Correlation of Hepcidin Level with Insulin Resistance and Endocrine Glands Function in Major Thalassemia

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

Abstract

Background. Hepcidin is a master regulator of iron metabolism that inhibits the transport of iron out of enterocytes and macrophages. Thalassemia major (TM) is associated with some of the endocrine disorders. However, studies have yet to be conducted on the correlation of hepcidin with hormone levels and insulin resistance (IR) in patients with TM.

Objectives. In the present study, the correlation of hepcidin level with some endocrine and biochemical parameters was investigated to determine the factors that mainly affect hepcidin correlation in patients with thalassemia. These factors include hormones, iron status, and IR parameters.

Material and Methods. Hepcidin and other measured biochemical parameters were compared between the TM patients (100) and healthy children (37).

Results. Serum thyroid-stimulating hormone (TSH) was positively correlated (p < 0.05) with hepcidin, iron, and ferritin. T4 hormone was correlated with ferritin only. Other hormones showed different correlation patterns with iron status parameters but were statistically insignificant (p > 0.05). The percentage of β-cell function was the only parameter among the IR parameters that showed a significant difference between thalassemic and control groups.

Conclusions. Thyroid and β-cells dysfunctions are common in TM patients with frequent blood transfusions. In addition, hepcidin and TSH levels can be predicted significantly using the most correlated factors with hepcidin. These factors, including ferritin, insulin and TSH were used to construct predicting equations: S. Hepcidin = 0.003*Ferritin + 3.02*TSH + 0.12*Insulin + 16.85 (± 7.78) and TSH = 0.0083 × Insulin + 0.0042 × Ferritin + 0.0937 × Hepcidin + 1.91 (± 1.373) (Adv Clin Exp Med 2015, 24, 1, 69–78).

Key words: thalassemia, hepcidin, insulin resistance, hormones.

β-Thalassemia is a congenital form of anemia that originated in the Mediterranean region; this disease is characterized by deficiency or lack of β-globin synthesis. Excessive iron absorption and iron concentration are the main characteristics of β-thalassemia; this condition can further lead to high mortality and morbidity whether or not patients receive regular transfusions [1]. Currently, thalassemias are managed with chelation therapy, but exogenous transferrin [2], exogenous hepcidin, [3], or hepcidin signaling agonists [4] may be effective options in the future.

In Iraq, thalassemia is a serious problem because of the lack of equipment and drugs in different periods of security issues and wars. In Najaf Governorate, with a population of approximately 1.2 million in 2012, a total of 612 patients have been treated since August 2012 in the Thalassemia Unit at AL-Zahra’a Teaching Hospital. Thalassemia has been studied in various cities in Iraq and in different fields of study [5, 6]. Studies have also shown that patients with β-thalassemia major are prone to metabolic complications, including endocrine dysfunction affecting single or multiple endocrine glands. Although the actual mechanism is not definitive, the most possible explanation is related to excess iron concentrations [7]. Furthermore, children who suffer from β-thalassemia...
major and undergo multiple transfusions may develop severe endocrine complications because of excess iron concentrations [8]. However, studies are yet to be conducted regarding the correlation of hepcidin with hormone levels and insulin resistance in patients with thalassemia major.

Hepcidin is a polypeptide produced by the liver, which is the principal regulator of iron metabolism. Hepcidin binds with ferroportin, the iron channel lies on the gut enterocytes and the membrane of reticuloendothelial system cells, and inhibits iron transport. The binding of hepcidin with ferroportin causes internalization and degradation, thereby exerting a general inhibitory effect on iron transport and release in the body [9]. Therefore, hepcidin maintains iron homeostasis.

Insulin resistance (IR) is a physiological condition in which the natural hormone insulin becomes less effective at lowering blood sugar levels. Blood glucose levels may exceed the normal range and cause adverse health effects depending on dietary conditions. If IR is observed, higher insulin concentration should be secreted by the pancreas. If this compensatory increase does not occur, blood glucose concentrations increase and type 2 diabetes occurs [10].

The present study is aimed to estimate and compare the endocrine function activity of patients with thalassemia with that of the control group. This study was also conducted to estimate the correlation between the levels of hormones and the parameters affecting iron concentrations, including hepcidin level.

