are the consequence of complex interactions of multiple pathophysiological factors: chronic anemia, ineffective erythropoiesis, hypercoagulability, and iron overload [1,2]. The treatment of NTDT relies on occasional or more frequent blood transfusions (during severe infection, pregnancy, and surgery), iron chelation, splenectomy, and hydroxyurea (HU). Gender medicine is a fascinating emergent approach which analyses the role of gender in several processes. It is increasingly being considered in all medical fields, including hemoglobinopathies. In sickle cell anemia and thalassemia major, several studies have demonstrated longer and better survival in females compared to males [3]. As of our knowledge there are no studies regarding NTDT. The aim of this retrospective study

was to verify whether there is a significant difference in NTDT-related complications and treatment among males and females.

Methods

In this study, we performed a re-analysis of samples evaluated in a previously published cross-sectional study, regarding 96 NTDT patients followed at the 'UOSD Malattie Rare del Globulo Rosso' Centre of the Cardarelli Hospital in Naples, Italy [4]. All medical records of NTDT patients were analyzed to collect data on the presence of common morbidities and disease complications. Complications were defined according to criteria reported in the OPTIMAL CARE study [5]. The study was approved by the Ethics Committee of the Cardarelli Hospital of Naples, Italy and all the enrolled patients signed the informed consent.

T2* Magnetic Resonance Imaging (MRI) measurements were available for 52 patients (54%) who underwent MRI within the Myocardial Iron Overload in Thalassemia (MIOT) network using a 1.5 T MR scanner

(GE Excite HD) and validated and standardized procedures. Briefly, for iron overload assessment, T2* gradient-echo multiecho sequences were acquired. For the liver, a mid-hepatic slice was obtained. T2* images analysis was performed using a custom-written, previously validated software (HIPPIOT®). Hepatic T2* values were calculated in a circular region of interest and were converted into liver iron concentration (LIC) using the Wood's calibration curve [6].

The data were collected in a specially prepared form, including age, sex, transfusion status, hydroxyurea (HU) treatment, splenectomy, besides the previously mentioned complications. The study population was divided by gender in order to establish a sex-dependent difference in complications. Forty-eight (51%) patients were under chelation therapy (31 desferrioxamine, 11 deferasirox, and 6 deferiprone). The biomarkers evaluated were hemoglobin (Hb), nucleated red blood cells (NRBC), creatinine, uric acid, soluble transferrin receptor (sTfR) and ferritin.

Statistical analyses were performed using MedCalc® (ver. 10.2.0.0, MedCalc software, bvba, Ostend, Belgium). Results for descriptive statistics were expressed as mean \pm standard deviation. Fisher's exact test was used to compare the incidence of different parameters between the two groups. Student's *t*-test was used to compare differences in parametric data. A *p*-value below 0.05 was considered statistically significant.

Results

Overall, 96 patients were included in the analysis (Table 1), 43 (45%) males and 53 (55%) females. Both groups showed comparable distribution within previously identified NTDT genetic entities [4]; the prevalence of males and females was respectively 51.2% vs. 43.4% ($p = 0.53$), 7% vs. 9.4% ($p = 0.73$), 27.9% vs. 30.2% ($p = 0.82$) and 13.9% vs. 17% ($p = 0.78$) considering homozygous or compound heterozygous state for β thalassemia, combination of a β defect plus a β chain variant, triplicated α genotype associated with β heterozygosity and HbH disease, respectively (data not shown). Median age was similar in the two groups ($p > 0.05$). Hemoglobin levels were significantly lower ($p < 0.01$) in females, while ineffective erythropoiesis and expanded erythropoiesis biomarkers, such as soluble transferrin receptor (sTfR) [7] and nucleated red blood cells (NRBC), were comparable. There was no significant difference for other laboratory parameters, except for serum creatinine and uric acid levels, which were lower in females, according to data reported in the general population. Despite the reduced hemoglobin level, occasional blood transfusions were significantly more frequent (75.5% vs. 41.9%, $p < 0.01$) in females than males. The use of HU,

iron chelation and splenectomy was comparable in the two groups. Iron overload assessment, including serum ferritin, LIC, hepatic and cardiac T2*, was comparable among genders. The two groups showed similar prevalence of all complications (Table 1): extramedullary hematopoiesis, osteoporosis, osteopenia, thrombosis, pulmonary hypertension, diabetes, cholelithiasis, hypothyroidism and nephropathy. The only complication that showed a significant difference among males and females was hypogonadism, with a higher prevalence in males ($p < 0.05$).

