Thrombosis Research 144 (2016) 119–122

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

# High incidence of silent cerebral infarcts in adult patients with beta thalassemia major

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ARTICLE INFO

*Article history:*
Received 16 April 2016
Received in revised form 10 June 2016
Accepted 12 June 2016
Available online 14 June 2016

*Keywords:*
Silent cerebral infarcts
Adult patients
Beta thalassemia major
Transfusion dependent
Iron chelation

ABSTRACT

*Objectives:* Survival of beta thalassemia major (TM) patients has improved significantly over the past few decades. Consequently, less commonly reported complications are now being recognized. An incidence as high as 60% of silent cerebral infarcts (SCI) has been demonstrated by brain Magnetic Resonance Imaging (MRI) studies in beta thalassemia intermedia (TI). The aim of this study was to determine whether regularly transfused TM adult patients experience less SCI, as compared to the incidence described in TI.
*Methods:* In this observational study, 28 transfusion dependent TM patients, >18 years of age underwent brain MRI studies.
*Results:* Focal bright foci in the cerebral white matter were demonstrated in 17 (60.7%) patients; most of them had multiple lesions. Elevated serum ferritin (SF), primarily 5 years Area Under the Curve, was found to have a significant association with the presence of SCI ($p < 0.031$). Similar results were found when 4 patients with intact spleen and 2 patients with splenules were excluded ($p = 0.027$). There was no significant association between number of SCI and clinical or other laboratory parameter evaluated.
*Conclusions:* The present study demonstrates a high rate of SCI in regularly transfused TM adult patients. Effective continuous iron chelation, preventive low dose aspirin and routine periodical brain MRI are recommended.


## 1. Introduction

Beta thalassemia is an inherited disorder resulting from mutations in the beta-globin gene and consequently impaired beta-chain production, alpha/beta-globin chain synthesis ratio imbalance, ineffective erythropoiesis, reduced red blood cell (RBC) survival, and subsequent hemolytic anemia [1,2]. Homozygous and compound heterozygous forms of beta-thalassemia have diverse phenotypes, traditionally defined as beta thalassemia major (TM) and beta thalassemia intermedia (TI) [1–3], and recently defined as transfusion dependent and nontransfusion dependent thalassemia [2,3].

Survival of transfusion dependent TM patients has improved significantly over the past few decades as better treatment became available [4]. Consequently, previously less commonly reported complications are now being recognized, including venous and arterial thromboembolic events [1,4–10].

Profound hemostatic changes and high incidence of thromboembolic events have led to the recognition of a chronic hypercoagulable state in TM and TI patients [1,6–8,10]. Mortality related to thromboembolic complications has been reported in up to 29.0% in TI, but only in 1.1–4.0% in TM [4,11–12]. A high incidence of asymptomatic silent cerebral infarcts (SCI) has been demonstrated by brain Magnetic Resonance Imaging (MRI) studies in patients with TI [13,14]. Supported by the fact that repeated transfusions significantly decrease the risk of thrombotic complications, it was assumed that in multi transfused TM patients, having less circulating pathological RBC and platelets, the incidence of SCI would be lower [9,11,13,15].

The aim of the present study was to determine whether transfusion dependent TM patients have less SCI compared to the high incidence described in TI.

## 2. Patients and methods

This observational study was performed in transfusion dependent TM patients, >18 years of age, followed and treated in the comprehensive center of thalassemia, hemoglobinopathies & rare anemias. All patients received regular blood transfusions every 2–3 weeks since early childhood. Patients with history of any thromboembolic event or on aspirin and/or anticoagulants were excluded. The study was approved by the Institutional Review Board of Rabin Medical Center and performed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, revised in 2008.

