Investigation of RBC Indices and HbA2 Levels in Parents of Beta-Thalassemia Patients: Impacts on Premarital Genetic Counseling

Mina Izadyar 1 M.D., Jila Dastan 2 M.Sc. M.D., Tayebeh Sabokbar 3 M.Sc., Solmaz Shoraka 3 M.D., Azadeh Shojaei 4 M.D., Habib Nastri 5 M.Sc., Saeed Reza Ghaffari 1,3,4,5 M.D. Ph.D.

1 Children Medical Center, Medical Sciences/ University of Tehran, Tehran, Iran.
2 Gene Clinic, Tehran, Iran.
3 Department of Genetics and Genomics, Cancer Research Center, Tehran, Iran.
4 Val-e-Asr Reproductive Health Research Center, Medical sciences /University of Tehran, Iran.
5 Department of Medical Genetics, Medical Sciences /University of Tehran, Iran.

Received October 2007; Revised and accepted December 2007.

Abstract
Objective: This study was designed to investigate RBC indices and HbA2 levels in parents of major beta-thalassemia patients to detect possible silent beta-thalassemia carriers and examine its potential impact on the premarital genetic counseling.

Materials and Methods: This cross sectional study was performed at Children Medical Center from 2004 to 2006. After genetic counseling and getting informed consent, peripheral blood sampling was carried out on 185 carrier parents of regularly blood transfused thalassemia children. Then RBC indices and HbA2 concentration were measured. Samples with MCV and MCH higher than and/or HbA2 lower than cut off values were rechecked.

Results: In one case, MCV and MCH indices were within the limits defined for non beta-thalassemia carriers. Furthermore, four other cases were found to have decreased values of MCV and MCH but normal HbA2 levels.

Conclusion: About 3% of beta-thalassemia carriers in our country may potentially be missed using current screening methods. Further studies are required to assess the need for presenting a new threshold for thalassemia carrier screening. Defining the causative mutations using molecular methods would pave the way for establishing a protocol for a premarital screening program in conditions when one of couples is a confirmed carrier.

Keywords: Thalassemia, Carrier detection, RBC indices, HbA2, Mutation analysis

Introduction
Thalassemias are the most common inherited disorders worldwide (1). Major beta-thalassemia patients are born healthy; however, symptoms such as anemia, hepatosplenomegaly, growth retardation, jaundice, and bone deformation, usually develop within the first year of life, thus making regular blood transfusion and iron chelating therapy necessary for their survival. These diseases and their treatments impose significant burdens on patients’ health as well as psychosocial and economic burdens on the patients, their families and the society. Bone marrow trans-
plantation and cord blood transplantation, the only available definitive cures for major beta-thalassemia, are possible only for the limited proportion of patients having HLA-matched sibling donors (1). Children affected with beta-thalassemia major or beta-thalassemia intermedia, typically have both parents affected with heterozygous beta-thalassemia, a condition that can be suspected on a standard complete blood count (CBC) and hemo-globin (Hb) studies. Detecting carrier couples by this simple blood test can prevent thalassemia, and at-risk couples can be identified and informed of their genetic risk before marriage and child bearing. One exception is the “silent” heterozygous beta-thalassemia associated with some specific mutations, especially in the promoter region of the beta globin gene (2).

A prevention program including population screening, counseling, and prenatal diagnosis would markedly reduce the birth prevalence of affected individuals. Primary care thalassemia prevention program of Iran was started in 1996. The program aimed to identify carrier couples before marriage and to offer counseling, thus providing them with the opportunity to separate or adopt a comprehensive preventive approach. In the process of screening, first, the man’s MCV (Mean corpuscular volume) and MCH (mean corpuscular hemoglobin) are checked. If the results are lower than cut off value (MCV < 27 pg or MCV <80 fl), the woman is tested. When both are microcytic, their HbA2 levels are measured. If both have a concentration above 3.5%, they are considered as beta-thalassemia carriers and referred to the local designated health post for genetic counseling (3). Microcytic individuals with a hemoglobin A2 concentration in the normal range are treated with Iron and their indices rechecked. All results are sent to the local genetic counseling team. Those who marry after counseling are referred to their local health post or health house for follow up until they have their family completed. This program has decreased the birth incidence of beta-thalassemia sharply, but has not eliminated the disease altogether. One possible explanation for the birth of new cases, in spite of the screening programs, could be the carriers with RBC indices within the normal limit (silent carriers) (4, 5). In this study, the RBC indices and HbA2 levels in 185 blood samples from parents of 105 Thalassemia patients are investigated.