We also tried to better investigate the difference in current phenotypes among females who had been pregnant (17 patients) and those who had not been (36 patients). Both groups were comparable for age: 46.41 years of age (range 24.68–65.93) and 35.08 years of age (range 22.33–78.8) for pregnant and non-pregnant, respectively ($p = 0.29$). Non-pregnant females, belonged more to the group with homozygous or compound heterozygous state for β thalassemia (55.6% vs. 17.6%, $p = 0.016$), were more often splenectomized (55.5% vs. 23.5%; $p < 0.05$) and had higher serum uric acid (5.2 ± 1.5 mg/dl vs. 4.3 ± 1.0 mg/dl; $p < 0.05$). Non-pregnant females had the tendency to have higher sTfR (7.8 ± 3.7 mg/ml vs. 6.2 ± 2.8 mg/ml, respectively; $p > 0.05$), NRBC ($11486.1 \pm 15806.5 \times 10^3/\mu\text{L}$ vs. $3549.1 \pm 11064.8 \times 10^3/\mu\text{L}$, respectively; $p > 0.05$), osteoporosis (27.8% vs. 5.9%; $p = 0.08$) and extramedullary hematopoiesis (38.9% vs. 11.8%; $p = 0.06$) and thus globally presented a more severe disease. On the other hand, all pregnant females received more blood transfusions (100.0% vs. 69.4%; $p < 0.05$), but less chelation (17.6% vs. 52.8%; $p < 0.05$) and had a slightly but not significantly lower LIC (4.4 ± 3.0 mg/g dry weight vs. 7.0 ± 8.12 mg/g dry weight, respectively; $p > 0.05$) as compared to non-pregnant ones.

Discussion

Overall, our data in a selected population, indicate that there is no significant difference in prevalence of common complications among male and female patients with NTDT, except for hypogonadism.

In healthy subjects, reference ranges for hemoglobin and ferritin in women of reproductive age are known to be lower than equivalent aged males. Interestingly, in our study NTDT patients show a similar pattern with respect to hemoglobin and ferritin values. Despite female NTDT patients having a history of more frequent transfusions compared to male patients, hemoglobin was significantly lower in females.

Several studies have reported better survival and a lower incidence of complications (in particular heart failure and arrhythmias) in female patients with thalassemia major and cardiac iron loading [3,8–10].

Table 1. Clinical and laboratory characteristics of the patients.

	Overall (n = 96)	Male (n = 43; 45%)	Female (n = 53; 55%)	p-value
Age (y) (range)	41.0 (17.9–78.8)	42.1 (17.9–74.4)	37.3 (22.3–78.8)	0.751
Hb (gr/dl) (SD)	9.6 (±1.0)	10.0 (±1.0)	9.2 (±0.9)	0.001*
Nucleated red blood cells ($\times 10^3/\mu\text{l}$) (SD)	11317.2 (±19613.7)	14246.9 (±24126.1)	8940.3 (±14826.5)	0.189
Creatinine (mg/dl) (SD)	0.7 (±0.2)	0.8 (±0.2)	0.6 (±0.1)	0.001*
Uric acid (mg/dl) (SD)	5.3 (±1.4)	5.8 (±1.3)	4.9 (±1.4)	0.004*
sTfR (mg/ml) (SD)	7.7 (±3.9)	8.3 (±4.3)	7.3 (±3.5)	0.198
Ferritin (ng/ml) (SD)	477.6 (±419.5)	550.8 (±347.7)	418.5 (±464.5)	0.129
T2* liver (ms) (SD)	11.7 (±8.1)	12.4 (±7.4)	10.7 (±9.0)	0.498
LIC (mg/g dry weight) (SD)	4.6 (±5.0)	3.6 (± 2.9)	6.1 (±6.7)	0.098
T2* heart (ms) (SD)	40.1 (±5.9)	39.1 (±5.6)	41.4 (±6.3)	0.288
Occasional blood transfusion (n, %)	58 (60.5%)	18 (41.9%)	40 (75.5%)	0.001*
Chelation therapy (n, %)	48 (51.0%)	26 (62.0%)	22 (42.0%)	0.100
Splenectomy (n, %)	44 (46.0%)	20 (48.0%)	24 (45.0%)	1.0
Hydroxyurea (n, %)	19 (20.0%)	9 (21.0%)	10 (19.0%)	0.803
No complications (n, %)	12 (12.5%)	6 (13.9%)	6 (11.3%)	0.762
Cholelithiasis (n, %)	56 (60.0%)	26 (63.0%)	30 (57.0%)	0.835
Osteopenia (n, %)	42 (47.0%)	21 (55.0%)	21 (40.0%)	0.412
Extramedullary hematopoiesis (n, %)	28 (35.0%)	12 (33.0%)	16 (36.0%)	0.825
Nephropathy (n, %)	17 (18.0%)	8 (20.0%)	9 (17.0%)	1.0
Osteoporosis (n, %)	15 (17.0%)	4 (11.0%)	11 (21.0%)	0.161
Hypothyroidism (n, %)	15 (16.0%)	5 (12.0%)	10 (19.0%)	0.404
Thrombosis (n, %)	10 (11.0%)	5 (12.0%)	5 (10.0%)	0.748
Hypogonadism (n, %)	7 (8.0%)	6 (14.0%)	1 (2.0%)	0.043*
Pulmonary hypertension (n, %)	5 (5.0%)	1 (2.0%)	4 (8.0%)	0.376
Diabetes mellitus (n, %)	2 (2.0%)	2 (5.0%)	0 (0.0%)	0.198

