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ORIGINAL ARTICLE

Prevalence of extramedullary hematopoiesis, renal cysts, splenic and hepatic lesions, and vertebral hemangiomas among thalassemic patients: a retrospective study from the Myocardial Iron Overload in Thalassemia (MIOT) network

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Abstract

We determined the prevalence of incidental extracardiac findings (IEF) at Magnetic Resonance Imaging (MRI) potentially related to anemia and hypoxia in age- and sex-matched populations ($N = 318$) with thalassemia major (TM) and thalassemia intermedia (TI) enrolled in the Myocardial Iron Overload in Thalassemia network. Overall, IEFs were detected in 33.3% and 25.8% of patients with TI and TM, respectively ($P = 0.114$). TI and TM patients had elevated but comparable prevalence of renal, splenic and liver cysts, and vertebral hemangiomas while TI patients had a significant higher frequency of extramedullary hematopoiesis (EMH) (15.1% vs 4.4%; $P = 0.002$). The prevalence of total IEFs increased with advancing age. TI non-transfusion-dependent patients had a significantly lower frequency of renal cysts than TI transfusion-dependent patients (8.8% vs 26.4%; $P = 0.005$). The prevalence of renal cysts in the thalassemic population was significantly higher than that in the general population (19.2% vs 1.9%; $P < 0.0001$). Our data on renal cysts indicate a significant higher prevalence of these IEFs compared to the general population, suggesting the role of the inappropriate activation of the hypoxia-inducible factor system linked to the chronic hypoxia. The significant prevalence of IEF in thalassemia patients undergoing MRI for iron quantification should prompt the discussion of the inclusion of IEF in the MRI report.

Keywords Thalassemia · Extracardiac findings · Magnetic resonance imaging

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Introduction

Magnetic Resonance Imaging (MRI) is increasingly being used for the assessment of cardiac and liver iron overload and cardiac function parameters in patients with thalassemia [1]. In recent years, the systematic use of MRI has contributed to improve the management and chelation treatment of thalassemic patients with and without iron overload, leading to a significant reduction of their morbidity and mortality [2, 3]. However, near to the heart and the liver, outside the target organs of interest, surrounding structures comprising the lungs, upper and part of lower abdomen, and thoracic spine are included in the scan area. As expected, this leads to the visualization of a significant volume of the patient and to the possibility of detecting extracardiac and incidental findings [4].

A variety of studies have previously explored the prevalence and clinical relevance of incidental extracardiac findings (IEF) in the general population [5, 6] undergoing cardiovascular

magnetic resonance (CMR) for common and/or non-thalassemic cardiac diseases. There are several types and sites of IEF: they can involve mostly neck, chest, lungs, bones, abdomen, and kidneys and may be clinically significant or not [7].

In a previous study from Myocardial Iron Overload in Thalassemia (MIOT) network, we have evaluated the prevalence of extramedullary hematopoiesis (EMH), an IEF quite common and closely linked to the pathogenesis of the disease in patients with thalassemia major (TM) [8]. In this study, the prevalence of EMH was higher with respect to previous assessment to the point that it seemed appropriate to suggest to search for EMH also in regularly transfused patients [9]. Patients with thalassemia syndromes are characterized by different degrees of ineffective erythropoiesis, peripheral hemolysis, anemia, and subsequent transfusion dependence. Furthermore, particularly in patients with thalassaemia intermedia (TI), the combination of ineffective erythropoiesis, anemia, and hypoxia induces the activation of hypoxia-inducible factors (HIFs) which in turn lead to a compensatory increase in serum levels of erythropoietin (EPO), to an expanded erythropoiesis, to a decrease in serum levels of hepcidin as well as to an increase in iron loading [10–12]. HIF factors are the pivotal intermediaries of the hypoxic response able to induce the expression of genes that arbitrate adaptation to low oxygen tensions. Consistently, high levels of HIF1a have been recently detected in the nuclear compartment of patients with thalassemia [13]. In congenital diseases such as von Hippel-Lindau disease and Chuvash policitemia, where HIFs are overexpressed, there is an increase in hypervascular and cystic lesions especially at kidney level and, in some cases, also in neoplastic lesions [14, 15].

