# Effects of Iron Overload and Treatment Methods on Serum Levels of IgG, IgM, and IgA in Beta Thalassemia Major (BTM) Patients

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## Abstract

This study is aimed to measure the serum levels of immunoglobulin A (IgA), (IgM), and (IgG) in BTM patients with respect to their iron overload, and methods of treatment. A total of 81 BTM patients were enrolled in the study among them 12 patients were just diagnosed under no treatment to serve as a second control group beside the first one which is composed of the healthy subjects. All of the study groups (patients on scheduled blood transfusions, subcutaneous (Sc.) Desferrioxamine (DFO) infusions beside blood transfusion, who were also subdivided into spleenectomized and non spleenectomized patients) were compared firstly with the healthy controls, secondly with the newly diagnosed thalassemics and a third type of comparisons was done among the study groups themselves. Blood samples were taken from every subject involved in the study to obtain the serum. The results showed that in comparison of the DFO infusioned thalassemics with the healthy controls a highly significant ($p>0.01$) increase in IgA, IgM, IgG were found**.** In comparing the spleenectomized patients with the healthy controls a highly significant increase were found in IgA, IgG, IgM, ($p>0.01$) while the non spleenectomized patients showed a highly significant increase in IgA, IgM($p>0.01$). blood transfused thalassemics when compared to the newly diagnosed thalassemics showed a significant decrease in IgG and IgM levels($p>0.05$)**.** DFO infusioned thalassemics when compared to the newly diagnosed thalassemics showed a significant decrease in IgM($p>0.05$)**.** In comparing blood transfused with the DFO infused thalassemics, the latter group showed a significant increase in IgA, IgG and IgM($p>0.05$).In comparing the newly diagnosed thalassemics with the healthy controls, a highly significant increase was found in IgG($p>0.01$), while IgM showed a significant increase($p>0.05$)**.** The spleenectomized patients showed a significant increase in IgG only ($p>0.05$) when compared to the non spleenectomized patients. No significant correlation was found between serum levels of ferritin and any of the three immunoglobulins.It is concluded that repeated blood transfusion, iron overload, and spleenectomy are interacting contributing factors to the immune abnormality in BTM patients.

## الخلاصة:

تهدف هذه الدراسة لقياس مستويات مصل الدم من الأميونوغلوبيولينات (A,G,M) لدى مرضى الثلاسيميا الكبرى نوع بيتا مع الأخذ بنظر الاعتبار مستوى زيادة الحديد وطريقة العلاج.العدد الكلي قدره٨١ مريض من بينهم ١٢ تم تشخيصهم توا و لم يستقبلوا علاجا من أي نوع ليكونوا فريق المقارنة الثاني إلى جانب الفريق الأول و المكون من أشخاص أصحاء.كافة المجاميع المدروسة (المرضى المعالجين بنقل الدم المنتظم، بنضح الديفروكسامين تحت الجلد و الذين تم تقسيمهم إلى أشخاص مستأصلي الطحال أو غير مستأصلي الطحال) تمت مقارنتها بالأشخاص الأصحاء أولا، و مع المرضى المشخصين حديثا ثانيا، إضافة إلى نوع ثالث من المقارنات بين مجاميع الدراسة نفسها. تم أخذ عينة دم من كافة المشاركين للحصول على المصل .أظهرت النتائج أنه في مقارنة المعالجين بنضح الديفروكسامين مع الأصحاء لوحظ فرق معنوي في الأميونوغلوبيولين G,M,A($p>0.01$). مقارنة مستأصلي الطحال مع الأصحاء