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http://dx.doi.org/10.1016/j.thromres.2016.06.010
0049-3848/

After obtaining a signed informed consent, and prior to blood transfusion, brain MRI studies were performed as previously published [16], using 3.0 Tesla (known for increased signal to noise ratio allowing for better resolution), sixteen channel head coil, Ingenia (Philips) system, in sagittal sections of 3 dimensional (3D) fluid-attenuated inversion recovery (FLAIR) (TR4800/TE324) voxel size 1 × 1 × 0.6 mm (for higher specificity and sensitivity), axial 4 mm sections in TSET2 (TR3000/TE80), SET1 (TR800/TE8) and Diffusion imaging. Susceptibility weighted image (SWI) sequence, used to assess visible artifacts due to iron deposition, was obtained in 140 axial sections (voxel size 1 × 0.9 × 0.9 mm). Studies were assessed by an experienced and highly qualified neuroradiologist. White matter lesions were defined as foci of increased signal intensity compared with normal intensity of the grey and white matter manifested in the thin slices of the 3DFLAIR but also in TSET2 sequences. The foci were numbered, measured and localized for each patient.

Clinical and laboratory data were retrieved from patients' medical files.

All patients underwent standard neurological examination tests, including motor and sensory systems, cranial nerves, reflexes, coordination, and mini mental status, by an experienced neurologist.

The statistical analysis was generated using SAS Software, Version 9.4. Continuous variables were presented by Mean ± SD, and median (IQR). Categorical variables were presented by (N, %). Area under the curve (AUC) for ferritin was calculated by the trapezoid rule. Correlation was assessed by the Spearman coefficient. Nonparametric Wilcoxon Rank-Sum test was used to compare variables between study groups and chi-square test was used to compare categorical variables. Association between SCI and continuous variables was assessed by ANOVA test.

## 3. Results

### 3.1. Patients' characteristics

Twenty-eight patients were included in the study, 15 males and 13 females. Demographic, clinical and laboratory characteristics are shown in Table 1. Their mean ± SD (range) age was 29.6 ± 6.0 (20–42) years. Twenty-four patients were splenectomized (SPX), two of them had one or more splenules demonstrated by abdominal ultrasonography, computed tomography or MRI. Neurological examination of all patients was normal, without any clinical consequences associated neurological deficits. All patients, as a group of transfusion dependent thalassemia patients, were treated with iron chelators, which include deferoxamine, deferiprone and deferasirox. In addition, patients were treated individually with a variety of medications, such as calcium and vitamin D supplement, thyroid hormone, and insulin.

Mean ± SD (range) pre-transfusion hemoglobin level was 99 ± 6.5 (87–110) g/L. Mean ± SD (range) platelet count was 520 ± 201 (146–1100) × $10^9$/L. SPX patients had a mean (range) platelet count of 568 (270–1100) × $10^9$/L, compared to 231 (146–320) × $10^9$/L in those with intact spleen.

Median (IQR) serum ferritin (SF) level for 3 months prior to MRI study and for 5 years prior to MRI study was 2687 (948–6134) pmol/L and 2946 (1719–6451) pmol/L, respectively. Median (IQR) area under the curve (AUC) for SF level over 5 year's period prior to MRI study was 5,336,117 (3,559,812–11,296,294). Complete workup for thrombophilia, inherited and acquired, including lupus anticoagulant and anti cardiolipin antibodies, was normal in all but 4 patients heterozygous for Factor V Leiden mutation. Mean ± SD (range) MRI T2* cardiac and hepatic values were 29.8 ± 17.8 (4.8–65.0) and 7.5 ± 7.6 (1.0–28.0) milliseconds, respectively.

### 3.2. MRI findings & patients' characteristics

MRI studies demonstrated focal bright foci, single or multiple (up to 73), in the cerebral white matter in 17 of 28 (60.7%) patients (95% CI 2.49–17.9, IQR 0–6.5). Most lesions were observed in frontal lobes of the brain, bilateral, with maximal diameter of 7 mm, as shown in Fig. 1. All lesions were negative in diffusion imaging. SWI sequence has not shown abnormality suspicious of sites of blood products. Eleven (39.3%) patients had normal MRI with no evidence of brain lesions.

