Immunological Assessment of \( \beta \)-thalassemic Major Children Aged 5-12 Years Old Attending Abd El-Aziz El-Rantisy Hospital in Gaza Strip

Gaza Şeridi’ndeki Abd El-Aziz El-Rantisy Hastanesi’ne Başvuran 5-12 Yaşlardaki \( \beta \)-Talasemi Major Hastalarının İmmünolojik Değerlendirmesi

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ABSTRACT

Immune abnormalities have been suggested as a precipitating factor for the fourth most common cause of death in \( \beta \)-thalassemia. These abnormalities have been attributed both to the disease itself and to the applied therapeutic intervention. In order to assess some immunological parameters in children aged 5-12 years old with \( \beta \)-Thalassemia major in Gaza Strip. This case-control study comprised 43 \( \beta \)-thalassemic major children aged 5-12 years old attending Abd El-Aziz El-Rantisy hospital and 43 healthy children served as controls. Cases and controls were matched for age and sex. Blood samples were collected just before a scheduled transfusion of packed red blood cells. Serum levels of interleukin-1-\( \beta \) (IL-1-\( \beta \)), interleukin-6 (IL-6) and tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)), were evaluated by ELISA using monoclonal antibodies, immunoglobulins IgG, IgA and IgM, complements C3, C4 were determined by turbidimetric assay. The average age of the study population was 7.9\( \pm \)2.2 years. Most patients 29 (67.4%) had allergic reactions. Discharge of desferrioxamine was intramuscular in 25 (58.1%) patients and subcutaneous in 17 (39.5%) patients and one patient (2.3%) refused iron chelation therapy. Complements 3 and 4 reduced significantly in thalassemic children compared to controls (118.7\( \pm \)12.4 vs. 136.6\( \pm \)23.3 mg/dL, \( p=0.000 \) and 49.3\( \pm \)13.4 vs. 62.3\( \pm \)21.6 mg/dL, \( p=0.001 \), respectively). Lower level of IgG was found in patients. When related to age, higher levels of IgG, IgM and IgA were found with increasing age. On the other hand, immunoglobulins had no relation with allergic reactions. Cytokines were normal that they almost showed undetected levels in controls and patients (TNF-\( \alpha \): 56.1 and 97.7%, IL-6: 95% and 88.4%, and IL-1\( \beta \): 100% for both controls and patients. Complements, Cytokines and Immunoglobulins should be evaluated to determine the immunological status of patients.

Key Words: \( \beta \)-thalassemia, Cytokines, Immunoglobulin's, Complement

ÖZET

İmmünitedeki anormallikler \( \beta \)-talasemiden ölülerin en sık dönemde sebeبيدir. Bu anormallikler hem hastalığın kendisine hem de uygulanan terapötik müdahaleye bağlıdır. \( \beta \)-talasemili çocuklarınimmünolojik parametreleri değerlendirilemek için, bu olgu-kontrol çalışma Gaza Şeridi’ndeki Abd El-Aziz El-Rantisy Hastanesi’ne başvuran 5-12 yaşlarındaki 43 \( \beta \)-Talasemi majorlu çocuk ve kontrol olarak 43 sağlıklı çocuktan oluşturuldu. Ölgüler ve kontroller yaş ve cinsiyet açısından eşleştirildi. Örneklere planlanan eritrosit transfüzyon öncesi alındı. Interleukin-1-\( \beta \) (IL-1-\( \beta \)), IL-6 and tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) serum düzeyleri ELISA, IgG, IgA, IgM, C3 ve C4 düzeyleri ise turbidimetrik yöntemle saptandı. Çalışma populasyonunun yaş ortalaması 7,9\( \pm \)2,2 yıldır. Hastaların 29’unda (%67,4) alerjik reaksiyonlar vardı. Desferioksamin uygulaması 25 hastada intramüsküler (%58,1), 17 hastada subkütan olarak uygulandi ve bir hasta demir şelasyon tedavisi ret etti. Kompleman 3 ve 4 düzeyleri kontollere göre
INTRODUCTION