To our knowledge, despite the growing number of incidentally diagnosed findings detected following the use of imaging techniques, such as ultrasound (US) [16], there is no study accurately evaluating the prevalence of IEF in patients with thalassaemia. Thus, the purpose of this study was to retrospectively evaluate "in real life" the profile of several IEF potentially linked to HIF overexpression in a large-sized cohort of age- and sex-matched populations with TM and TI undergoing CMR within the MIOT network.

Methods

Patients

The MIOT project is an Italian network built in 2006 and constituted by 70 thalassemia centers, and 9 MRI centers where MRI exams are performed using homogeneous and standardized procedures. All centers are linked by a web-based database, configured to collect and share patient data [17].

We selected the first 159 TI patients (34.71 ± 9.20 years; 86 females) consecutively enrolled in the project between April 2006 and May 2014. Moreover, a cohort of 159 TM patients was randomly selected on the basis of a 1:1 matching by age and gender.

Finally, 159 patients without thalassemia referred to the core lab of the MIOT network (Pisa, Italy) for clinically indicated CMR were enrolled retrospectively in this study on the basis of a 1:1 matching by age and gender for the evaluation of IEF prevalence in the general population.

Kidney function was assessed by measuring the glomerular filtration rate (GFR), estimated by means of the modification of diet in renal disease (MDRD) formula [18].

The study complied with the Declaration of Helsinki. All patients gave written informed consent to the protocol. The institutional review board approved this study.

Magnetic Resonance Imaging

MRI exams were performed using a 1.5 T scanner. An 8-element cardiac phased-array receiver surface coil with breath holding in end expiration and ECG gating were used for signal reception.

Multiplane steady-state free precession (SSFP) or spoiled gradient-recalled (SPGR) localizers were used.

After localization of cardiac axes, a standardized CMR protocol including the following sequences was applied:

- cine SSFP images in the two standard long-axis planes of the left ventricle (LV): horizontal long axis or 4-chamber view and vertical long axis or 2-chamber view;
- stack of parallel short axis cine SSFP acquisitions with coverage from the atrio-ventricular ring to the apex for the study of cardiac function;
- T2* gradient–echo multiecho images in three parallel short-axis views of the LV (basal, medium, and apical) for the study of myocardial iron [3, 19, 20].
- late gadolinium enhancement (LGE) images acquired replicating the same imaging planes from the cine SSFP sequences 10–18 min after Gadobutrol (Gadovist®; Bayer Schering Pharma; Berlin, Germany) intravenous administration at the standard dose of 0.2 mmol/kg to detect the presence of myocardial fibrosis [21].

LGE images were not acquired in patients with a glomerular filtration rate < 30 ml/min/1.73 m^2 and in patients who refused.

Moreover, a single mid-hepatic transverse slice was acquired for the quantification of hepatic iron [22]. Te T2* value was calculated in a circular region of interest (ROI) of standard dimension, chosen in a homogeneous area of parenchyma without blood vessels [23]. As recommended [24], liver

T2* values were converted into liver iron concentration (LIC) values using the calibration curve introduced by Wood [25].

All the acquired images were retrospectively reviewed by two radiologists experienced in cardiac MRI, who investigated the presence of IEF such as extramedullary hematopoiesis, renal cysts, splenic and hepatic lesions, and vertebral hemangiomas.

Statistical analysis

All data were analyzed using SPSS version 13.0. Continuous variables were described as mean ± standard deviation (SD) and categorical variables as frequencies and percentages.

The normality of distribution of the parameters was assessed by using the Kolmogorov–Smirnov test. For continuous values, comparisons between groups were made by independent-sample *t* test or by Wilcoxon's signed rank test. The χ^2 test was used for non-continuous variables.