Material and Methods

Patients

A total of 100 Arabic Iraqi patients with thalassemia (50 boys and 50 girls) aged 3 years to 11 years participated in the present study. These patients were registered as patients with thalassemia in Thalassemia Unit at Al-Zahra’a Teaching Hospital in Najaf City, Iraq. Consent was obtained from the patients’ first-degree relatives (mother or father). These patients were also informed that the results of the study would be provided to them as free useful laboratory tests. The patients were diagnosed with β-thalassemia major as recorded in their files. Diagnosis was established by observing clinical symptoms and conducting hematological and hemoglobin (Hb) HPLC analysis. Hb HPLC was conducted using HPLC instrument (VARIANT™ β-Thalassemia Short Program). All of the patients were subjected to a blood transfusion as part of their treatment. Serum C-reactive protein (CRP) was negative in all of the samples (CRP < 6 mg/L). A normal CRP is necessary to ensure that no inflammation affects serum ferritin or hepcidin [11].

Exclusion Criteria: the present study excluded patients with apparent diabetes mellitus, infection and inflammation, and heart diseases; patients from a non-Arabic ethnic group were also excluded.

Control group: 37 healthy children participated in the present study as the control group with their age range comparable to that of the patients. None of these controls was anemic or manifested an evident systemic disease.

Methods

A-Assays

Iron status, in terms of Hb level, was estimated colorimetrically according to Drabkin’s method by using a Spinreact® kit (Spain). Serum iron was measured using the colorimetric ferrozine method in the Randox® kit (UK). Total iron-binding capacity (TIBC) was measured by spectrophotometer using a Randox® kit (UK). The following method was performed. An excessive amount of iron ions was added to serum iron to saturate transferrin. The unbound iron was precipitated with basic magnesium carbonate. After centrifugation, iron in the supernatant was determined. Estimation of ferritin quantitatively was performed using a solid-phase enzyme-linked immunosorbent assay (ELISA) supplied by (BioCheck®, USA). To calculate Transferrin iron saturation percentage (TISP), serum iron concentration was divided by TIBC [10]. Transferrin concentration was calculated from serum iron; transferrin saturation percentage was determined using the following equation: serum iron (μmol/L)/transferrin (g/L) × 3.98. This equation is based on the maximum binding capacity of two moles Fe³⁺ per mole of transferrin and the molecular weight of 79, 570 dalton for transferrin [11]. All of the hormones were estimated in the serum by the ELISA technique with commercially available kits (Monobind® Inc., USA) according to the manufacturer’s instructions.

B-Insulin Resistance Status

Insulin resistance parameters were calculated from insulin and fasting glucose levels by using HOMA calculator software (http://www.dtu.ox.ac.uk/homacalculator/download.php). This software was used to generate IR index (HOMA2IR), insulin sensitivity (HOMA%β), and β-cell function
Endocrines as a Function of Hepcidin in Thalassemia

Normal-weight individuals aged less than 35 years exhibited a HOMA2IR of 1 mol µU/L² and HOMA%S and β-cell function of 100% [12]. A subject was considered as IR if HOMA2IR > 3 [13].

Statistical Analysis

The types of distribution of the variable results were examined using Kolmogorov-Smirnov test. The results of the analysis were calculated by dividing the variables into 2 classes depending on the statistical distribution: normally distributed and non-parametric variables.

For normally distributed variables, the results were stated as mean ± standard deviation. Pooled t test was used to compare patients and the control groups. Pearson’s correlation coefficient (r) was computed to determine the correlation between parameters.

For non-parametric variables that are not normally distributed, the results were expressed as median in addition to mean ± standard deviation. Mann-Whitney U test was utilized to compare the patients and the control groups. Spearman’s correlation coefficients (ρ, rho) were calculated to determine the correlation between parameters.

Statistical analysis was performed in SPSS version 19.0.1 multilingual program (2010; IBM, USA). A forecasting study was performed using “Regression Forecasting Model” software (Business Spreadsheets, USA).