However, heart iron detected by T2* MRI was comparable between the sexes, speculating that females tolerate iron toxicity better, as an effect of reduced sensitivity to chronic oxidative stress [3]. For other complications such as diabetes, thrombosis, hypothyroidism, and hypogonadism no association between genders has been reported [9].

This is also true for our study in a population of patients with a different hemoglobinopathy with the only exception of hypogonadism, which is more common in our male population. The OPTIMAL CARE study which also analyses a large population of NTD patients found a significant difference in thrombosis, cholelithiasis, osteoporosis, and hypogonadism, all more common in females. However, these patients were more heavily iron overloaded and transfused compared to ours; furthermore, the study did not specify distribution of males and females among different genotypes. Therefore the two populations and the respective results are not comparable [5].

In our series where cardiac iron loading is not encountered, a difference in cardiac complications could not be detected. In NTD patients, the lower levels of hemoglobin found in females do not seem to indicate a higher prevalence of complications. This data should be considered in studies with experimental treatments aiming to correct anemia and should probably be extrapolated to consider different transfusion regimens among genders. In fact, in transfusion-dependent patients (TDT), there is evidence that current standard transfusion regimens are associated with personalized levels of erythroid activity, indices of erythropoiesis and the presence of extramedullary

erythropoiesis that may also differ in relation to gender, being higher in males [11–13]. Further prospective studies are needed to verify if it would be safe to use higher pre-transfusion hemoglobin level in male TDT patients in order to normalize this difference and probably reduce the observed gap in morbidity and life expectancy [14].

Considering the significantly higher transfusion requirement of female patients, likely secondary to pregnancy, we would have expected a higher iron overload and therefore a higher incidence of iron-related complications (endocrinopathies), which was not found.

However, we must consider that pregnancy generally in our series occurred in females with fewer complications and with less severe forms of NTD (mostly those with triplicated α genotype associated with β heterozygosity, data not shown) and consequently with lower intestinal iron absorption. Furthermore, this may be partially explained by iron demand of the fetus. The transitory regular transfusions regimen does not seem to have a significant impact on overall prognosis in these patients and iron overload during pregnancy seems well-tolerated.

Of course, the retrospective nature of the study and the small patient population do not allow us to draw general conclusions; further studies are needed to verify these finding in a larger and differently composed population of NTD.

The data presented somehow confirm a better iron toxicity toleration in females, as reported in the thalassemia major population [14], possibly as an effect of decreased sensitivity to chronic oxidative stress. Further studies are needed to clarify if the observed

reduced prevalence of hypogonadism could be ascribed to a direct effect of female hormones.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Paolo Ricchi  <http://orcid.org/0000-0001-7361-3308>

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