ASSOCIATION BETWEEN THALASSEMIA TRAIT AND INSULIN RESISTANCE

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ABSTRACT

Introduction: One of the subjects discussed in the patients with thalassemia major and thalassemia intermedia is whether excess iron deposition in the liver causes insulin resistance or not. As well, patient group with thalassemia trait (minor) is considered as iron deficiency anemia erroneously in daily practice and this group is subjected to excess iron treatment unnecessarily. In this study, if unnecessary iron load in the patients with thalassemia minor had any effect on insulin resistance was assessed.

Materials and methods: A two-hour oral glucose tolerance test (OGTT) was performed in 30 thalassemia carrier patients and 30 gender-age-history matched healthy individuals. Glucose and insulin levels were measured at time zero, 30, 60, 90 and 120 minutes. Homeostasis model assessment of insulin resistance (HOMA-IR), whole-body insulin sensitivity index (WBISI), were calculated and the association between acute phase reactant C-reactive protein (CRP) and insulin resistance was investigated.

Results: There was no significant difference between study group and control group with respect to age, gender and body mass index (BMI) parameters. Insulin resistance (IR) parameters were compared within two groups and no statistical significance was determined. Groups were classified in two subgroups according to BMI value: Subgroups with BMI >25 kg/m² and subgroups with BMI<25 kg/m². When considered with respect to same BMI values, there was no difference between patient and control groups regarding glucose metabolism parameters and CRP levels. Insulin resistance was more evident inherently regarding BMI difference.

Conclusion: It was concluded that glucose metabolisms in patients with thalassemia trait did not show difference compared to BMI-matched healthy individuals. Detecting insulin resistance to be increased in both groups as BMI increased was suggestive of main factor causing disturbances in glucose metabolism in the patients thalassemia trait was obesity.

Keywords: Insulin resistance, thalassemia minor, thalassemia trait.

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Introduction

“Thalassemia” represents a group of inherited blood disorders. Hemoglobin which is oxygen-carrying component of red blood cells is a protein made up four heme groups surrounding a globin group. Globin consists of four polypeptide chains: two alpha and two non-alpha polypeptide chains. Each polypeptide chain is made up iron (Fe²⁺) attached to the protoporphyrin-IX ring⁶. The normal adult erythrocyte contains three forms of hemoglobin (Hb): HbA (96%), HbA2 (2% to 3%), and HbF (< 2%). The most common form of hemoglobin is HbA2 and it is composed of two alpha and two beta chains.

Expressing small quantities of hemoglobin causes hypochromia in red blood cells and erythrocytosis in heterozygotes. Clinical picture is determined by disequilibrium between globins. In absence of complementary binding chain, aggregation and precipitation occur in the cytoplasm. These aggregates damage cell membrane and cause early cell destruction⁷. Lack of globin chain synthesis in thalassemia pathophysiology shows a benign course in compound heterozygotes for alpha and beta thalassemia which have a course of an imbalance of alpha and beta chains. Alpha chain deposition occurs in homozygous beta thalassemia with lack of globin chain synthesis. Alpha-chain aggregates form insoluble inclusion bodies by precipitating in
erythroid precursors\(^4\). These inclusion bodies prevent the survival of erythroid precursors and cause ineffective erythropoiesis\(^5\). Only 15-30% of red blood cells produced in homozygous patients can pass through peripheral blood and the remaining ones are destroyed (ineffective erythropoiesis)\(^5\). The rate of DNA synthesis in G2 phase was decreased in thalassemic normoblasts\(^6\). This contributes to ineffective erythropoiesis. Defective cells in globin synthesis phase are destroyed before passing through peripheral blood. If the cell synthesizes relatively more gamma chain, less inclusion body occurs and so cell survival becomes longer\(^7\).

When erythrocytosis, reduction of mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) without iron deficiency anemia is seen or in the presence of abnormal hemoglobin fractions on electrophoresis, thalassemia minor can be considered. While MCV is 70-80 fL in alpha-thalassemia minor, it is generally <70 fL in beta-thalassemia minor\(^8\). Morphological changes is less important in alpha-thalassemia minor and basophilic stippling is rarely seen. Diagnosis of alpha-thalassemia minor is made with family screening or demonstration of HbH inclusion bodies in peripheral red blood cells by using Brilliant-Cresyl Blue. Hb Bart’s level is between 2-10%. HbA2 and HbF levels are lower than normal.