Beta thalassemia is the result of deficient or absent synthesis of beta globin chains, leading to excess alpha chains. Beta-thalassemias are the most important among the thalassemia syndromes because they are so common and usually produce severe anemia in their homozygous and compound heterozygous states. Beta-thalassemia has become a worldwide clinical problem due to an increasing immigrant population\(^1\)\(^-\)\(^3\). Individuals with thalassemia major usually come to medical attention within the first 2 years and require regular blood transfusion to survive. Iron overload constitutes a significant problem for beta-thalassemia patients. The precipitation of hemicromes, heme disintegrates, and toxic non-transferrin-bound iron (NTBI) species leads to the formation of toxic free radicals from superoxide and hydrogen peroxide. Lipid peroxidation then occurs, disrupting red blood cell membranes\(^4\). However, repetitive transfusions lead to iron-overload continuous allo-antigenic stimulation and have been associated with autoimmune hemolysis\(^5\). At the same time, they are followed by the risk of transmission of viruses with immunosuppressive properties.

Several studies on immune competence in beta-thalassemia have revealed numerous quantitative and functional defects, involving T and B lymphocytes, immunoglobulin production, changing the pattern of cytokine production, neutrophils and macrophages, chemotaxis, and phagocytosis, as well as the complement system\(^6\)\(^-\)\(^10\). Immune abnormalities have also been held responsible for the frequent occurrence of malignancies in beta-thalassemia, especially leukemia and lymphomas. Thus, surveillance for infections in patients with beta-thalassemia is crucial, while further studies are warranted on immune function abnormalities and the implicated mechanisms\(^5\).

Tovo et al.\(^11\) studied IgG, IgA and IgM levels in 187 homozygous beta-thalassemic patients and compared them with age-matched normal control subjects. They not yet transfused and the polytransfused splenectomized patients showed a significant increase of all Ig classes. The polytransfused splenectomized patients showed a significant increase only of IgG and IgA. The splenectomized patients, when compared to the nonsplenectomized ones, showed a significant increase of IgG, of IgA in the elder ones and a significant reduction of IgM.

The activity of the complement system were examined in sera from 24 thalassemic patients in a study to explore the basis for suffering from frequent and serious infections in patients with thalassemia major especially after splenectomy. Mean concentrations of C3, factor B, properdin, and immunoglobulins were normal. The result showed deficient activity of the alternative pathway of complement in beta-thalassemia major, especially in conjunction with asplenia\(^12\).

The unstimulated and induced production of granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), IL-3, IL-6, stem cell factor (SCF), IL-1\(\beta\), TNF-\(\alpha\), TNF-\(\beta\), interferon-\(\gamma\) (IFN-\(\gamma\)) and transforming growth factor-beta (TGF-beta) were determined after culture of
blood mononuclear cells from 22 patients with severe β-thalassaemia in a regular transfusion programme, five non-regularly transfused patients with β-thalassaemia intermedia and nine normal persons. A distinct pattern of cytokine production in thalassaemic patients was detected, namely a low unstimulated production of all cytokines and a significant increase in the stimulated production of IFN-γ, TNF-α and IL-1β; these abnormalities were more pronounced in the more heavily transfused older patients. The increased production of the above cytokines, which usually characterize the acute response to infectious agents and have a negative effect on erythropoiesis, may explain the deterioration of anemia found in thalassaemic patients during acute infections.

A small scale screening study for β-thalassemia trail has been carried out in the Gaza Strip involving 1650 secondary schools healthy students, 16-18 years old and from both sexes. The overall prevalence of β-thalassemia in the Gaza Strip was 4.3%. It was concluded that both β-thalassemia and microcytosis anemias are major health problems in the Gaza Strip.