As shown in Table 1, median (IQR) SF level for 3 months and 5 years prior to MRI study was 1966 (733–6359) pmol/L and 2130 (1371–6898) pmol/L, respectively, among patients without SCI, and 2696 (2029–5907) pmol/L and 3146 (2741–5651) pmol/L, respectively, among patients with SCI. Median (IQR) AUC for SF level over 5 year's period prior to MRI study was 3,910,636 (2,608,199–12,381,024) among patients without SCI, and 6,199,749 (4,998,137–9,631,505) among patients with SCI. Elevated SF, primarily 5 years AUC, was found to have a significant association with the presence of SCI ($p < 0.031$). Similar results were found when data was evaluated after exclusion of 4 patients with intact spleen as well as exclusion of 2 patients with splenules ($p = 0.027$). There was no significant association ($p > 0.05$) between the number of SCI and clinical or other laboratory parameter evaluated.

## 4. Discussion

The pathophysiology of hypercoagulability in thalassemia, resulting in symptomatic venous as well as arterial thromboembolic complications in various organs including the brain, is multi factorial, including procoagulant activity of increased numbers of circulating pathological RBC and platelets, alterations in markers of coagulation activation and natural anticoagulant proteins, as well as endothelial, monocyte, and granulocyte activation [1,5–10].

Disintegration of unstable alpha globin chains in RBC, resulting in accumulation of intracellular labile iron, and formation of free oxygen radicals, induce oxidation of membrane lipids and proteins resulting in more rigid and deformed RBC, with consequent premature destruction [1,5,7,8,10,22]. This process is associated with loss of the normal asymmetrical distribution of the RBC membrane phospholipids and exposure of negatively charged phospholipids, such as phosphatidylserine(PS), on the external surface of the RBC membrane, by a “flip-flop”

**Table 1**
Clinical and laboratory parameters of 28 patients with beta thalassemia major.

| Parameter | All patients N = 28 (100%) | Patients without SCI N = 11 (39.3%) | Patients with SCI N = 17 (60.7%) | p value |
|---|---|---|---|---|
| Age (years) mean ± SD (range) | 29.6 ± 6.0 (20–42) | 31.6 ± 6.1 (20.0–42.0) | 28.2 ± 5.7 (21.0–37.0) | ns |
| Gender M:F (%) | 15:13 (54:46%) | 6:5 (55:45%) | 9:8 (53:47%) | ns |
| Splenectomy (SPX): Intact spleen (%) | 24:4 (86:14%) | 9:2 (82:18%) | 15:2 (88:12%) | ns |
| Hemoglobin (g/L) mean ± SD (range) | 99.0 ± 6.5 (87.0–110.0) | 98.4 ± 5.5 (90.0–108.0) | 99.4 ± 7.2 (87.0–110.0) | ns |
| Platelet (× $10^9$/L) mean ± SD (range) | 520 ± 201 (146–1100) | 474 ± 195 (146–784) | 549 ± 205 (164–1100) | ns |
| Ferritin (pmol/L), 3 months median (IQR) | 2687 (948–6134) | 1966 (733–6359) | 2696 (2029–5907) | ns |
| Ferritin (pmol/L), 5 years median (IQR) | 2946 (1719–6451) | 2130 (1371–6898) | 3146 (2741–5651) | ns |
| Ferritin, 5 years AUC median (IQR) | 5,336,117 (3,559,812–11,296,294) | 3,910,636 (2,608,199–12,381,024) | 6,199,749 (4,998,137–9,631,505) | < 0.031 |
| MRI T2* Heart mean ± SD (range) | 29.76 ± 17.80 (4.80–65.00) | 30.99 ± 18.78 (6.00–65.00) | 28.93 ± 17.74 (4.80–57.00) | ns |
| MRI T2* Liver mean ± SD (range) | 7.51 ± 7.64 (1.0–28.0) | 9.41 ± 9.75 (1.50–28.00) | 6.25 ± 5.89 (1.00–19.50) | ns |