Microcytosis, hypochromia, target cells and basophilic stippling at peripheral blood smear is seen in beta-thalassemia heterozygotes. Usually increased HbA2 levels are rarely associated with increased HbF levels. Red blood cell counts are normal but HbA2 level is increased in beta-thalassemia heterozygote individuals carrying a gene for alpha-thalassemia\(^9\). HbA2 level is normal but red blood cell count is not affected in δ-thalassemia\(^10\). Similarly, a reduction of HbA2 level can be seen also in iron deficiency anemia. Therefore, it is necessary to measure HbA2 level after correction of iron deficiency\(^11\). Red blood cell count and HbA2 level measurement are used as screening tests in beta-thalassemia minor. There are tiny differences in routine blood tests for differentiation of these two diseases\(^12\).

The simple one is while MCV/RBC count ratio is below 13 in thalassemia, it is above 13 in iron deficiency anemia\(^13\). Also red cell distribution width (RDW) is different in both conditions, it is increased in iron deficiency anemia and decreased in beta-thalassemia minor\(^14\).

Insulin resistance (IR) is defined as reduction of insulin efficacy and not obtaining biological response at physiological doses. Since absolute IR is not compatible with life, this is a partial condition\(^8\). At a certain blood glucose level, in case of finding insulin concentration level to be higher than usual level, then it can be mentioned about hyperinsulinemia and IR\(^16\). IR was defined as “loss of sensitivity of target tissues to the effects of insulin in diabetic patients” by Himswart in 1939. Currently, it is defined as impaired biological response of the body to exogenous and endogenous insulin. Impaired biological response can be metabolic (carbohydrate, lipid and protein metabolism) or mitogenic (like slowed growth, differentiation, DNA synthesis and impaired gene transcription)\(^17\).

Every kind of disorder that can occur at steps beginning from insulin synthesis to onset of insulin action in target cells causes insulin resistance\(^18\).

In order to be able to show biological effect of insulin, insulin should be released from the pancreatic beta cells, enter blood circulation through the liver, pass through interstitial fluid and bind to specific receptors located in tissue membranes. Impairment that could develop in each of these steps results in IR\(^19\). IR mechanisms are divided into 3 groups: pre-receptor causes, abnormal insulin and insulin antibodies, blood flow disorder; receptor-related causes, reduced receptor number and affinity; post-receptor causes, abnormal signal transmission and phosphorylation.

**Materials and methods**

**Patients**

Thirty patients with diagnosis of thalassemia minor presenting to Outpatient Clinic of General Internal Medicine of Department of Internal Medicine of Istanbul School of Medicine between 2013 and 2014 and 30 healthy individuals were included in the study. Inclusion criteria were determined to be aged 18-65 years, female and male and to have diagnosis of thalassemia trait (alpha or beta). Exclusion criteria were presence of type 1 or 2 diabetes and iron deficiency anemia.

This study was approved by Istanbul School of Medicine Ethics Committee and written informed consent form was received according to the Declaration of Helsinki.
Methods

A total of 60 individuals comprising of 30 patients with thalassemia minor and 30 healthy individuals were included in this study. Ages, additional diseases of the patients, diabetes and diabetes-related diseases in the family history, currently used drugs and comorbid diseases were interrogated. Heights, weights and waist circumferences of all patients were measured. Waist circumference measurement was performed between the lower end of the 12th rib and the iliac crest in the midline and at the horizontal plane by using a tape measure and while the patient in a standing position and distributing his/her weight equally on both feet. Fasting venous blood samples were taken into the tubes containing ethylenediamine tetraacetic acid (EDTA), lithium heparin and gel in the morning and hemogram, glycated haemoglobin (HbA1c), iron, total iron binding capacity (TIBC), ferritin, c-reactive protein (CRP), triglyceride, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol values were measured. An oral glucose tolerance test was performed with 75 g glucose in all participants. Glucose and insulin levels were measured at time zero, 30, 60, 90 and 120 minutes. Serum glucose, CRP, iron, TIBC, triglyceride, HDL cholesterol, LDL cholesterol were determined using latex particle enhanced immunoturbidimetric assay kit (Kamiya Biomedical Company, Seattle, WA 98188) and read on the Roche/Hitachi 911 (Roche Diagnostics, Indianapolis, IN 46250) and HbA1c levels were measured with cation-exchange high performance liquid chromatography (HPLC) with Bio-Rad Turbo II (Bio-Rad, Richmond, California, USA). Ferritin and insulin levels were measured with electrochemiluminescence immunoassay method by using a Modular Analytics E170 immunoassay analyzer. Total blood count was performed with laser optic method by using a Beckman Coulter LH 780 Hematology Analyzer.

In the patients with thalassemia minor, first of all iron deficiency diagnosis was excluded, then diagnosis was confirmed by checking hemoglobin amount, MCV value and red blood cell count.