Results

Comparison Between Patients with Thalassemia and Control Group

Table 1 shows the expected status of anemia associated with thalassemia as indicated by a decrease (p < 0.05) in Hb concentration and pack cell volume (PCV). Table 1 also shows the significantly high concentration of ferritin and other iron status parameters in patients with thalassemia compared with the control group. Endocrine changes in Table 1 shows a higher serum TSH level (p < 0.05) and a significant decrease (p < 0.05) in serum cortisol, thyroxine (T4), and prolactin in the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Thalassemia group (n = 100)</th>
<th>Control group (n = 37)</th>
<th>Significance p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>7.88 ± 1.30</td>
<td>13.07 ± 1.76</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>PCV %</td>
<td>24.65 ± 3.88</td>
<td>40.20 ± 5.29</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Ferritin (pmol/L)</td>
<td>(588, 297–1178)</td>
<td>(130, 97–288)</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>EIBS (mmol)</td>
<td>(84.13, 57–127 )</td>
<td>(18.62, 14.24–27.11 )</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>S.Iron (umol/L)</td>
<td>24.68 ± 8.09</td>
<td>11.58 ± 5.35</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TIBC (umol/L)</td>
<td>69.44 ± 14.76</td>
<td>61.00 ± 11.45</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TS %</td>
<td>36.49 ± 14.08</td>
<td>19.20 ± 9.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>0.18 ± 0.04</td>
<td>0.15 ± 0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>UIBC (umol/L)</td>
<td>45.50 ± 16.22</td>
<td>49.42 ± 11.15</td>
<td>ns.</td>
</tr>
<tr>
<td>Hepcidin (nM)</td>
<td>51.25 (14.15–71.42)</td>
<td>11.47(0.94–19.12)</td>
<td>0.041</td>
</tr>
<tr>
<td>GH (ng/mL)</td>
<td>(3.88, 0.21–5.43)</td>
<td>(3.10, 0.43–5.71 )</td>
<td>ns.*</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>(0.23, 0.12–1.8 )</td>
<td>(0.98, 0.38–2.1 )</td>
<td>ns.*</td>
</tr>
<tr>
<td>T3 (ng/mL)</td>
<td>(1.07, 0.43–1.40)</td>
<td>(0.79,0.62–1.92 )</td>
<td>0.039*</td>
</tr>
<tr>
<td>T4 (µg/dL)</td>
<td>6.12 ± 2.79</td>
<td>7.73 ± 1.81</td>
<td>0.001</td>
</tr>
<tr>
<td>TSH (µIU/mL)</td>
<td>5.48 ± 1.86</td>
<td>4.36 ± 1.36</td>
<td>0.002</td>
</tr>
<tr>
<td>Cortisol (µg/dL)</td>
<td>(2.90, 1.94–4.98)</td>
<td>(6.55, 3.91–9.82)</td>
<td>0.006*</td>
</tr>
<tr>
<td>PRL (ng/mL)</td>
<td>(3.94, 3.18–6.7)</td>
<td>(4.06, 3.33–8.79)</td>
<td>0.044*</td>
</tr>
</tbody>
</table>

* Using Mann-Witney U test for nonparametric variables, other p-values due to t-test. ns.: p > 0.05.
thalassemic group compared with the healthy control group.

The percentage of β-cell function (HOMA%B) measured by HOMA calculator was the only parameter among the IR state parameters that showed a significant difference (p = 0.037) between the thalassemic and control groups (Table 2). Serum glucose concentration was slightly and insignificantly higher in patients than in the controls (p > 0.05).

Comparison between Male and Female Patients

Significant increases (p < 0.05) in Hb, PCV, T3 and testosterone in male patients compared with female thalassemic patients were observed, and the following values were obtained: Hb, male = 8.30 ± 1.44 ng/mL and female = 7.46 ± 0.99 ng/mL, p = 0.12; PCV, male = 25.90 ± 4.318 ng/mL and female = 23.40 ± 2.98 ng/mL, p = 0.011; testosterone, male = 0.64 ng/mL and female = 0.37 ng/mL, p = 0.004; T3, male = 1.09 ± 0.94 ng/mL and female = 0.61 ± 0.36 ng/mL, p = 0.002.

Effect of Number of Transfusions

The numbers of transfusion was investigated for correlation with all the measured parameters, and results revealed a significant correlation of transfusion number with some measured parameters; (Iron (p = 0.373, p = 0.018), TIBC (p = -0.321, p = 0.029), ferritin, (p = 0.411, p = 0.007), EIBS (p = 0.411, p = 0.007), T3 (p = -0.334, p = 0.042), Cortisol (p = -0.353, p = 0.035), and HOMA%B (p = -0.256, p = 0.048), whereas others showed no significant correlation. The results generally showed a positive correlation between the number of blood transfusions and iron overload parameters.

