Plasma chitotriosidase activity in β-thalassemia major: a comparative study between Sicilian and Sardinian patients

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Abstract

Background: Chitotriosidase is a functional chitinase secreted by activated macrophages, which is extremely increased in plasma of patients with Gaucher disease (β-glucocerebrosidase deficiency). Recently, we found that chitotriosidase plasma levels were increased to a variable extent in Sicilian patients with β-thalassemia major. The aim of this study is to elucidate the possible mechanisms underlying chitotriosidase overproduction in β-thalassemia major.

Methods: Plasma chitotriosidase was measured in 134 patients with β-thalassemia major (64 from Sardinia and 70 from Sicily), which are treated chronically by blood transfusions leading to systemic iron overload. They all have peripheral anemia and enormous enlargement of the reticulo-endothelial system.

Results: Plasma chitotriosidase activity was found most frequently elevated among Sardinian (48.4%) than Sicilian (17.1%) patients. In either group, the highest levels of plasma chitotriosidase were observed in patients with the highest degree of iron overload, suggesting that this factor could trigger chitotriosidase overproduction.

Conclusions: The higher rate of subjects with increased plasma chitotriosidase values among Sardinian than Sicilian could be related to distinct molecular basis of β-thalassemia and environmental features. © 2001 Published by Elsevier Science B.V.

Keywords: Chitotriosidase; β-Thalassemia; Genotype; Infectious diseases; Macrophage
1. Introduction

Chitotriosidase (EC 3.2.1.14) is a functional chitinase that is able to cleave chitin, a major structural component of fungi and various pathogens [1,2]. Chitotriosidase is synthesised by specifically activated macrophages and precursors of neutrophils [3,4]; it is encoded by a gene on chromosome 1q31 [4–6], whose activity is regulated by a promoter with an as yet unclear molecular mechanism [7]. Chitotriosidase gene mutations are responsible for chitotriosidase deficiency, which is encountered in almost 6% of all ethnic groups [6,7].

Plasma chitotriosidase activity is extremely increased in patients with Gaucher disease [3], a lysosomal disorder characterized by the storage of uncleaved β-glucocerebroside in macrophages (Gaucher cells) [8]. In addition to Gaucher disease, increased plasma chitotriosidase activity has been observed in some other lysosomal diseases [9] and various immunological disorders in which activated macrophages are involved [3,9,10]. Elevated plasma chitotriosidase levels have been found in various infectious diseases including neonatal systemic candidiasis [11] and recently, it has been demonstrated that intraperitoneal chitotriosidase injections strongly enhance the survival of experimental animals systemically infected with pathogenic fungi. Such findings have been related to the high chitinase activity of chitotriosidase against chitin-containing fungal cell walls [12].

β-Thalassemia major is a genetic defect of β-globin chain synthesis, characterized by unproductive erythropoiesis, peripheral anemia and expansion of the reticulo-endothelial system. Patients need regular and assiduous blood transfusions leading to systemic iron overload [13]. Recently, we found that plasma chitotriosidase was increased to a variable extent in 18% of 70 Sicilian patients with β-thalassemia major, suggesting that the increase of plasma chitotriosidase in β-thalassemia might reflect a macrophage activation associated with intracellular iron overload and deposition of erythrocyte membrane degradation products [14]. The aim of this study is to elucidate the possible mechanisms underlying chitotriosidase overproduction in β-thalassemia major. For this purpose, we measured plasma chitotriosidase in Sardinian and Sicilian β-thalassemia major patients who have distinguishing genetic and environmental features.

2. Patients and methods

This study included 64 Sardinian and 70 Sicilian patients with β-thalassemia major, coming from North Sardinia area (Sassari) and from East Sicily area (Catania), respectively. Sardinian subjects were 30 males and 34 females, with age ranging from 20 to 40 years (median: 29 years). Sicilian subjects were 27 males and 43 females, with age ranging from 2.5 to 41 years (median: 21 years). All patients are regularly transfused, according to the high transfusion regimen and undergo long-term iron chelation therapy with desferoxamine (DFX) by continuous subcutaneous infusion [15].

Clinical and laboratory findings of the patients were collected from the clinical files at the Thalassemic Center, General Hospital of Sassari and at the Thalassemic Center, University of Catania.

Fresh EDTA blood was obtained from the patients preceding blood transfusion. Plasma samples were kept frozen at −20°C until assayed. For chitotriosidase assay, the samples, in dry ice, were sent by fast air mail service to the Laboratory for Metabolic Diseases, Division of Pediatric Neurology, University of Catania. Chitotriosidase activity was measured by fluorimetric method, as previously described, and it was expressed as nmol/h/ml [14]. Samples with chitotriosidase activity > 120 were re-assayed after dilution of 10- or 50-fold with distilled water.