In all tests, $P < 0.05$ was considered to be statistically significant.

Results

Comparison between thalassemia patients and general population

Incidental findings were found in 94/318 (29.6%) of patients with thalassemia, for a total of 106 IEFs. The most common site was the kidney (61/106, 57.5%). Thalassemia patients without IEF were significantly younger than patients with IEF (33.21 ± 9.09 years vs 38.30 ± 8.40 years; $P < 0.0001$). Incidental findings were found in 39 males, and 55 females and the percentage of males was comparable between patients without and with IEF (47.8% vs 41.4%; $P = 0.305$).

Incidental findings were found in 14/159 (8.8%) of patients without thalassemia. Non-thalassemia patients without and with IEF showed comparable age (34.68 ± 9.33 years vs 35.43 ± 7.90 years; $P = 0.798$) and percentage of males (45.5% vs 50.0%; $P = 0.748$).

The incidence of collateral findings, considered all together, was significantly higher in thalassemia patients (29.6% vs 8.8%; $P < 0.0001$). In the subgroup of patients with IEF, no significant difference of age was detected between thalassemia and non-thalassemia patients (38.30 ± 8.40 years vs 35.43 ± 7.90 years; $P = 0.207$).

The frequency of renal cysts was significantly higher in thalassemia than in non-thalassemia patients (19.2% vs 1.9%; $P < 0.0001$) while no significant difference was found in the frequency of hepatic lesions (0.9% vs 3.8%; $P = 0.066$), splenic lesions (1.6% vs 0.6%; $P = 0.669$), and vertebral hemangiomas (1.9% vs 2.5%; $P = 0.651$). EMH was absent in the non-thalassemia population.

Incidental findings in TI and TM patients

Table 1 shows the comparison between TI and TM patients age- and sex-matched. TI patients made their first transfusion significantly later and had significantly lower serum ferritin and hemoglobin levels. The incidence of collateral findings, considered all together, was comparable between TI and TM patients. Moreover, TI and TM patients showed a comparable frequency of renal cysts, liver lesions, splenic lesions, and vertebral hemangiomas. Conversely, the EMH frequency was significantly higher in TI patients.

Among the TI patients, the group without collateral IEF was significant younger than the group with IEF (33.72 ± 9.09 years vs 36.71 ± 9.18; $P = 0.016$) while no influence of gender was detected. Analogously, among the TM patients, the age was significantly lower for the group without IEF (32.75 ± 9.10 years vs 40.36 ± 6.84 years; $P < 0.0001$).

Renal cysts

The prevalence of renal cysts increased with age. It was significantly lower in patients under the age of 25 years and in the age group 25–35 years than in patients in the age groups 35–45 years and 45–55 years (Fig. 1a).

The prevalence of renal cysts tended to be higher among the females, but the statistical significance was not reached (Fig. 1b).

The GFR was comparable between patients without and with renal cysts (144.93 ± 47.44 ml/min/1.73 m^2 vs 147.27 ± 47.66 ml/min/1.73 m^2; $P = 0.703$).

Among TM patients, those with renal cysts showed, when compared to those without renal cysts, significantly lower MRI LIC values (4.69 ± 4.56 mg/g dw vs 8.29 ± 11.43 mg/g dw; $P = 0.043$) and an higher frequency of EMH, although the statistical significance was not reached (9.7% vs 3.1%, $P = 0.135$).

Among TI patients, those with and without renal cysts had comparable MRI LIC values (9.10 ± 9.14 mg/g dw vs 9.84 ± 9.06 mg/g dw; $P = 0.642$) and frequency of EMH (13.3% vs 15.5%; $P = 0.765$).

Correlation between MRI findings and transfusion dependency in TI

Thalassemia intermedia patients were divided into two groups according to their transfusional regimen: non-transfusion dependent (NTD) and transfusion dependent (TD).

The NTD group (68/159 = 42.8%) included the TI patients who have never been transfused or who have received occasional RBC transfusions (< 6/year).