Glucose and insulin levels were measured at time zero minute and homeostasis model assessment of insulin resistance (HOMA-IR) value was calculated to determine insulin resistance. A HOMA-IR value of ≥ 2.7 was considered to be an indicator of insulin resistance (20).

Additionally, whole-body insulin sensitivity index (WBISI) value evaluating all glucose and insulin values in oral glucose tolerance test which was considered to be able to provide more information about insulin resistance was calculated (21).

\[ \text{WBISI} = \frac{\text{glucose} \times \text{insulin}}{\text{oral glucose} \times \text{oral insulin}} \]

Statistic analysis

Normality was tested by using Shapiro-Wilk tests and histogram graphs. Descriptive statistics were shown as mean, standard deviation, median, minimum, maximum, frequency and percentage. Independent Samples t test was used for the intergroup comparisons of parameters with normal distribution and Mann Whitney U test was used for the intergroup comparisons of parameters without normal distribution. Difference against time within groups was determined by using Friedman test. Multiple comparisons were performed after Friedman test by using Bonferroni-corrected Wilcoxon test. Nominal variables were compared by using Fisher’s exact probability test, and Yates corrected chi square \( (x^2) \) test. P value of two way and one way significance was considered to be <0.05. Analyses were performed by using “Statistical Package for Social Sciences for Windows version 21”.

Results

Clinical and laboratory features of the cases were shown in Table 1. Fasting plasma glucose and insulin values were seen to be within normal ranges in both groups. Difference between mean waist circumferences and mean heights of control and patient groups was not found to be statistically significant \( (p=0.763, p=0.083) \). Also difference between median age, weight and BMI of control and patient groups was not found to be statistically significant \( (p=0.859, p=0.673, p=0.367) \). When considered with respect to main factors which could affect insulin resistance, there was no difference between two groups. While difference between mean RBC and MCV values showed statistical significance \( (p=0.763, p=0.083) \) inherently, also difference between median Hb values was statistically significant \( (p=0.000) \) (Table 1).

When two groups were evaluated together, difference between median HOMA-IR \( (p=0.451) \), median WBISI \( (p=0.152) \) was not statistically significant (Table 2).
Patients and control cases were classified in two more subgroups according to BMI value: Subgroups with BMI < 25 kg/m² and subgroups with BMI > 25 kg/m². These new subgroups were compared with each other again with respect to HbA1c, mean glucose, mean insulin values and difference in HOMA-IR parameters.

When the patient and control groups with BMI > 25 kg/m² in both group were compared with each other, no difference was determined between glucose metabolism (GM) and CRP values (Table 3). Similarly, no difference was determined between glucose metabolism values also in the patient and control groups with BMI < 25 kg/m² (Table 4). As it was expected, marked statistical differences were determined in the comparisons of the patient and control groups with different BMI values (Table 5, Table 6). This last condition showed that the methods used were sensitive.

When CRP value was compared between two groups; it was determined that there was no statistical significance, but in the comparison performed between subgroups CRP level was increased proportionally to BMI. While CRP value was 0.59±0.3 in control subgroup with BMI < 25 kg/m², it was 4.08±2.9 in control subgroup with BMI >25 kg/m² (p=0.014). While mean CRP value was 0.94±1.0 in patient subgroup with BMI < 25 kg/m², it was 2.08±1.5 in patient subgroup with BMI >25 kg/m² (p=0.010).

### Table 1: Demographic characteristics of the study group. Median [range].

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control</th>
<th>Patient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE</td>
<td>36.5 (23-59)</td>
<td>34.5 (18-70)</td>
<td>&lt;0.859</td>
</tr>
<tr>
<td>WEIGHT</td>
<td>69 (47-94)</td>
<td>69 (49-98)</td>
<td>&lt;0.673</td>
</tr>
<tr>
<td>WAIST</td>
<td>83.77±11.1</td>
<td>82.87±84-108</td>
<td>&lt;0.763*</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>160.87±7.0</td>
<td>164.33±11.8</td>
<td>&lt;0.083*</td>
</tr>
<tr>
<td>CRP</td>
<td>2.4±0.7</td>
<td>1.47±1.3</td>
<td>&lt;0.444</td>
</tr>
<tr>
<td>RBC</td>
<td>4.6±1.4</td>
<td>5.3±0.5</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>HG</td>
<td>13.4±1.2</td>
<td>11.95±4.2</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>MCV</td>
<td>88.2 (79.3-98.8)</td>
<td>65.5 (56.4-76.3)</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>RDW</td>
<td>15.45±1.4</td>
<td>17.95±2.3</td>
<td>&lt;0.000</td>
</tr>
<tr>
<td>BMI</td>
<td>26.10±5.8</td>
<td>24.48±4.8</td>
<td>&lt;0.367</td>
</tr>
</tbody>
</table>

*indicates the ones conform to a normal distribution and use parametric test.