**Materials and methods**

This cross-sectional study was performed at Children Medical Center from 2004 to 2006. Among the patients receiving regular blood transfusion at the Transfusion unit of the Children Medical Center, 150 children were registered as having Beta-thalassemia. Three-Hundred parents were supposed to enroll in this study. The objectives and process of the study were presented to the families by a trained genetic counselor at the transfusion clinic. After clarifying the impacts of study on their family and community in private and group sessions, 105 families accepted to take part in the study. Both parents were available in 80 families and only one parent in 25 families. A 10 ml sample of peripheral blood was obtained from each parent and divided into 2 parts, one for hematologic tests, and the other for DNA extraction and storage in DNA bank for subsequent molecular analysis. Pedigree construction and genetic counseling were carried out in the same session. Then red cell indices (MCV and MCH) were measured and Hb electrophoresis was done on all samples using standard protocols.

**Results**

Data obtained from hematologic tests and Hb electrophoresis was analyzed. It was expected that obtained results be concordant with beta-thalassemia carriers pattern (MCV < 27 pg or MCV < 80 fl and HbA2>3.5). After double check with a parallel reference lab, results obtained from 180 samples for the beta-thalassemia parents were in accordance with the carrier status. However, the results of 5 remaining samples were found to have RBC indices and/or HbA2 levels incompatible with the defined pattern for carriers. One sample, out of five, had MCV and MCH values within the normal limits (Table 1). Furthermore, for 4 other cases, decreased values of MCV and MCH but normal HbA2 levels were present (Table 2).

**Discussion**

In this study, RBC indices and HbA2 levels of 185 healthy parents of regularly transfused beta-thalassemia major patients were analyzed. Obtained results showed that values detected for MCV and MCH in one person was unexpectedly higher than cut off value for carriers of beta-thalassemia. Furthermore, HbA2 concentrations of four cases were below 3.5%.

National screening programs for beta-thalassemia have been established in several countries, but success rate is variable among them. For example Cyprus
screening program, established in 1979, lead to no birth of affected children in the last 5 years (6). Conversely, births of affected neonates have not been stopped in Taiwan, in spite of execution of similar carrier screening program (7). One of the probable explanations would be the marriage of the carriers with persons of other ethnic groups (1) which could generate similar problem in our country. Other factors have been cited as the possible causes of the failure of the screening programs to completely eliminate the disease in Iran (8).

Silent beta-thalassemia carriers pose an important pitfall to screening program (3). These individuals in spite of having mutations in their beta-globin gene have normal RBC indices and/or HbA2 concentration. According to the results obtained from this study, about 3% (5 out of 185) of carriers are silent. Therefore it is crucial for genetic counselors to know how to approach with such cases. Defining the causative mutations using molecular methods would pave the way for establishing a protocol for a pre-marital screening program in conditions that one of couples is a confirmed carrier.

References

Table 1: Results obtained from a sample with unexpected high MCV and MCH

<table>
<thead>
<tr>
<th>Sample</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>HbA2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90.8</td>
<td>27</td>
<td>5.1</td>
</tr>
</tbody>
</table>

Table 2: Results obtained from samples with unexpected low HbA2

<table>
<thead>
<tr>
<th>Sample</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>HbA2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>18</td>
<td>3.4</td>
</tr>
<tr>
<td>2</td>
<td>67.1</td>
<td>17.4</td>
<td>3.1</td>
</tr>
<tr>
<td>3</td>
<td>77.1</td>
<td>23.9</td>
<td>3.1</td>
</tr>
<tr>
<td>4</td>
<td>64.9</td>
<td>22.1</td>
<td>2.8</td>
</tr>
</tbody>
</table>