*short report*
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# SERUM INTERLEUKIN-8 LEVELS IN THALASSEMIA INTERMEDIA

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**ABSTRACT**

**In this study we determined serum IL-8 levels in 18 untransfused patients with β-thalassemia intermedia and in 14 subjects affected by HbH disease. As reported in polytransfused homozygous β-thalassemia, untransfused β-thalassemia and HbH disease show significantly ($p<0.005$) higher serum IL-8 levels than normal controls. Our data suggest that there could be an intrinsic cause for the IL-8 increase in thalassemia intermedia. We think that the hyperactivity of thalassemic macrophages related to chronic hemolysis is the main cause for the increment in cytokines, such as IL-8, found in thalassemic syndromes.**

*Key words: thalassemia intermedia, IL-8*



Human interleukin-8 (IL-8) is a cytokine produced by peripheral blood mononuclear cells, endothelial cells and fibroblasts. It is a chemoattractant protein for neutrophils that stimulates chemotaxis and degranulation, but it may also have a complex function in the regulation of the inflammatory process. Recently, the serum levels of different cytokines, such as IL-8,[1] IL-2,[2,3] IL-6[3] and TNF,[3,4] were determined in patients affected by β-thalassemia major to investigate their role in the immune alteration reported in this disease.

In particular, a high serum concentration of IL-8 was observed in the majority of homozygous transfused β-thalassemic patients, suggesting that high production of this cytokine could be related to early chronic transfusion therapy.[1]

In the present study we determined the IL-8 serum concentration in untransfused patients affected by β-thalassemia intermedia and HbH disease in order to verify the possible primary role of this disease in increasing serum IL-8 levels.

## *Patients and Methods*

We examined 18 untransfused patients with β-thalassemia intermedia (12 males and 6 females; mean age 32.4 years, range 18-49) and 14 with HbH disease (7 males and 7 females; mean age 27.8 years, range 7-63.

In the β-thalassemic group the mean Hb level was 9.6±0.78 g/dL and the serum ferritin level 639±500 ng/mL.

Eleven patients underwent splenectomy at the mean age of 17 years when they developed a clinical picture of hypersplenism.

Although not regularly, 66% of all patients received iron-chelating therapy with desferrioxamine that was begun when the serum ferritin level was above 1500 ng/mL.

In the HbH group the mean Hb level was 9.4±0.68 g/dL; none of them had been splenectomized and none of them had been treated with iron-chelating therapy since their mean ferritin serum level was 178±154 ng/mL.

At the time of this study, all the patients were apparently free from infection.

Serum levels of IL-8 were measured on frozen (–20°) samples using a commercially available immunoenzymetric assay (IL-8 EASIA Medgenix Diagnostics, Brussels, Belgium); the precision (CV) was 4% intra-assay and 3.8% inter-assay.

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Fifteen healthy blood donors, matched for sex and age, were used as normal controls.

Statistical assessment was carried out after logarithmic transformation of IL-8 values using analysis of variance; Tukey's test was then employed for mean separation. Significance was taken as $p<0.05$.

Student's t-test and Spearman's correlation coefficients were used for the other statistical analyses.

### *Results*

Figure 1 reports the distribution of serum IL-8 concentrations in normal controls, β-thalassemia intermedia patients and in HbH disease.

The median concentration of serum IL-8 levels in the normal controls was 34.2±32 (range 3-96) pg/mL. There was no difference between males and females.

In the β-thalassemic patients the mean values of serum IL-8 levels was 366±294 pg/mL and in HbH patients 137±61 pg/mL.

Both groups of thalassemic subjects showed significantly ($p< 0.005$) higher serum IL-8 levels than normal controls.

No significant difference was found between β-thalassemia and HbH patients. IL-8 concentrations were not related to sex, age, splenectomy, iron overload, chronic hepatitis or disease duration in either the α or the β-thalassemic patients.

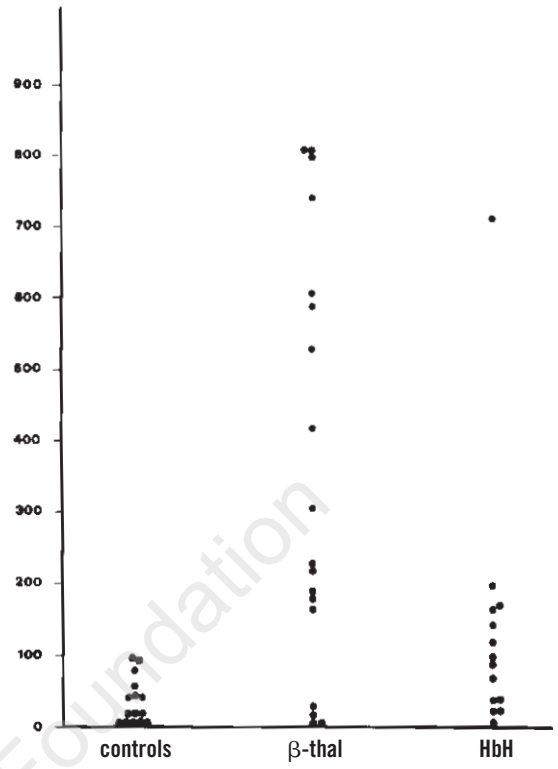


Figure 1. Serum IL-8 concentrations in normal controls, in patients with β-thalassemia and HbH disease . The two groups of patients show a significant difference with respect to normal controls ($p< 0.005$).

### *Discussion*

Increased serum levels of IL-8[1] and TNF-α[3-4] were recently reported in homozygous polytransfused β-thalassemia major. In these studies the authors suggested that the main causes for the rise in these cytokines were macrophage activation due to iron overload and the antigenic stimulation related to chronic transfusion therapy.

As reported for polytransfused patients, we found that untransfused β-thalassemia intermedia and HbH disease are also characterized by significantly higher serum IL-8 levels than normal controls.

These untransfused patients constitute a natural form of thalassemic disease without the severe clinical complications and functional immunological defects caused by chronic transfusion therapy. Therefore, in the absence of any relationship between IL-8 levels and the various clinical features examined (such as splenectomy and iron overload), our data suggest that there could be an intrinsic cause for IL-8 elevation in untransfused thalassemia syndromes. It is known that the anemia of thalassemia is a consequence of the accumulation of excess globin chains in erythroblasts and erythrocytes which causes membrane damage,[5] and that the mononuclear phagocytes are of major importance in the destruction of red blood cells. In particular, it has been demonstrated that thalassemic erythrocytes are phagocytosed by activated human[6] and mouse macrophages *in vitro*,[7] and the mean

number of β-thalassemic cells ingested by monocytes was found to be approximately 30% higher than that removed by normal monocytes.[8] All these data indicate that in the thalassemia syndromes there is a macrophage hyperactivity related to the chronic hemolysis.

The HbH patients showed lower IL-8 levels, although not significantly, than the β-thalassemia intermedia subjects.

We think that this difference could be explained by a lesser susceptibility to attack by macrophages of the α-thalassemic red cells, which are characterized by a lighter precipitation of excess globin chains in the bone marrow[9] and by different membrane damage with respect to β-thalassemia.[5]

Since it has also been reported that during erythrophagocytosis activated monocytes may produce different cytokines to enhance their phagocytic function[10] and that IL-8 may increase in response to endogenous stimuli such as tumor necrosis factor and IL-1,[11] we think that the elevated serum IL-8 levels found in untransfused thalassemia syndromes could be related to the phagocytosis of red blood cells by hyperactive monocytes.

The potential role of IL-8, and the interactions between different cytokines in thalassemic erythrophagocytosis require further investigation.



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