لوحظ فرق معنوي في الأميونوغلوبيولين(p›0.01)A ,G ,M، أما مقارنة الغير مستأصلين الطحال مع الأصحاء لوحظ فرق معنوي في الأميونوغلوبيولين(p›0.01)M,A. عند مقارنة المعالجين بنقل الدم المنتظم مع المشخصين حديثا لوحظ فرق معنوي في الأميونوغلوبيولين G و M فقط(p›0.05) ، في حين مقارنة المعالجين بنضح الديفروكسامين مع المشخصين حديثا لوحظ فرق معنوي في الأميونوغلوبيولين M(p›0.05). مقارنة المعالجين بنقل الدم المنتظم مع المعالجين بنضح الديفروكسامين لوحظ فرق معنوي في الأميونوغلوبيولين G,M,A (p›0.05) أما مقارنة المشخصين حديثا مع الأصحاء لوحظ فرق معنوي الأميونوغلوبيولين (p›0.05 ,M G(p›0.01).في مقارنة مستأصلي الطحال مع الغير مستأصلي الطحال لوحظ فرق معنوي في الأميونوغلوبيولين G (p›0.05 ). لم توجد علاقة ملحوظة بين مستوى الفيرتين و أي من الأميونوغلوبيولينات.تم الأستنتاج أن نقل الدم المتكرر و تراكم الحديد و أستئصال الطحال هي عوامل متداخلة تسهم في الخلل المناعي لهؤلاء المرضى.

## Introduction

The term “thalassaemia” refers to a group of blood diseases characterized by decreased synthesis of one of the two types of polypeptide chains (α or β) that form the normal adult human haemoglobin molecule (HbA, $\alpha_2\beta_2$), resulting in decreased filling of the red cells with haemoglobin, and anemia [1]. Regular red blood cell (RBC) transfusions eliminate the complications of anemia and compensatory bone marrow expansion will permit normal development throughout childhood and extend survival [2]. In parallel, blood transfusions result in a "second disease" while treating the first, that has resulted from the inexorable accumulation of tissue iron, further altering the prognosis of thalassemia major over the last 20 years has been progressed in the development of iron-chelating therapy for iron overload [3].

In patients with β-thalassemia in whom yearly transfusion requirements exceed 200 ml packed cells per kilogram body weight, spleenectomy should significantly diminish RBC requirements and iron accumulation [4].

The major causes of morbidity and mortality in this disease are anemia and iron overload [5].

Studies of immune cell function in clinical situations of iron overload seem to have emerged from two major interests: an interest in the greater susceptibility to infections found in spleenectomized patients with iron overload due to β-thalassaemia major and an interest in the modulating interactions of iron with cells of the immune system [6]. Iron overload or repeated blood transfusions were not found to down-regulate the humoral immune system of thalassemic patients [7]; however, consumption of high-iron diets in mice caused a marked accumulation of iron in the liver and a twofold reduction in the IgM antibody response without alteration in the IgG response [8]. Direct evidence implicating iron overload in immune system abnormalities is provided by the fact that intensive chelation therapy with DFO has been shown to improve some of these symptoms [9].

This study aims to investigate the extent to which chelation therapy improves the immunity of BTM patients and to investigate whether there is a relationship between iron overload and immunoglobulin production to know if iron overload contributes to the immunosupression of those patients.

## Materials and Methods

It is a case-control study that was done in The Inherited Blood Diseases Center in Babylon Maternity and Children Hospital.

a total of 81 β-thalassemia major patients (42 males and 39 females) were enrolled in this study. age range (1.5 to 19 years) (8.37±5.63). They were subdivided according to the method used in their treatment into: the newly diagnosed thalassemic patients who still did not receive blood transfusion nor DFO therapy, the patients treated by scheduled blood transfusion to maintain their Hb levels around 9.5 mg/dl, the patients treated by scheduled blood transfusion and to treat their transfusional iron overload they receive DFO (Desferal®) in vials that contain 500mg from Novartis, Switzerland, as Sc. infusions, the mean dose of DFO was 20-40 mg /kg / infusion over 8-12 hour, 5-6 days per week.

Patients were excluded from the study if they had one or more of the following conditions: hepatitis B or C, a history of positive HIV test, chronic renal failure, heart failure, and endocrine complications. 5 ml of blood were taken from each participant to obtain the serum by centrifugation to assess the immunoglobulins levels using the commercially available single radial immunodiffusion plates (SRID) from LTA, Italy. Serum ferritin were measured by minividas from Biomerieux, France. It was measured to all the study groups.

The serum samples were frozen at -20 ?C until used. All values were expressed as means ± standard deviation (μ±S.D). The data were analyzed by using a computerized SPSS program. Student's t –test was used to examine the differences between any two groups while ANOVA test were used to examine the relations within the groups.

Also a study for the regression and correlation was also done between serum ferritin and immunoglobulins levels. A p‹0.05 denoted by (*) is considered to be significant and a p ‹ 0.01 denoted by (**) is considered to be highly significant **(17).** clinical features of the study groups are illustrated in table (1).

