Effect of Iron Overload on Function of Endocrine Glands in Egyptian Beta Thalassemia Patients

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ABSTRACT

Thalassemia is a hereditary disorder involving deficient synthesis of the hemoglobin polypeptide chains, its treatment depends on regular blood transfusions and chelation therapy. Repeated transfusions and poor compliance to therapy and chronicity of the disease lead to iron overload related complications including endocrine complications (insufficiency of the pituitary, gonads and less commonly thyroid gland). Early recognition of any defect in the activity of these glands is mandatory for the improvement of the quality of life of these patients. This study was performed to evaluate of thyroid and gonadal functions in transfusion-dependent beta-thalassemia patients. It was conducted on 54 patients with beta-thalassemia. In addition to 37 apparently healthy subjects matching age and gender served as a control group. According to age and gender each of patients and controls were divided into 4 subgroups. Complete blood count and serum iron, ferritin, thyroid function tests (TSH, FT3, and FT4), FSH, LH, estradiol, testosterone and progesterone hormones, were measured for all participants in the study. The study revealed that there were a significant elevation of serum iron and ferritin in patients compared to controls. The thyroid function tests showed no significant difference between patients and controls. Meanwhile serum levels of gonadotropins and sex hormones were significantly lower in patients as compared with controls. The results of this study reinforced the concept that iron overload in transfusion-dependent patients with β-thalassemia deserves careful attention, and regular endocrine screening during the first years of life in these patients is necessary for early detection and management of associated complications.

Key words: thalassemia, ferritin, endocrine glands.

Introduction

Thalassemia is hemoglobin (Hb) disorder typed as quantitative disorder that passed down through families in which the body makes an abnormal balance of hemoglobin, a disorder results in excessive destruction of red blood cells (RBCs) and anemia(Cunningham, 2008).

Beta thalassemia may be the most well-known type of thalassemia. It causes variable anemia that can range from moderate to severe, depending in part on the exact genetic change underlying the disease. Beta thalassemia can be classified into thalassemia minor, intermedia and major (Galanello and Origa, 2010).

Treatment of thalassemia major includes regular blood transfusions and chelation therapy, the combination of blood transfusion and chelation therapy has dramatically prolonged the life expectancy of these patients, thus transforming thalassaemia from a rapidly fatal disease of childhood to a chronic disease compatible with a prolonged life (Borgna-Pignatti et al., 2004; Khan, 2006).

However, frequent blood transfusions, iron overload, poor compliance to therapy and chronicity of the disease have in turn contributed to a whole spectrum of complications including cardiac problems, hypogonadism, hypothyroidism, hyperparathyroidism and other endocrine and metabolic problems (Satwani et al., 2005).

Since several endocrine glands may be affected in patients with beta thalassemia major - and because their life expectancy is now much longer - it is important that physicians be aware of the endocrine abnormalities that may develop. One important aspect of management in polytransfused thalassemic patients is early recognition and treatment of these endocrine dysfunctions (Abdulazahra et al., 2011; Pirinçcioğlu et al., 2011).

The aim of this study was to evaluate thyroid and gonadal functions in transfusion-dependent beta-thalassemia patients.

Materials and Methods

The study was conducted on 91 subjects; 54 patients (group I) with beta thalassemia, attending the out patients clinic, Pediatric Department, National Liver Institute, Menoufia University in the period from February...
to June 2012. Their diagnosis was based on marked reduction of RBCS count and Hb concentration, CBC picture (low MCV, MCH, and MCHC beside high reticulocytic count) and Hb electrophoresis (elevated levels of HbA2 and HbF with low level or absent HbA). They were on regular blood transfusion, and received suboptimal iron chelating therapy started many months after the onset of blood transfusion. They have serum ferritin values (>2000 ng/ml) which are much likely to be an indicator of iron overload state. In addition to 37 apparently healthy control subjects (groupII) matching age and gender of the patient group. Informed consent was obtained from all participants in this work or from their parents, all members were subjected for: complete history taking, clinical examination, laboratory investigations including: estimation of serum ferritin, thyroid function tests; thyroid stimulating hormone, free thyroxin and free triiodothyronine (TSH, FT4, and FT3), anterior pituitary hormones; luteinizing hormone and follicle stimulating hormone (LH and FSH), gonadal hormones; estradiol (E2), testosterone and progesterone using, chemiluminescent immunoassay based IMMULITE 1000 ANALYZER. Siemens, USA, serum iron using the Architect automated immunoanalyzer (Abbott Laboratories, Abbott Park, IL.) USA. And CBC using Sysmex KX-21, Japan.