MATERIALS AND METHODS

Patients and study design

The present study is a case control study enrolled 43 β-thalassemia major children (24 males and 19 females, mean age 10 years; range, 5-12, with serum ferritin level above 3000ng/ml. Patients were selected from Hematology Department, Abd El-Aziz El-Rantisy Specialized Pediatric Hospital. All patients are in hypertransfusion, to maintain the hemoglobin level above 9g/dl at a mean transfusional interval of 14 days, and they received regular subcutaneous or intramuscular iron-chelating therapy with desferrioxamine according to their weight and level of serum ferritin, ranging between 25-50 mg/kg.

Patients were excluded from this study if they had one of the following criteria: a history of hepatitis B or C infection, a history of positive HIV test, chronic renal or heart failure, recent or active infection at the time of blood sampling.

Collection of blood Samples and processing

Blood samples were collected from each thalassemic children just before a scheduled transfusion of packed red blood cells and allowed to clot, and then clear serum samples were obtained by centrifugation, aliquoted and stored at -20 ºC until the time of performing the analysis.

Clinical Laboratory Parameters

The frozen serum samples were thawed at 4-8°C then mixed by gentle shaking at room temperature prior to use. Then, the serum samples were used to determine serum levels of IL-1β, IL-6 and TNF-α cytokines using commercially available kits of enzyme-linked immunosorbent assay (IBL International GmbH Kit Flughafenstr. 52A, D-22335 Hamburg, Germany) according to the instructions of the manufacturer.

Immunoglobulins (IgG, IgA, IgM), complements 3 and 4 were measured by quantitative turbidimetric assay (Globe Diagnostics S.r.l. Via Galileo Galilei 38, 20096 Seggiano di Pioltello (Milan), Italy).

Statistical analysis

Data were computer analyzed using SPSS/ PC (statistical package for social science Inc. Chicago, Illinois USA, version 13.0) statistical package. Simple distribution of the study variables and the cross tabulation were applied. Means were compared by independent-sample t-test. Percentage difference was calculated according to the formula:

\[
\text{Percentage difference} = \frac{\text{mean of patients} - \text{mean of controls}}{\text{mean of control}}
\]

Probability values (p) were obtained from the students table of "t" and significance was at p< 0.05.

RESULTS

The average age of the study population was 7.9±2.2 years. Study population comprised 24 (55.8%) males and 19 (44.2%) females. Table 1.

<table>
<thead>
<tr>
<th>Character</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allergic reaction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>29</td>
<td>67.4</td>
</tr>
<tr>
<td>No</td>
<td>14</td>
<td>32.6</td>
</tr>
<tr>
<td><strong>Iron chelator</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>17</td>
<td>39.5</td>
</tr>
<tr>
<td>Intramuscular</td>
<td>25</td>
<td>58.1</td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Table 1. Allergic reaction and iron chelator therapy in the cases (n=43)
illustrates allergic reaction and the way of discharge of iron chelator. Most of cases 29 (67.4%) had allergic reactions. Discharge of iron chelator was intramuscular in 25 (58.1%) patients and subcutaneous in 17 (39.5%) patients. However, only one patient (2.3%) refused iron chelation therapy.

Serum levels of C3 and C4 are presented in Table 2. β-thalassemic major children demonstrated significantly lower mean values than those observed in the controls. controls (118.7±12.4 mg/dL VS 136.6±23.3 mg/dL, difference of -13.1% p=0.000). Similarly, C4 showed a significant decrease in cases as compared to controls (49.3±13.4 Vs 62.3±21.6 mg/dL, % difference= -20.9 and p=0.001).

Table 3 illustrates the mean concentrations of IgG, IgM and IgA in cases and controls. The concentrations of IgG were significantly decreased in cases compared to controls, showing percentage difference of -16.7 and p=0.002 (1783.6±335.7 Vs 2141.2±661.6). On the other hand, no significant change was observed in the mean levels of IgM and IgA between cases and controls. (228.8±62.1 Vs 230.5±74.2 mg/dL and 297.9±121.9 Vs 307.9±106.3 mg/dL, p=0.909 and p=0.684, respectively).