**Table 1** clinical features of the study groups

| The study group | Sample size | Age(years) | | | Sex | |
|---|---|---|---|---|---|---|
| | | Range | Mean | SD | Male | Female |
| 1-Healthy controls | 10 | 2-20 | 10.8 | 6 | 5 | 5 |
| 2-Newly diagnosed thalassemics | 12 | 1.5-2 | 1.2 | 1 | 4 | 8 |
| 3-Blood infused thalassemics | 19 | 1.5-4 | 3.2 | 2.5 | 8 | 11 |
| 4- DFO infused thalassemics | 50 | 4-20 | 10.6 | 5.1 | 30 | 20 |
| 4a-Spleenectomized | 24 | 5-19 | 12.6 | 5.5 | 15 | 9 |
| 4b-non spleenectomized | 26 | 4-19 | 8.8 | 4.4 | 13 | 13 |

## Results

By comparing the three study groups with the healthy controls, the newly diagnosed thalassemics showed a highly significant increase (p‹ 0.01) in IgG levels, and IgM levels significantly increased (p‹ 0.05). No significant differences were found between the blood transfused thalassemics and the healthy controls while DFO infused thalassemics

showed highly significant increase (p‹ 0.01) in serum levels of IgA, IgG, and IgM. All these results are illustrated in table (2).

By comparing blood transfused thalassemic patients, and thalassemic patients treated with DFO with the newly diagnosed thalassemic patients, blood transfused thalassemic patients showed a significant decrease in serum levels IgG (p‹0.05), and a highly significant decrease in serum levels IgM (p‹0.01), while DFO infused thalassemics showed a significant decrease (p‹0.05) in serum levels of IgM as shown in table (2).

When comparing blood transfused thalassemics with DFO infused thalassemics the latter group showed a significant increase (P‹ 0.05 ) in serum levels of IgA, IgG, and IgM as shown in table (2).

**Table 2** Comparison of serum levels of IgG, IgM, and IgA of the study groups.

| Immunoglobulin serum levels | Healthy controls N=10 | newly diagnosed thalassemics N=12 | Blood transfused thalassemics N=19 | DFO infused thalassemics N=50 |
|---|---|---|---|---|
| IgA (mg/dl) | 185±99 | 282.2±134.8 | 187.8±165.5 | 346.5±207.6 A**,C* |
| IgG (mg/dl) | 1170±342 | 1964.4±888.1 **A | 1122.2±510.6 B* | 1620.8±646.1 ,C***A |
| IgM (mg/dl) | 125±155 | 356±107.1 *A | 191.5±90.4 B** | 253.8±89.6 ,B*,C***A |

(A)Indicates a significant difference between the mentioned group and the healthy controls, (B) indicates a significant difference between the mentioned group and the newly diagnosed thalassemics, and (C) indicates a significant difference between the last two groups,(*) means p<0.05,(**) means p<0.01.

The patients receiving Sc. DFO infusions were also subdivided according to the spleen state into spleenectomized and non spleenectomized. By comparing those two groups with the healthy controls, the spleenectomized patients showed a highly significant increase (p‹ 0.01 ) in serum levels of IgG, IgM, and IgA. The non spleenectomized patients showed a highly significant increase (p‹ 0.01 ) in serum levels of IgM, and IgA. In the comparison of the spleenectomized and non spleenectomized patients with the newly diagnosed thalassemic patients, no significant differences were found between the newly diagnosed thalassemics and either of the spleenectomized patients and the non spleenectomized patients. In comparison of the non

spleenectomized patients with the spleenectomized patients, the latter group showed a significantly higher ($p<0.05$ ) serum levels of IgG . These results are illustrated in table (3).

**Table 3** Comparsion of the spleenectomized and the non spleenectomized patients with the healthy controls and the newly diagnosed thalassemics.

| Immunoglobulin serum levels | Healthy controls N=10 | Newly diagnosed thalassemics N=12 | spleenectomized N=24 | non spleenectomized N=26 |
|---|---|---|---|---|
| IgA (mg/dl) | 185±99 | 282.2±134.8 | 401.4±252.2 A** | 304.1±160.2 **A |
| IgG (mg/dl) | 1170±342 | 1964.4±888.1 | 1942.1±628.7 **A | 1419.1±584.9 C* |
| IgM (mg/dl) | 125±155 | 356±107.1 | 245.2±99.6 **A | 267.1±83.2 **A |

(A)Indicates a significant difference between the mentioned group and the healthy controls, and (C) indicates a significant difference between the last two groups,(*) means $p<0.05$,(**) means $p<0.01$.