Correlation Between Hormones and Iron Parameters

The results of the r and p values for the relationships between every hormone and all the iron status parameters in thalassemic patients indicated that the most significant positive correlations were observed in TSH with hepcidin (r = 0.404, p = 0.001), serum iron (r = 0.258, p = 0.049), and ferritin (r = 0.305, p = 0.018). T4 hormone was correlated with ferritin T4 (Ferritin r = 0.430, p = 0.001). The other results showed various correlations between hormones and iron parameters but no significance was found (p > 0.05).

Correlation Between IR Parameters and Iron Status Parameters

The results in Table 3 presented the correlation coefficients (p) and p-values for the relationship between IR state parameters and each parameter of iron status in thalassemic patients. The results in Table 3 are very interesting. Insulin showed a significant positive correlation with hepcidin (p = 0.288, p = 0.033) and ferritin (p = 0.263, p = 0.042), and negative relation between hepcidine and HOMA%S (r = -0.255, p = 0.049). Furthermore, ferritin and subsequently, estimated iron body storage, showed a significant correlation with insulin (r = 0.263, p = 0.042), insulin/glucose ratio (r = 0.265, p = 0.042), and HOMA%B (r = 0.266, p = 0.041).

Forecasting Regression for Factors that Correlated with Hecpiddin

The following showed correlation with hepcidin: thyroid-stimulating hormone (TSH, r = 0.404, p = 0.001), insulin (r = 0.288, p = 0.033), and ferritin (r = 0.297, p = 0.041). Considering the importance

Table 2. Parameters of insulin resistance in thalassemic and control groups (median, range in brackets)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Thalassemia group (n = 100)</th>
<th>Control group (n = 37)</th>
<th>Significance p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (µlU/mL)</td>
<td>(5.52, 3.87–11.75)</td>
<td>(5.86, 3.49–13.74)</td>
<td>n.s.a</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.07 ± 1.82</td>
<td>5.65±1.13</td>
<td>n.s.</td>
</tr>
<tr>
<td>Ins/Glu</td>
<td>(1.02, 0.87–1.92 )</td>
<td>(0.94, 0.87–2.15)</td>
<td>n.s.</td>
</tr>
<tr>
<td>HOMA2IR</td>
<td>(0.81, 0.49–1.87 )</td>
<td>(0.80, 0.38–1.58 )</td>
<td>n.s.</td>
</tr>
<tr>
<td>HOMA%B</td>
<td>(70.80, 57.44–82.56 )</td>
<td>(66.65, 62.66–97.84 )</td>
<td>0.037*</td>
</tr>
<tr>
<td>HOMA%S</td>
<td>86.45 ± 58.89</td>
<td>84.09 ± 54.13</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* Using Mann-Wittney U test for nonparametric variables. While other p-values due to t-test. ns. = p > 0.05.
Table 3. Correlation between IR and iron status parameters in thalassemic patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Insulin</th>
<th>Glucose</th>
<th>Ins/Glu</th>
<th>HOMA2IR</th>
<th>HOMA%S</th>
<th>HOMA%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV%</td>
<td>r-value</td>
<td>–0.14</td>
<td>0.12</td>
<td>–0.04</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.30)</td>
<td>(0.36)</td>
<td>(0.77)</td>
<td>(0.99)</td>
<td>(0.78)</td>
</tr>
<tr>
<td>Hb(g/L)</td>
<td>r-value</td>
<td>–0.14</td>
<td>0.12</td>
<td>–0.04</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.29)</td>
<td>(0.35)</td>
<td>(0.78)</td>
<td>(0.99)</td>
<td>(0.78)</td>
</tr>
<tr>
<td>Iron</td>
<td>r-value</td>
<td>0.19</td>
<td>0.16</td>
<td>0.10</td>
<td>0.22</td>
<td>–0.15</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.15)</td>
<td>(0.22)</td>
<td>(0.47)</td>
<td>(0.09)</td>
<td>(0.24)</td>
</tr>
<tr>
<td>TIBC</td>
<td>r-value</td>
<td>0.09</td>
<td>0.19</td>
<td>0.22</td>
<td>0.24</td>
<td>–0.24*</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.48)</td>
<td>(0.15)</td>
<td>(0.11)</td>
<td>(0.07)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>Ferritin</td>
<td>r-value</td>
<td>0.26*</td>
<td>–0.23</td>
<td>0.27</td>
<td>0.11</td>
<td>–0.23</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.04)</td>
<td>(0.08)</td>
<td>(0.42)</td>
<td>(0.08)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>EIBS</td>
<td>r-value</td>
<td>0.26*</td>
<td>–0.23</td>
<td>0.27*</td>
<td>0.11</td>
<td>–0.23</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.04)</td>
<td>(0.08)</td>
<td>(0.42)</td>
<td>(0.08)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>UIBC</td>
<td>r-value</td>
<td>0.22*</td>
<td>0.08</td>
<td>0.13</td>
<td>0.10</td>
<td>–0.12</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.06)</td>
<td>(0.54)</td>
<td>(0.34)</td>
<td>(0.43)</td>
<td>(0.35)</td>
</tr>
<tr>
<td>TS%</td>
<td>r-value</td>
<td>0.17</td>
<td>0.10</td>
<td>–0.05</td>
<td>0.04</td>
<td>–0.02</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.21)</td>
<td>(0.46)</td>
<td>(0.73)</td>
<td>(0.74)</td>
<td>(0.98)</td>
</tr>
<tr>
<td>Transferrin</td>
<td>r-value</td>
<td>–0.07</td>
<td>–0.08</td>
<td>0.19</td>
<td>0.21</td>
<td>–0.19</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.59)</td>
<td>(0.57)</td>
<td>(0.14)</td>
<td>(0.12)</td>
<td>(0.14)</td>
</tr>
<tr>
<td>Hepcidin</td>
<td>r-value</td>
<td>0.29*</td>
<td>0.14</td>
<td>0.19</td>
<td>0.21</td>
<td>–0.26*</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>(0.03)</td>
<td>(0.28)</td>
<td>(0.15)</td>
<td>(0.11)</td>
<td>(0.04)</td>
</tr>
</tbody>
</table>