Patients and controls were divided according to their ages into four subgroups:

- **Group IA**: included 19 thalassemic males with age ranged from 3.4 to 12 years.
- **Group IIA**: included 10 control males with age ranged from 4 to 12.4 years.
- **Group IB**: included 13 thalassemic males with age ranged from 14 to 18 years.
- **Group IIB**: included 8 control males with age ranged from 13.8 to 17.7 years.
- **Group IC**: included 10 thalassemic females with age ranged from 4 to 9.9 years.
- **Group IIC**: included 8 control females with age ranged from 3 to 10 years.
- **Group ID**: included 12 thalassemic females with age ranged from 12 to 18 years.
- **Group IID**: included 11 control females with age ranged from 12.5 to 18 years.

**Statistical analysis:**

Data was statistically analyzed using SPSS (statistical package for social science) program. All data are presented as mean ± standard deviation (SD), setting p < 0.05 for significance.

**Results:**

The mean age of thalassemic patients was 11.4±5.2 years, 32 (59.3%) were males, the mean age of controls was 12.5±4.7 years, 18 (48.6%) were males. They were age and gender matched. Hb concentration and RBCs counts decrease significantly in patient group compared to controls (p<0.05) while the mean serum levels of iron and ferritin increase significantly in patients as compared with controls (p<0.05). The thyroid functions tests (TSH, FT3 and FT4) showed no significant difference between patients and controls (p>0.05) Table (1).

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Group I (n=54)</th>
<th>Group II (n=37)</th>
<th>Test of significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.4 ±5.2</td>
<td>12.5 ±4.7</td>
<td>U-test 1.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>32/22</td>
<td>18/19</td>
<td>X² 0.99</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>7.29 ± 1.26</td>
<td>12.76 ± 1.34</td>
<td>t-test 19.89</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>RBCs (million/ul)</td>
<td>3.25 ± 0.92</td>
<td>4.93 ± 0.45</td>
<td>t-test 10.32</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Iron (ug/dl)</td>
<td>182.13 ± 95.03</td>
<td>65.46 ± 30.48</td>
<td>U-test 6.83</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>4485.91 ± 2820.85</td>
<td>60.86 ± 43.95</td>
<td>U-test 8.07</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Free T3(pg/ml)</td>
<td>3.61 ± 0.88</td>
<td>3.6 ± 1.02</td>
<td>t-test 0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Free T4 (ng/dl)</td>
<td>1.01 ± 0.12</td>
<td>1.07 ± 0.16</td>
<td>t-test 2.12</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>TSH (μIU/ml)</td>
<td>3.18 ± 1.93</td>
<td>3.39 ± 2.46</td>
<td>U-test 0.09</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

U-test= Mann Whitney test; P.value> 0.05 = non-significant; Hb= hemoglobin; RBCs= red blood cells count; Free T3= free triiodothyronine; Free T4=free thyroxin and TSH= thyroid stimulating hormone
Regarding male groups, there was a significant elevation of the mean serum levels of iron and ferritin and a significant decrease in the mean serum level of FSH (p value <0.05) in group IA compared to group IIA. Meanwhile, no significant difference was detected between the two groups regarding LH (Table 2).

Table 2: Comparison between group IA and group IIA regarding some studies parameters.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Group IA (n=19)</th>
<th>Group IIA (n=10)</th>
<th>Test of significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.43 ± 3.21</td>
<td>9.28 ± 2.19</td>
<td>U-test 1.45</td>
<td>0.15</td>
</tr>
<tr>
<td>Iron (μg/dl)</td>
<td>161.53± 72.65</td>
<td>50.8 ± 28.11</td>
<td>U-test 3.44</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>3896.68±2203</td>
<td>73.9±50.77</td>
<td>U-test 4.36</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LH (μIU /ml )</td>
<td>0.13 ± 0.1</td>
<td>0.38 ± 0.49</td>
<td>U-test 0.46</td>
<td>0.65</td>
</tr>
<tr>
<td>FSH (μIU /ml )</td>
<td>0.45 ± 0.24</td>
<td>1.55 ± 1.11</td>
<td>U-test 3.19</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

U-test= Mann Whitney test; LH=luteinizing hormone and FSH=follicle stimulating hormone

Also, there was a significant elevation of the mean serum levels of iron and ferritin and a significant decrease in the mean serum level of LH, FSH and testosterone (p value <0.05) in group IB compared to group IIB Table (3).