Table 2 shows the comparison between TI-NTD and TI-TD patients. TI-NTD had significantly lower serum ferritin levels. The incidence of collateral findings, considered all together,

Table 1 Comparison between TI and TM patients

	TI ($N = 159$)	TM ($N = 159$)	P
Sex (M/F)	73/86	73/86	1.000
Age (years)	34.71 ± 9.20	34.71 ± 9.18	0.960
Age at first transfusion (years)	9.82 ± 10.71	1.72 ± 1.75	< 0.0001
Chel. starting age (years)	14.21 ± 11.26	6.18 ± 5.56	< 0.0001
Mean serum ferritin (ng/l)	958.79 ± 859.09	1386.59 ± 1378.99	0.004
Pre-transfusion hemoglobin (g/dl)	9.09 ± 1.06	9.66 ± 0.57	< 0.0001
MRI findings, *N* (%)	53 (33.3)	41 (25.8)	0.114
Kidney cysts, *N* (%)	30 (18.9)	31 (19.5)	0.879
Liver lesions (cysts and hemangiomas), *N* (%)	0 (0.0)	3 (1.9)	1.000
Splenic lesions (cysts and hemangiomas), *N* (%)	4 (2.5)	1 (0.6)	0.213
Vertebral hemangiomas, *N* (%)	3 (1.9)	3 (1.9)	1.000
Extramedullary erythropoiesis, *N* (%)	24 (15.1)	7 (4.4)	0.002

was comparable between the two groups. Moreover, TI-NTD and TI-TD patients showed a comparable frequency of liver lesions, splenic lesions, vertebral hemangiomas, and EMH. Conversely, the frequency of renal cysts was significantly lower in TI-NTD patients.

Considering only the TI-NTD group, patients without and renal cysts showed comparable age (33.14 ± 9.04 years vs 35.31 ± 15.14 years; $P = 0.601$) and frequency of males (54.8% vs 16.7%; $P = 0.101$). Considering only the TI-TD group, patients without renal cysts were significantly younger than patients with renal cysts (34.76 ± 8.83 years vs 38.49 ± 8. 34 years; $P = 0.030$) while no significant difference was detected in the frequency of males (44.8% vs 33.3%; $P = 0.329$).

Discussion

Today, MRI has become a fundamental procedure in the management of patients with thalassemia [26]. Patients with thalassemia frequently undergo MRI for iron overload evaluation [19, 27–29] but also for EMH detection [30, 31]. However, guidelines of CMR do not currently include the assessment of extracardiac pathologies and no data are available for patients with thalassemia [28, 32]. Our data firstly show that IEF are well-recognized features in patients undergoing MRI for heart and liver iron evaluation, being renal cysts, EMH, and vertebral hemangiomas the most frequently observed.

The prevalence of vertebral hemangiomas was not significantly different between thalassemia patients and our control population and seemed to suggest a not accidental association. Further studies are needed to better assess the real prevalence of these IEF with proper and dedicated MRI anatomical sequences.

Our data among thalassemic patients showed a significant role for advancing age in the development of renal cysts and a significant higher frequency of renal cysts in comparison to the control population. It is worth noting that our results are in line with those of a recent study showing that the prevalence of renal cysts was significantly higher in sickle cell disease (SCD) patients than in the control subjects across all age groups [33]. The authors hypothesized the involvement of HIF overexpression in their clinical findings. Recently, also the pathogenesis of multiple bilateral renal cysts, characteristic of polycystic kidney disease (PKD), has been linked to overactivation of hypoxia-inducible factor 1-alpha (HIF-1α) [34]. HIF-1 is a protein with DNA-binding activity consisting of an O2-sensitive α-subunit and a constitutively expressed β-subunit. Three HIF α-subunits are known, HIF-1α, HIF-2α, and HIF-3α, together with HIF-2α (also known as endothelial PAS domain protein 1 (EPAS1) or HIF-like factor, (HLF); HIF-1α facilitates O2 delivery and cellular adaptation to hypoxia by stimulating a wide spectrum of biological processes encompassing cell growth, erythropoiesis, iron loading, glucose metabolism, and angiogenesis [35]. Theoretically, in TM patients which receive regular transfusions to improve hemoglobin levels and to suppress bone marrow activity, the homeostatic response to hypoxia should be reduced as compared to TI ones. But, in the study of Ferro et al. [13], it was showed firstly that the persistent hypoxia of untransfused patients was not appropriately reflected by HIF1a and Glut1 expression, highlighting the differential impact of initial versus chronic response in the HIF1a vs HIF2a response, respectively, as also previously hypothesized by Holmquist–Mengelbier et al. [36]. In our series, patients with renal cysts were significantly older and in TM group patients with renal cysts showed significantly lower levels of liver iron overload and a tendency to develop EMH more frequently. Despite lower Hb levels and higher EMH, although not statistically significant, TI-NTD patients had a significant lower frequency of renal cysts compared with