(r) – Pearson’s correlation coefficient, (ρ) – Spearman’s correlation coefficient. (*) – significant correlation (p < 0.05).

Fig. 1. Correlation between actual and the predicted hepcidin levels from the regression equation.
of the parameters that significantly correlated with hepcidin in the consequences of iron overload, forecasting analysis was performed for the regression of these parameters with hepcidin. The following equations showed only the predictable factors obtained from the forecasting analysis:

**Forecasting of Serum Hepcidin**

The following equation was obtained using the forecasting regression software:

\[ S. \text{ Hepcidin} = 0.003 \times \text{Ferritin} + 3.02 \times \text{TSH} + 0.12 \times \text{insulin} + 16.85 \ (\pm 7.78) \]

When the serum hepcidin values, calculated using the above equation, are plotted against the actual hepcidin, the results indicate a good correlation \( R^2 = 0.3327 \) between the actual and calculated serum hepcidin levels.

**Forecasting of S.TSH**

The following equation was obtained using the forecasting regression software:

\[ S. \text{TSH} = 0.0083 \times \text{Insulin} + 0.0042 \times \text{Ferritin} + 0.0937 \times \text{Hepcidin} + 1.91 \ (\pm 1.373) \]

When the TSH values calculated by using the above equation are plotted against the actual, measured TSH, the results indicate a good correlation \( R^2 = 0.3056 \) between the actual and calculated TSH levels.

**Discussion**

**Comparison Between Patients with Thalassemia and Control Group**

**Iron Status and Endocrine Glands Function**

The results in Table 1 indicated that iron overload was detected in patients. Serum ferritin is a marker of iron overload in thalassemia. In β-thalassemia major, repeated blood transfusions, ineffective erythropoiesis, and increased gastrointestinal iron absorption lead to iron overload in the body [14]. With repeated transfusion, excess iron is sequestered intracellularly as rapidly mobilizable, dispersed, and soluble ferritin iron or as aggregated and insoluble hemosiderin iron for long-term storage [15]. This condition is toxic to tissues probably because oxidative stress is partially induced [16]. Iron accumulation in the body over time as a consequence of continuous red blood cell transfusions causes hepatic, endocrine, and cardiac complications [17, 18].