Plasma chitotriosidase activity was also evaluated in plasma of 90 healthy Sardinian subjects, with age (median 30; range 21–42) and sex distribution (42 males and 48 females), and in 100 healthy Sicilian subjects, with age (median 29; range 20–41) and sex distribution (55 males and 45 females). Chitotriosidase was considered elevated if it was higher than 2 standard deviation of the mean value for control subjects for each group.

3. Statistical analyses

The results are expressed as median and range or mean ± standard deviation. Sample means were...
compared by using the Student $t$-test ($p < 0.05$); sample variances were compared by the $U$-test of Mann–Whitney ($p < 0.05$).

4. Results

Chitotriosidase plasma levels in Sardinian and Sicilian patients with β-thalassemia major are shown in Fig. 1. Plasma chitotriosidase was significantly increased in 31 out of 64 (48.4%) Sardinian β-thalassemia patients (median 864; range:153–4143) with respect to control subjects (median 42; range: 4–222). It was normal in 33 patients (51.5%), whereas one subject had very low chitotriosidase activity in plasma (chitotriosidase deficiency). Among healthy controls, 4/90 (4.4%) were deficient in chitotriosidase.

In Sicilian thalassemia subjects, plasma chitotriosidase was increased in 12 out of 70 (17.1%)

Table 1

<table>
<thead>
<tr>
<th>Clinical and laboratory parameters of Sicilian and Sardinian patients with β-thalassemia major</th>
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<tbody>
<tr>
<td>Sardinian patients ($n = 64$)</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Age at diagnosis (months)</td>
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<tr>
<td>Age at the first transfusion (months)</td>
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<tr>
<td>Gender (m/f)</td>
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<tr>
<td>Splenectomy</td>
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<td>Pre-transfusional Hb level (g/dl)</td>
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<tr>
<td>Blood consumption (ml/kg/year)</td>
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<tr>
<td>Serum ferritin (ng/ml) (normal range: 22–322)</td>
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<td>Chronic hepatitis</td>
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patients (median 542; range 147–9282), while in the healthy control subjects, the median value was 37.5 (range 6–100). Only 2 out of 70 (2.85%) patients had chitotriosidase deficiency, while 4 out of 100 (4%) healthy control subjects were deficient in chitotriosidase.

In Table 1, the clinical and laboratory findings of Sardinian and Sicilian β-thalassemia patients are reported. Mean age and serum ferritin levels were significantly higher in Sardinian than Sicilian patients; plasma chitotriosidase levels were not significantly related with clinical findings including splenectomy, reduced glucose tolerance, diabetes, chronic liver disease and cardiomyopathy.

Based on their plasma chitotriosidase activity, three groups of patients might be detected both in Sardinian and Sicilian groups (Table 2): the groups with the highest chitotriosidase levels (> 1000) also had significantly higher mean serum ferritin level in comparison to that observed in patients with moderately increased values (values comprised between 150 and 1000) or normal chitotriosidase levels (< 150). In both groups, there was no correlation between plasma chitotriosidase and other laboratory parameters reflecting the status of the disease, namely mean blood yearly consumption and pre-transfusional Hb levels.

5. Discussion

Based on the present findings in Sardinian and Sicilian β-thalassemia patients, we argue that an increase of plasma chitotriosidase, a macrophage marker, might be a feature of β-thalassemia major. In either group, the highest levels of plasma chitotriosidase were observed in patients with the highest degree of iron overload, as judged by their serum ferritin levels. Therefore, it appears that the increase of plasma chitotriosidase in β-thalassemia major might reflect an iron-mediated damage of lysosomes leading to leakage of enzymes out of the cells.

Some important differences do occur between Sardinian and Sicilian patients with regard to plasma chitotriosidase activity. This was found more frequently elevated among Sardinian (48.4%) than Sicilian (17.1%) patients. On the whole, Sardinian patients are older and have higher mean serum ferritin levels than Sicilian subjects. However, it is noteworthy that mean serum ferritin levels are significantly higher among Sardinian patients than Sicilian, also in the groups with normal or moderately increased plasma chitotriosidase. Such findings suggest that an extreme iron overload, as that seen in β-thalassemia patients with the highest plasma chitotriosidase activity, could be a feature of β-thalassemia major.