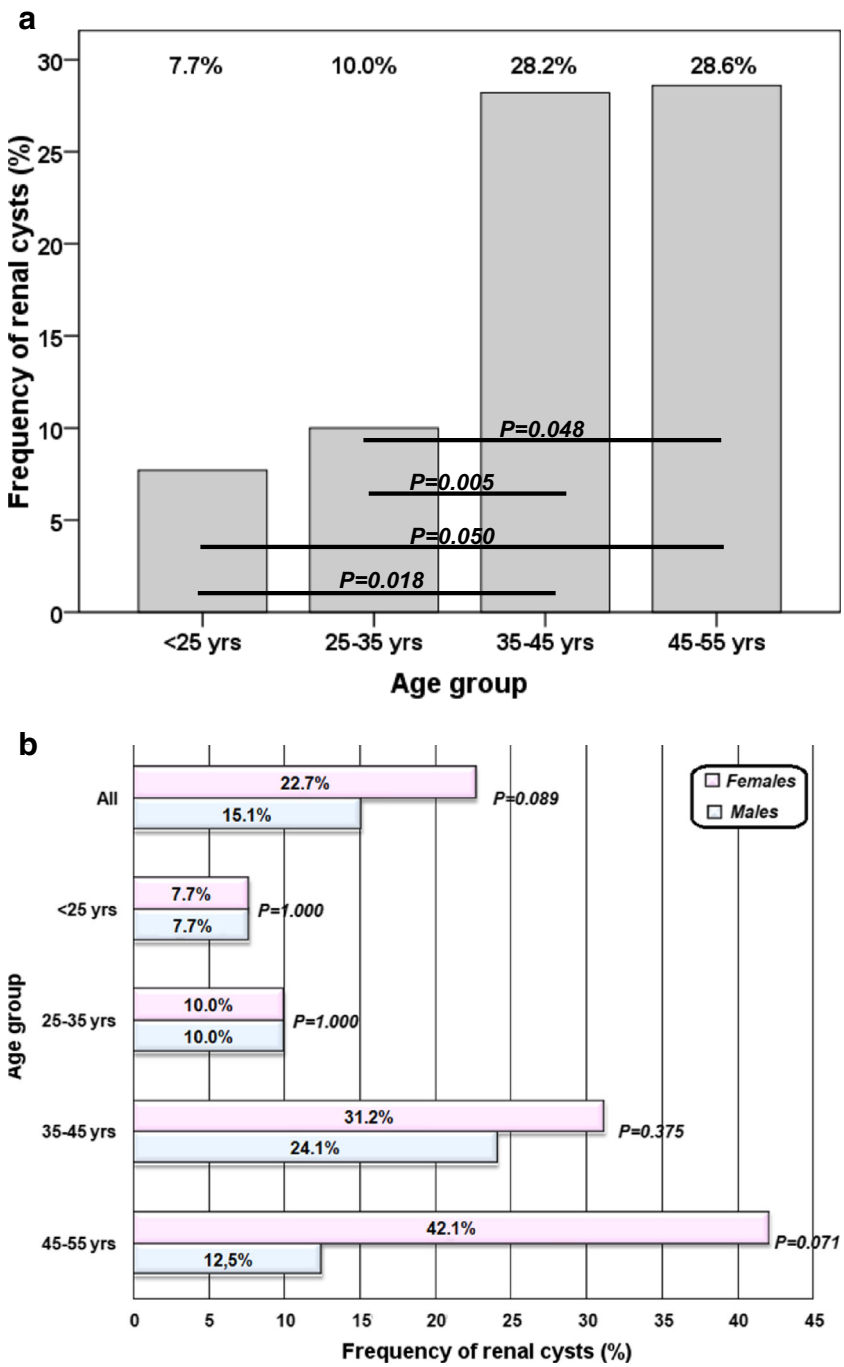

Fig. 1 **a** Frequency of renal cysts in different classes of age. The horizontal lines indicate a significant difference between two classes. **b** Frequency of renal cysts in males and females for all thalassemia patients and for each class of age

TI-TD. Overall, such radiological evidence could suggest from a pathogenetic point of view a role of HIF-overexpression in promoting both EMH and renal cysts and a complex, time-dependent, and probably organ-specific relationship between hypoxia, anemia, and the induction of different HIF signaling. Further studies are needed to establish at molecular level how HIF promotes erythropoiesis and coordinates cell type-specific hypoxia responses [37] in patients with thalassaemia and the concurrent role of unrecognized factors in this *scenario*. The complex interplay of cellular factors regulating HIFs in vivo and to what extent the activation of this molecular pathway play a role in promoting each IEF described need to be further elucidated. Likely, it may be pathogenetically involved also in the recently described increase in clear cell renal cancer and angiomiolipoma prevalence among thalassemic patients; tumors where HIF overexpression and expanded erythropoiesis are well recognized features, respectively [16, 38, 39]. Renal disease is an emerging morbidity in thalassemic patients [40]. It

Table 2 Comparison between TI-NTD and TI-TD patients

	TI-NTD ($N = 68$)	TI-TD ($N = 91$)	P
Sex (M/F)	35/33	38/53	0.224
Age (years)	33.34 ± 9.59	35.74 ± 8.81	0.074
Ferritin (ng/l)	718.40 ± 613.28	1139.08 ± 970.19	0.008