Under secondary iron overload conditions caused by chronic transfusion therapy as in thalassemia major, plasma hepcidin levels are increased, resulting in ferroportin degradation [19]. Hepcidin reduces serum iron by decreasing iron...
absorption and discontinuing macrophages from releasing iron, thereby causing iron sequestration. Increased iron content likely upregulates hepcidin, which then decreases iron and vice versa. Three relevant sites involved in hepcidin regulation are kidney, bone marrow, and liver cells [20]. Hepcidin is finally induced during infection, thereby decreasing the available host iron pool essential for the survival of invading pathogens [21].

The increase in TSH level in thalassemic patients in Table 1 may indicate a decrease in the function of the thyroid gland and the subsequent increase in TSH secretion from the pituitary gland to compensate for the decrease in thyroid hormone secretion in the thalassemic group. Furthermore, these results indicated that the adrenal cortex and prolactin-producing cells in the pituitary gland are also involved. The significant decrease in thyroid hormones is consistent with that found in the published results of other studies [22]. The cause of these changes may be attributed to the sensitivity of endocrine cells or glands to iron overload in patients with thalassemia. The precipitation of different forms of iron in these tissues may lead to tissue necrosis and destruction of endocrine cells. Iron overload complications in endocrine glands result in insufficient secretion of many glands including pituitary, thyroid, parathyroid, and adrenal glands [23].

Changes in the level of cortisol between thalassemic and control groups revealed unique results describing the involvement of the adrenal cortex. However, stimulation tests and large scale studies are necessary to confirm this involvement. Most studies supposed that adrenal insufficiency may be caused by impaired hypothalamic-pituitary-adrenal axis (HPA) axis function, and secondarily, hemochromatosis of adrenal or pituitary glands [24]. However, the possibility of chronic liver disease occurring because of iron overload may reduce the synthesis of hepatic cortisol-binding globulin, which can result in abnormally low serum cortisol [25].

**Insulin Resistance Parameters**

The change in the function of pancreatic β-cell function (Table 2) during adolescence or later may result in hyperinsulinemia, IR, and failure of normal glucose tolerance to β-cells and the development of insulin-dependent diabetes mellitus. Another endocrine disorder that may affect thalassemic patients is the primary hypothyroidism which usually affects patients from the second decade of life onward. Despite treatment, the involvement of the endocrine system still affects the life quality of those patients. Developing therapeutics would reduce morbidity and mortality of thalassemic patients and enhance the functions of endocrine disorders [26].

**Comparison between Male and Female Patients**

The change in Hb, PCV, and testosterone can be easily explained by the genetic difference between females and males. Males, in general, have a higher Hb than females in healthy subjects, and the present findings indicate the same profile of change. Adult women have less stored iron, which depends on the duration of menstruation, pregnancy, child birth, lactation, and iron intake [27]. Total iron body store in normal adults is the result of the balance between iron absorbed from the diet and the lost iron. However, the difference in T3 is difficult to explain and a larger sample size is necessary to explain this finding.

**Effect of Number of Transfusions**

The positive correlation between the iron overload parameters and the number of blood transfusions is due to the fact that these parameters are indicators of stored iron and iron overload that increases with increasing number of blood transfusions. Regular transfusion and chelation therapy have improved the lifespan and life quality of patients, but many patients suffer from clinical complications. Patients affected by the most severe forms of thalassemia require chronic blood transfusions to sustain life and chelation therapy to prevent iron overload. If regular transfusions are required, as in β-thalassemia major patients, this doubles the rate of iron accumulation. In addition to the transfusion-related iron overload, increased iron absorption also participates in β-thalassemia major, and its importance is inversely related to Hb levels [28, 29]. Iron overload is a consequence of multiple blood transfusions and an inappropriate increase in iron absorption due to the ineffective erythropoiesis. The long-term consequences of iron toxicity, including endocrine disorders, are preventable and mostly reversible by effective iron chelation therapy [1].