Table 2
Clinical and laboratory features in three groups of Sardinian and Sicilian patients with β-thalassemia major according to their plasma chitotriosidase activity

<table>
<thead>
<tr>
<th></th>
<th>Sardinian thalassemia patients</th>
<th>Sicilian thalassemia patients</th>
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<tbody>
<tr>
<td>Plasma chitotriosidasea</td>
<td>59 (5–149)</td>
<td>288 (153–897)</td>
</tr>
<tr>
<td></td>
<td>n = 33</td>
<td>n = 17</td>
</tr>
<tr>
<td>Age at diagnosis (months)</td>
<td>8.12 ± 3.78</td>
<td>7.41 ± 3.78</td>
</tr>
<tr>
<td></td>
<td>n = 14</td>
<td>n = 14</td>
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<tr>
<td>Age at the first transfusion (months)</td>
<td>11.09 ± 7.17</td>
<td>10.25 ± 6.14</td>
</tr>
<tr>
<td>Spleenectomy</td>
<td>5 (15.1%)</td>
<td>7 (41.1%)</td>
</tr>
<tr>
<td>Pre-transfusional Hb level (gr/dl)</td>
<td>10.53 ± 0.48</td>
<td>10.41 ± 0.46</td>
</tr>
<tr>
<td>Blood consumption (ml/kg/year)</td>
<td>156.21 ± 31.1</td>
<td>147.25 ± 29.5</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml) (normal range: 22–322)</td>
<td>4892 ± 3417</td>
<td>4205 ± 2750</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>10 (27%)</td>
<td>7 (46.6%)</td>
</tr>
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Student’s t-test. * vs. ′*: p = ns; ′*: ′′*: p = 0.0001; ′′*: ′′′*: p = 0.0001. ○ vs. ○○: p = ns; ○○ vs. ○○○: p = 0.016; ○ vs. ○○○: p = 0.0001. ′*: ′′*: p = 0.0001; ′′*: p = 0.074; ′′′*: p = 0.007.

a Median (range within parentheses).
totriosidase values (> 1000), might trigger chitotriosidase overproduction. On the other hand, iron storage alone might not be a sufficient condition for bringing about changes in plasma chitotriosidase levels, as seen in β-thalassemia.

It could be suggested that different genetic and environmental factors might influence the ability of individual subjects to overproduce chitotriosidase in some conditions. With regard to this, it should be mentioned that in the Sardinian population, the most common type of β-thalassemia is the β0 variety, which is characterized by the absent production of β-globin chains. The Sardinian β-thalassemia population is homogeneous in its genetic composition with an absolute prevalence of the codon β-39 nonsense mutation, which accounts for 95.7% of the β-thalassemia chromosomes [16]. Conversely, a wide genetic heterogeneity with five more common mutations underlies β-thalassemia in Sicily. In such populations, patients with β-thalassemia major have been found homozygous for a severe β0- or β+ allele or were compound heterozygotes for two severe β-thalassemia genes [17]. Therefore, it might be possible that distinct molecular bases of β-thalassemia between Sardinian and Sicilian patients might influence macrophage activation and, hence, chitotriosidase overproduction.

Moreover, it appears evident that chitotriosidase produced by human macrophages might play a role in defense mechanisms against fungi and chitin-containing pathogens; plasma chitotriosidase was found normal in patients with tuberculosis and leprosy, while it was elevated in subjects with leishmaniasis [1,9,10], where the “systemic” protozoan infection leads to an enormous expansion of the reticulo-endothelial system and macrophage activation and possibly, chitotriosidase overproduction.

If it is true, in addition to different genotype, the higher rate of subjects with increased plasma chitotriosidase values among Sardinian than Sicilian patients could also be related to a diversity in some environmental factors between the two populations. The prevalence of β-thalassemia in areas with past malarial endemicity pointed to a presumptive protective role of such disease against malaria [18]. Sardinian β-thalassemia patients come from a geographical area (Sassari) with a past intermediate malarial endemicity, in comparison to East Sicily, where malaria was less endemic; patients from Sardinia might then have acquired some protective features against malaria. Therefore, it might be possible that Sardinian β-thalassemia patients, genetically prone to overproduce chitotriosidase under specific circumstances, have been selected for by malaria. Also, the difference between the two groups of Sardinian and Sicilian healthy control (p < 0.0001) suggest a different genetic composition of Sardinian population. Such observations concur with recent studies suggesting that genetic variation in cytokine promoter regions influences susceptibility to infections [19].

A better knowledge of molecular mechanisms regulating chitotriosidase gene transcription, including gene promoter polymorphism studies, could provide insights on the meaning of chitotriosidase overproduction in patients with β-thalassemia. The relationship between β thalassemia and malaria and increased plasma chitotriosidase levels could be elucidated by the assessment of plasma chitotriosidase levels and chitotriosidase gene promoter studies in β-thalassemia patients from countries with a distinct past malaria endemicity.

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References