Hemoglobin (g/dl)	8.98 ± 1.40	9.18 ± 0.70	0.068
MRI findings, *N* (%)	19 (27.9)	34 (37.4)	0.212
Kidney cysts, *N* (%)	6 (8.8)	24 (26.4)	0.005
Liver lesions (cysts and hemangiomas), *N* (%)	0 (0.0)	0 (0.0)	1.000
Splenic lesions (cysts and hemangiomas), *N* (%)	1 (1.5)	3 (3.3)	0.636
Vertebral hemangiomas, *N* (%)	0 (0.0)	3 (3.3)	0.261
Extramedullary erythropoiesis, *N* (%)	14 (20.6)	10 (11.0)	0.094

is currently believed that patients with TM develop renal dysfunction related to anemia and hypoxia and to the effects of iron overload and iron chelation therapy. In patients with TI, the more severe anemia leading to hyperfiltration and to hyperdynamic circulation is responsible in the long term of a progressive increased creatinine clearance [41]. However, according to our serum creatinine findings, in this retrospective study, the presence of renal cysts was not associated to renal impairment. So, further longitudinal studies are needed to evaluate if their presence is a prognostic marker of progressive renal injury or of an increased sensitivity to nephrotoxic effect of iron chelation therapy.

In conclusion, the significant prevalence of IEF in thalassemia patients undergoing MRI for heart and liver iron quantification could support the inclusion of IEF in the MRI report. The survey of these additional IEF may help to go further inside the understanding of physiopathology of the disease suggesting the occurrence of an inappropriate activation of HIF system linked to the chronic anemia.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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