Table 3: Comparison between group IB and group IIB regarding some studies parameters.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Group IB (n=13)</th>
<th>Group IIB (n=8)</th>
<th>Test of significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.66 ± 1.93</td>
<td>16.3 ± 1.41</td>
<td>t-test 0.81</td>
<td>0.43</td>
</tr>
<tr>
<td>Iron (μg/dl)</td>
<td>192.15 ± 40.52</td>
<td>58.5 ± 18.29</td>
<td>t-test 8.73</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>5398.54± 3873.85</td>
<td>65.2 ± 48.88</td>
<td>U-test 3.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LH (μIU /ml )</td>
<td>1.13 ± 1.28</td>
<td>5.39 ± 2.15</td>
<td>U-test 5.72</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FSH (μIU /ml )</td>
<td>1.24 ± 1.04</td>
<td>5.61 ± 5.86</td>
<td>U-test 2.66</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>66 ± 123.66</td>
<td>331.98 ± 173.76</td>
<td>U-test 4.11</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

U-test= Mann Whitney test; LH=luteinizing hormone and FSH=follicle stimulating hormone

Regarding female groups, the mean serum level of LH showed a significant decrease in group IC as compared with group IIC, however the mean serum level of FSH showed no significant difference between the two groups (Table 4). Furthermore, there were significant decreases in the mean serum levels of LH, FSH, progesterone and estradiol hormones (E2) in group ID compared to group IID (Table 5). The age of all patients showed a significant positive correlation with ferritin, and a significant negative correlation with FT4. Also, a significant negative correlation was noticed between ferritin and each of free T4 and FSH (data were not shown in the tables).

Table 4: Comparison between group IC and group IIC regarding some studies parameters.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Group IC (n=19)</th>
<th>Group IIC (n=10)</th>
<th>Test of significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.0 ± 2.11</td>
<td>7.16 ± 2.82</td>
<td>U-test 0.40</td>
<td>0.69</td>
</tr>
<tr>
<td>Iron (μg/dl)</td>
<td>167.7 ± 88.42</td>
<td>77 ± 36.64</td>
<td>U-test 2.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>3626.8 ± 1509.8</td>
<td>78.86 ± 43.85</td>
<td>U-test 3.55</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LH (μIU /ml )</td>
<td>0.11 ± 0.09</td>
<td>0.15 ± 0.06</td>
<td>U-test 2.41</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FSH (μIU /ml )</td>
<td>1.54±0.94</td>
<td>2.33 ± 2.09</td>
<td>U-test 0.62</td>
<td>0.53</td>
</tr>
</tbody>
</table>

U-test= Mann Whitney test; LH=luteinizing hormone and FSH=follicle stimulating hormone
Table 5: Comparison between group ID and group IID regarding some studies parameters.

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Group ID (n=12)</th>
<th>Group IID (n=11)</th>
<th>Test of significance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16.6 ± 2.65</td>
<td>16.52 ± 2.34</td>
<td>t-test 0.08</td>
<td>0.94</td>
</tr>
<tr>
<td>Iron (μg/dl)</td>
<td>215.92 ± 156.2</td>
<td>75.45 ± 31.45</td>
<td>U-test 3.51</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>5155.83 ± 3071.72</td>
<td>32.77 ± 17.45</td>
<td>U-test 4.06</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LH (μIU /ml)</td>
<td>1.23±0.98</td>
<td>9.93±7.21</td>
<td>U-test 3.57</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>FSH (μIU /ml)</td>
<td>2.56±1.96</td>
<td>4.95±3.57</td>
<td>U-test 2.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>2.21±4.41</td>
<td>4.09±4.67</td>
<td>U-test 2.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>22.91±17.41</td>
<td>108.17±107.45</td>
<td>U-test 2.86</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

U-test= Mann Whitney test; LH=luteinizing hormone; FSH=follicle stimulating hormone and E2 = estradiol

**Discussion:**

Thalassemia patients are dependent on blood transfusions to maintain the levels of hemoglobin and packed cell volume in their blood. Transfusion and iron-chelation therapy have prolonged and improved the quality of life in these patients (Borgna-Pignatti et al., 2004). Such a treatment, however, leads to chronic iron overload affecting the endocrine glands (Abdulzahra et al., 2011).