Table 4 shows the serum cytokines levels: TNF-α, IL-6 and IL-1β were mostly undetectable in both cases and controls. The numbers of cases with undetected TNF-α and IL-6 were 42 (97.7%) and 38 (88.4%) compared to the controls of 28 (65.1%) and 41 (95.3%). Interleukin-1β showed completely undetectable levels in both cases and controls.

Allergic reactions and complements and immunoglobulins are presented in Tables 5 and 6. Independent sample t-test showed no significant relations between allergic reactions and the mean concentrations of complements and immunoglobulins (p>0.05). Table 7 illustrates the mean concentrations of complements C3 and C4 of cases in relation...
to different age groups. The mean concentration of C3 and C4 didn’t significantly change with increasing age.

As indicated in table 8 independent t-test showed that the level of all immunoglobulin studied in age group 9-12 years were higher than that in age group 5-8 years. IgM and IgA showed significant increase (262.1±50.9 and 342.6±85.6 Vs 204.8±59.1 and 265.7±135.0, t=3.314, p=0.002 and t=2.124, p=0.040, respectively). However such increase in IgG was not significant

Table 5. Allergic reactions in relation to complements C3 and C4 (mg/dL)

<table>
<thead>
<tr>
<th>Allergic reaction</th>
<th>Complement</th>
<th>Yes (n=29)</th>
<th>No (n=14)</th>
<th>T</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>119.6±12.1</td>
<td>116.8±13.3</td>
<td>-0.689</td>
<td>0.495</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>49.8±11.2</td>
<td>48.2±17.6</td>
<td>-0.358</td>
<td>0.722</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ±SD.
p> 0.05: not significant.

Table 6. Allergic reactions in relation to IgG, IgM and IgA concentrations

<table>
<thead>
<tr>
<th>Allergic reaction</th>
<th>Immunoglobulin</th>
<th>Yes (n=29)</th>
<th>No (n=14)</th>
<th>T</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>1747.9±249.1</td>
<td>1857.4±471.0</td>
<td>1.002</td>
<td>0.322</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>226.6±63.4</td>
<td>233.3±61.6</td>
<td>0.326</td>
<td>0.746</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>294.6±86.7</td>
<td>304.6±178.2</td>
<td>0.248</td>
<td>0.805</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ±SD.
p> 0.05: not significant.

Table 7. Age of patients in relation to complement component C3 and C4

<table>
<thead>
<tr>
<th>Age/ year</th>
<th>Complement</th>
<th>5-8 (n=25)</th>
<th>9-12 (n=18)</th>
<th>T</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3</td>
<td>117.6±13.5</td>
<td>120.1±10.9</td>
<td>-0.639</td>
<td>0.526</td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>48.6±14.0</td>
<td>50.3±12.9</td>
<td>-0.410</td>
<td>0.684</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ±SD.
p> 0.05: not significant.

Table 8. Age of patients in relation to IgG, IgM and IgA

<table>
<thead>
<tr>
<th>Age/ year</th>
<th>Immunoglobulin</th>
<th>5-8 (n=25)</th>
<th>9-12 (n=18)</th>
<th>T</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>1722.6±399.7</td>
<td>1868.3±199.6</td>
<td>1.421</td>
<td>0.163</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>204.8±59.1</td>
<td>262.1±50.9</td>
<td>3.314</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>265.7±135.0</td>
<td>342.6±85.6</td>
<td>2.124</td>
<td>0.040</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean ±SD.
p> 0.05: not significant.
p<0.05: significant.
DISCUSSION

This is the first study to assess some immunological parameters in children aged 5-12 years old with β-Thalassemia major in Gaza Strip. The thalassemic children in the present study were on long-term hypertransfusion therapy and on iron chelation with desferrioxamine. The majority of them had allergic reactions which could be attributed to: 1) hypersensitivity to soluble allergens found in the transfused blood component, 2) production of antibodies against RBCs by the recipients and 3) formation of multiple alloantibodies to common Rh, Kell, Kidd, or other blood group antigens. Several authors reported that regular blood transfusions are associated with alloimmunization15-17. Furthermore, thalassemic children differ in the way of chelating the iron with desferrioxamine, they received it either subcutaneous 17 (39.5%) or intramuscular 25 (58.1%). One patient (2.3%) refused chelation therapy.