### Table 2: Insulin resistance parameters of patient and control groups. *indicates the ones conform to a normal distribution and use parametric test.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Patient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>1.05±0.5</td>
<td>1.1±0.5</td>
<td>0.451</td>
</tr>
<tr>
<td>HOMA-IR%</td>
<td>86.09±27.9</td>
<td>99.11±34.2</td>
<td>0.145</td>
</tr>
<tr>
<td>HOMA-S%</td>
<td>118.33±53.8</td>
<td>113.59±60.5</td>
<td>0.451</td>
</tr>
<tr>
<td>WBISI</td>
<td>4.04±2.3</td>
<td>4.87±2.6</td>
<td>0.152</td>
</tr>
</tbody>
</table>

### Table 3: Comparison of GM parameters in BMI >25 kg/m² in both group.

<table>
<thead>
<tr>
<th>BMI &gt;25 kg/m²</th>
<th>Patient &gt;25 kg/m²</th>
<th>Control &gt;25 kg/m²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1c</td>
<td>5.44±0.3</td>
<td>5.76±0.5</td>
<td>0.051</td>
</tr>
<tr>
<td>Mean glucose</td>
<td>103.4±2.5</td>
<td>100±27.5</td>
<td>0.965</td>
</tr>
<tr>
<td>Mean insulin</td>
<td>100±35</td>
<td>97.34±32.7</td>
<td>0.93</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.39±0.5</td>
<td>1.28±0.7</td>
<td>0.348</td>
</tr>
<tr>
<td>WBISI</td>
<td>3.45±1.3</td>
<td>2.99±1.4</td>
<td>0.359</td>
</tr>
</tbody>
</table>

### Table 4: Comparison of GM parameters in BMI <25 kg/m² in both group.

<table>
<thead>
<tr>
<th>BMI&lt;25 kg/m²</th>
<th>Patient &lt;25 kg/m²</th>
<th>Control &lt;25 kg/m²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1c</td>
<td>5.34±0.56</td>
<td>5.76±0.47</td>
<td>0.016</td>
</tr>
<tr>
<td>Mean glucose</td>
<td>80.29±17.4</td>
<td>80.29±17.4</td>
<td>0.843</td>
</tr>
<tr>
<td>Mean insulin</td>
<td>74.61±24.1</td>
<td>73.85±19.3</td>
<td>0.843</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.87±0.5</td>
<td>0.82±0.3</td>
<td>0.937</td>
</tr>
<tr>
<td>WBISI</td>
<td>6.1±2.9</td>
<td>5.1±2.6</td>
<td>0.268</td>
</tr>
</tbody>
</table>

### Table 5: Comparison of GM parameters of control subgroups.

<table>
<thead>
<tr>
<th>BMI</th>
<th>&lt;25 kg/m²</th>
<th>&gt;25 kg/m²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1c</td>
<td>5.10±0.4</td>
<td>5.44±0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean glucose</td>
<td>80.3±21.4</td>
<td>103.40±27.41</td>
<td>0.009</td>
</tr>
<tr>
<td>Mean insulin</td>
<td>74.61±24.1</td>
<td>100.86±35.5</td>
<td>0.014</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.87±0.4</td>
<td>1.39±0.5</td>
<td>0.005</td>
</tr>
<tr>
<td>WBISI</td>
<td>6.1±2.9</td>
<td>3.45±1.3</td>
<td>0.012</td>
</tr>
</tbody>
</table>

### Table 6: Comparison of GM parameters of patient subgroups.
Discussion

Due to alterations in red blood cell morphology, patients with thalassemia minor are frequently confused with iron deficiency anemia and so they are subjected to excess iron treatment. In differential diagnosis of thalassemia minor and iron deficiency anemia, simple tests as MCH/RBC count ratio, MCV/RBC count ratio (Meintzer ratio), red cell distribution width (RDW) and red blood cell count are used for screening. Definite diagnosis can be made by performing family screenings and by measuring HGB A2 levels.