The results of blood transfusion also showed that the thyroid and adrenal cortex gland functions are correlated with the number of transfusions and subsequent iron overload state. Many studies showed that the strongest predictor for development of hypothyroidism is duration of transfusion therapy [22], which clarifies the thyroid dysfunction in most of the patients in the present study (77% of the thyroid disorder patients received the highest number of blood transfusion).
Correlation Between Hormones and Iron Parameters

The correlations between the measured hormones and iron status parameters may not be completely excluded because the number of patients was relatively small and all patients were taking drugs in the medical centers under the supervision of expert physicians. However, the results indicate that the most sensitive gland to the iron overload state is the thyroid gland. Furthermore, secondary hypothyroidism is uncommon in thalassemic patients [30] and the disorders are mostly due to iron deposition in the thyroid gland. A previous study [6] conducted in the same city (Najaf-Iraq) showed that growth hormone in thalassemic patients is significantly lower than that in healthy subjects, whereas in the present study, no significant difference was found between the two groups.

Correlation Between IR Parameters and Iron Status Parameters

The results in Table 4 are very interesting. Insulin showed a significant positive correlation with hepcidin. While a negative correlation with HOMA%S. Furthermore, ferritin and, subsequently, estimated iron body storage showed a significant positive correlation with insulin (r = 0.263, p = 0.042), insulin/glucose ratio (r = 0.265, p = 0.042), and HOMA%B (r = 0.266, p = 0.041). IR, impaired glucose tolerance test, and diabetes mellitus later in life are frequent complications in patients with hemoglobinopathy that require repeated blood transfusion states, including those with thalassemia [31]. In some studies, the strongest predictor for the development of diabetes was the duration of transfusion therapy, with every decade of transfusion exposure increasing the odds of developing diabetes by a 2.5 more times [32]. McClain et al. [33] have shown a high prevalence of abnormal glucose homeostasis in hemochromatosis patients, as well as impaired insulin secretion and IR [33]. The mechanisms for this resistance may be iron overload causing resistance by itself or related hepatic dysfunction [34]. Elevation of the level of iron and ferritin may cause iron toxicity in the liver and pancreas leading to insulin abnormalities and subsequently causes impaired glucose metabolism in thalassemic patients.

Forecasting Regression for Factors that Correlated with Hepcidin

From the overall correlations between hepcidin hormone and the measured parameters, forecasting models were constructed from multiple regression analyses using “Regression Forecasting Model” software bought from Business Spreadsheets, USA.

Forecasting of Serum Hepcidin

From the above regression equation (actual serum hepcidin = (predicted serum hepcidin -24.136)/0.3345), 33.27% of the change in serum hepcidin levels can be explained by the change in the 3 independent variables with standard error equal to ± 7.78 based on the result of regression equation at 95% confidence.

Multivariate analysis (F = 9.311) and critical Durbin-Watson statistic values (lower (Dl) =1.48; upper (Du) = 1.69) were obtained, indicating that a negative autocorrelation may be present at 95%. Therefore, the analysis is significant. After adjustment for sample size bias, serum hepcidin level was found to be correlated with TSH (r = 0.55, p < 0.001), insulin (r = 0.22, p = 0.205), and ferritin (r = 0.18, p = 0.493).

Forecasting of S.TSH

From the best fit equation (actual TSH = (predicted TSH–3.1404)/0.568), 30.56% of the change in TSH levels can be described by the change in the three independent parameters with standard error equal to ± 1.373 based on the result of regression equation at 95% confidence. After adjusting for sample size bias, TSH level was found to be correlated with hepcidin (r = 0.55, p < 0.001), whereas no correlation was found with insulin (r = 0.12, p = 0.961) and ferritin (r = 0.162, p = 0.569).

Forecasting equations for insulin and ferritin showed poor values (predicting values 9.22% and 11.12%, respectively), and thus, the equations are not cited here.

The authors concluded that the presence of some endocrine dysfunctions, including IR condition, in the present study may be a result of poor disease control in early life, in which irreversible tissue damage occurs because of iron overload. In addition to iron overload parameters, cortisol, HOMA%B, and T3 were correlated with the number of transfusion units. Hepcidin and TSH levels can be predicted significantly using the factors most correlated with hepcidin, namely, ferritin, insulin, and TSH.
References


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