A study of regression and correlation of all the parameters with the level of serum ferritin had shown a non significant negative relationship with IgA, a non significant positive relationship with IgG, and a non significant positive relationship with IgM, the significance and r-values for this study are shown in table (4).

**Table 4** A regression and correlation study of serum ferritin and immunoglobulins levels.

| Immunoglobulin type | r value | Significance |
|---|---|---|
| IgA (mg/dl) | 0.075- | 0.76 |
| IgG (mg/dl) | 0.065 | 0.79 |
| IgM (mg/dl) | 0.077 | 0.75 |

## Discussion

By comparing the newly diagnosed thalassemic patients with the healthy controls, we found a highly significant increase in IgG, with a significant increase in serum levels of IgM, this disagrees with the results found by

[10] who found a significant increase in serum levels of IgA, IgG and IgM using the same method of analysis. This difference may be attributed to the differences in the patients age, the sample size, in addition to a number of the individual variations such as the nutritional state and the presence or absence of complications from iron overload which are known to be increased with age.

Patients were treated by Sc. DFO infusion when compared with the healthy controls showed a highly significant increase in the three classes of immunoglobulins (IgA, IgM, IgG). This occurs mainly due to allogenic stimulation by blood transfusion with the resultant exposure to foreign antigens [11].

Comparing patients on blood transfusion with the untreated thalassemic patients group, the results showed a significant decrease in serum levels of IgG, with a highly significant decrease in serum levels of IgM. This might indicate the impact of iron overload on the immune system.

Patients on Sc. DFO infusions when compared with the newly diagnosed thalassemic patients, showed a significant decrease in serum levels of IgM. DFO has been reported to be associated with immune abnormalities in iron loaded thalassemic patients [12].

Patients who receive Sc. DFO infusions to treat their transfusional iron overload when compared with the patients treated by scheduled blood transfusion showed a significant increase in serum levels of IgA, IgM and IgG, this indicates the positive effects of DFO on the immune system.

The spleenectomized patients when compared with the healthy controls showed a highly significant increase in serum levels of IgA, IgM and IgG, this disagrees with [13] who found that the three immunoglobulin subclasses increased but not significantly, this result refers to the fact that the spleen alters the immune status of thalassemic patients but the exact mechanism by which this occurs is still unclear. We can also consider this increase as transfusion related .

The non spleenectomized patients when compared with the healthy controls showed a highly significant increase in serum levels of IgA, and IgM which disagrees with the findings of [14] who reported a significant increase in all of the three subclasses.

The spleenectomized patients, when compared with the newly diagnosed thalassemic patients, showed a non significant increase in serum levels of IgA associated with a non significant decrease in serum levels of IgG, and IgM. In BTM patients the blood transfusion, iron overload, spleenectomy and chronic infections can lead to immunologic abnormalities [15].

The non spleenectomized patients, when compared with the newly diagnosed thalassemic patients, showed a non significant increase in serum levels of IgA, a non significant decrease in serum levels of IgG and IgM .

The spleenectomized patients when compared with the non spleenectomized patients showed a significant increase in serum levels of IgG. This agrees with the results found by [16],who attributed the increase in IgG to an increased liver damage after spleenectomy and\or a decreased catabolism after the removal of the spleen.

No statistically significant correlation was found between serum ferritin and all immunoglobulins this agrees with [11] who reported that the immune abnormality could be attributed to defects in the complement

system or spleenectomy. Our results showed a non significant positive correlation between serum levels of IgM and ferritin, However, clinically the correlation between serum levels of immunoglobulins and ferritin is significant.

## Conclusions

Iron overload can affect the humoral immunity in BTM patients clinically although it is not significant statistically, also spleenectomy can affect the humoral immunity , and finally DFO does not alleviate the immunologic abnormality seen in BTM patients to a great extent

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