Association between thalassemia minor and insulin resistance became subject of many studies in the literature. In the study named “Insulin sensitivity assessment with euglycemic insulin clamp in adult β-thalassemia major patients” and performed by Apostolos T. et al., association between insulin resistance and elevated serum ferritin levels was shown\(^{(25)}\). In the study named “Insulin resistance and hyperinsulinemia in patients with thalassemia major treated by hypertransfusion” and performed by Merkel PA et al., association between insulin resistance and frequent transfusion and thalassemia major was investigated and this association was found to be significant\(^{(26)}\). In the study named “Pancreatic iron and glucose dysregulation in thalassemia major” and performed by Leila J. et al., the deposition and toxicity of iron in beta cells was accused of insulin resistance in thalassemia major\(^{(27)}\).

There is limited number of studies about association between thalassemia minor and insulin resistance. In the study named “C-Reactive Protein and Insulin Resistance in Subject with Thalassemia Minor and a Family History of Diabetes” and performed by Peter Y.N. Tang et al., it was predicted that insulin resistance was increased in the patients with thalassemia minor and this could be associated with increased CRP as inflammation marker\(^{(28)}\).

Starting from association between iron and insulin resistance, our aim in this study was to determine whether IR was more common in thalassemia patients or not. Different evidences were demonstrated about presence of association between iron stores and insulin resistance in the literature. In the study performed by Fernandez et al., serum ferritin levels were determined to be higher in type 1 and 2 DM patients with poor metabolic control. It was seen that higher ferritin level was marker of HBA1c and metabolic control independently of blood glucose\(^{(29)}\). Iwasaki et al. suggested that excess ferritin expression in individuals with insulin resistance could be an adaptive defense mechanism against iron-induced oxidative damage seen in adipocytes and this explains the positive relationship between obesity, insulin resistance and ferritin\(^{(30)}\).

Elevated ferritin levels are between 6-33% in Type 2 DM patients. Probably there are 3 mechanisms than can explain this condition. Firstly, elevated ferritin levels may reflect increased iron stores; secondly, as an acute phase reactant it may be marker of underlying inflammation and finally, ferritin levels may elevate due to delayed clearance of glycolized ferritin from the circulation\(^{(28)}\). Transferrin saturation percentage is determined to be increased in conditions where elevated ferritin levels developing secondary to iron deposition. However, transferrin saturation percentage is found less in hyperferritinemia determined in case of inflammation. In the studies performed, it was suggested that lower transferrin saturation percentage could be associated with diabetes. Consequently, it was come out that hyperferritinemia in diabetes patients was secondary to inflammation\(^{(29,30)}\).

Literature data show that iron and iron metabolism-related factors have a direct role in association between adipocyte IR and adiponectin. Insulin resistance in adipocyte cell is seen in early phase of type 2 DM pathogenesis\(^{(31)}\). Iron metabolism-related factors may contribute to IR in muscle, liver and adipocytes and consequently cause impaired glucose metabolism and hyperglycemia\(^{(28)}\).

Patients without diagnosis of thalassemia minor and presenting to Outpatient Clinic of General Internal Medicine of Department of Internal Medicine of Istanbul School of Medicine and who had lower levels of HB, HCT, MCV and higher red blood cell counts were also included in the patient group of the study by considering thalassemia minor. Similarly, healthy individuals considered that they might have insulin resistance were included in the study as control group.

During selection of groups, both group was tried to include same parameters which could affect insulin resistance. Thus, patient and control groups not showing statistical difference were obtained. However, negative aspect of this meticulous approach was to study with smaller number of patient group.

For the diagnosis of insulin resistance, very different measurements ranging from simple clini-
cal observation to hyperinsulinemic euglycemic clamp test were used. HOMA is one of the most commonly used practical methods for this purpose in clinical practice. HOMA is not very sensitive in borderline patients and this is limitation of the method. Therefore, we used insulin responses to OGTT in order to be able to evaluate the patients in detail.

When we assessed whole group, no significant difference was determined between parameters affecting glucose metabolism. Moreover, when we divided both groups into two subgroups as BMI <25 kg/m² and BMI >25 kg/m² and compared them diagonally, no difference was determined between patient and control groups according to BMI.

When subgroups within groups were evaluated with themselves, they showed difference inherently. This was a data indicating that tests and methods used were sensitive.

Independent from thalassemia minor, increased weight and so increased BMI is the most important step of mechanisms causing development of insulin resistance.

Different from type 2 DM developing secondary to the proven iron deposition in thalassemia major and intermedia forms, insulin resistance was observed to be increased in the patients with thalassemia minor only when BMI was >25 kg/m². This finding shows that development of insulin resistance in the patients with thalassemia minor does not show difference compared to the patients without thalassemia minor, insulin resistance can develop as obesity increases in this patient group as it is in control patients and a disease-related resistance development is not in question. Also increase of CRP as weight increases independently in thalassemia minor supports this view.

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