The present results revealed that the mean levels of complements 3 and 4 were significantly decreased in cases as compared to control. Similar result was found by James et al.12. The decrease in complements 3 and 4 can be attributed either to reduced their synthesis or increased their consumption; the latter is more probable with the increase rate of infection 18. In our patients the decrease noted in complements 3 and 4 may be due to reduced their synthesis rather than increased their consumption particularly in young children where the rate of infection is expected to be low.

As depicted from the present result, the mean concentration of immunoglobulin G was significantly decrease in cases compared to controls. Amin et al.18 showed an increase level in IgG in thalassemic patients with increasing age. They attributed this increase to repeated infection which was not the case of our patients. In another study Vergin et al19 showed that IgG level was within normal limit in thalassemic patients. This controversy may be due to marked heterogeneity of the patients in different studies. Immunoglobulin M and A concentrations were not significantly change in cases compared to controls, suggesting that anaphylactic reactions due to IgA deficiency is excluded 3. Such finding was in agreement with that reported by Ezer et al.20 and Tourkantoni et al.21.

In general cytokines were mostly undetectable in both cases and controls. The numbers of cases with undetected TNF-α and IL-6 were 42 (97.7%) and 38 (88.4%) compared to the controls of 28 (65.1%) and 41 (95.3%). Interleukin-1β showed completely undetectable levels in both cases and controls. Based on the procedure used in the present study the obtained undetectable result means that cytokines in both controls and patients are normal i.e. normally cytokines are undetectable. However, this depends on the immunological status of the patient implaying that our patients had low level of immunological complications related to cytokines. Elevated human cytokines levels depend on the type of immunological disorder. The present finding was in agreement with the study carried out by Gharagozloo et al.22 who revealed no significant difference in serum TNF-α levels in the thalassemia and control groups. Similarly El Nawawy et al.23 reported that IL-1β and TNF-α concentrations did not differ significantly between patients and controls. In contrast to our findings, Kyriakou et al.24 and Aggeli et al.25 showed values of TNF-α, IL-1β and IL-6 were higher in thalassemic patients compared to controls. Jacob et al.26 mentioned that the broad distribution of TNF-α concentrations accords with the significant differences among individuals in the levels of TNF-α synthesis by stimulated mononuclear cells observed by Jacob et al.26 in healthy subjects. Moreover the heterogeneity of thalassemia patients regarding genotype, age, number of transfusions, frequency of infections, and iron overload in the various reports makes comparison of the results in the various studies sometime unreliable24.

Data presented in this study showed that the ferritin level was higher in allergic patients indicating that iron overload may play a role in immunological complication observed in the present study27. The complements 3, C4, IgG, IgM and IgA concentrations didn't significantly change between the allergic and none allergic patients. This means that allergic reactions may be related to immunoglobulin E rather than the studied ones28.

There was no significant relationship between age and complement 3 and C4. Such results are in agreement with that reported by Amin et al.,
High levels of IgG, IgM and IgA were also observed in age group 9-12 years compared to age group 5-8 years with significant difference for IgM and IgA. This result is in congruence to that of Amin et al. who found that the elder patients had significantly higher values of the three major classes of serum immunoglobulins (IgG, IgM and IgA) than younger group. They suggested that repeated blood transfusion in β-thalassemia patients will result in a continuous exposure to various antigens and will lead to increased levels of serum immunoglobulins.

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REFERENCES