In the current study a significant decrease was observed in Hb concentration and RBCs count and a significant increase in the mean serum levels of iron and ferritin in patients as compared with control. These findings are in agreement with Charles and Linker, (2005); Irshaid and Mansi, (2009) where they reported that Hb concentration values in thalassemia patients are significantly lower than controls.

Similarly, Abdulzahra et al., (2011); Patil and Mujawar, (2010); Vahidi, et al., (2003) stated that iron indices were markedly increased in thalassemia patients, and the mean serum level of ferritin was many times higher than controls.

Thyroid dysfunction is known to occur frequently in thalassemia major, but its prevalence and severity varies in different cohorts and the long-term natural history is poorly understood. Thyroid dysfunction is very important, because hypothyroidism could be associated with growth problems so commonly seen in these patients (Abdulzahra et al., 2011; Pirinççioğlu et al., 2011).

In the current study, thyroid function tests (free T4, free T3 and TSH) showed no significant difference between thalassemic patients and controls.

These results agreed with findings reported from previous studies (Abdulzahra et al., 2011;Irshaid and Mansi, 2009; Ong et al., 2008).

On the contrary, hypothyroidism was detected in different ratios in other studies done by Kurtoglu et al., (2012); De Sanctis et al., (2006) as they found hypothyroidism in 12.8%. 2.1% and 11% of their patients respectively.

The diversity of these results could be explained by that the thyroid dysfunction in thalassemic patients is depending on many factors such as the age of studied population, the duration of receiving blood transfusions, the amount of iron overload, the dosage of iron-chelating agent, and the procedure used for evaluation (Aruratanasirikul et al., 2007).

The present study detected a significant positive correlation between age and ferritin and a significant negative correlation between ferritin and free T4. So, thyroid function tests might be affected with progress of age of those patients and increased their serum ferritin level.

These findings are in line with Hashemizadeh and Norri, (2012); AL-Hader et al., (1993) where they found that impaired thyroid function was associated with iron overload (expressed by ferritin). Also, Irshaid and Mansi, (2009) reported that it is anticipated that the rate of thyroid dysfunctions increases steadily with advancing age.

Controversially, Abdulzahra et al., (2011); Zervas et al., (2002) stated that no statistically significant correlation was found between serum ferritin levels and thyroid functions.

Pituitary and gonadal dysfunctions; most frequently hypogonadism, which lead to growth retardation are common problems in beta-thalassemia major patients. They are caused by iron overload (Al-Rimawi et al., 2005;Papadimas et al., 1996). The current study detected a significant elevation of the mean serum levels of each of iron and ferritin and a significant decrease in the mean serum level of FSH (p value <0.005) in group IA compared to group IIA. Meanwhile, no significant difference was detected between the two groups regarding LH.
Similarly, Dundar et al., (2007) found that the serum level of FSH in male thalassemia patients was significantly lower compared to controls. Also, Masala et al., (1984) noticed that no significant difference was found in the mean serum levels of LH.

In this work the mean serum levels of LH, FSH and testosterone were significantly lower in GIB compared to GHB.

In agreement with these results, Anoussakis et al., (2008); Dundar et al., (2007); Vahidi et al., (2003) reported that patients with beta-thalassemia had lower serum LH, FSH and testosterone hormones levels than the controls.

The mean serum levels of LH was significantly lower in GIC than GIIC (p<0.05). Meanwhile, the mean serum level of FSH showed no significant difference between the two studied groups. Results which were in accordance with the results of a study done by Dundar et al., (2007) on 25 patients with beta thalassemia major as they found that the mean serum levels of LH was significantly lower in patients than controls.

In the current study, female thalassemia patients in GID had a significant decrease in the mean serum levels of LH, FSH, progesterone, and E2 as compared with controls in GIDH.

These findings matched results of a study done by Liavdas et al., (2008) as they found that the basal LH values were lower in the thalassaemic patients than in the controls and did not increase significantly after Luteinizing hormone releasing hormone (LHRH) administration. The FSH values were also lower in the thalassaemic group compared to the controls, and increased only slightly after LHRH administration, and explained these finding by presence of pituitary deficiency; mostly on gonadotrophs.

In a study done by Mula-Abed et al., (2008) hypogonadotropic hypogonadism was detected in 15 (50.0%) of thalassemic patients (7 female and 8 male) with significant low levels of FSH and low LH with low E2 (in females) or testosterone (in males).

The aforementioned results demonstrated that there was a significant alteration in the activity of gonadotrophs in the anterior pituitary gland (LH and FSH), which takes place early in life and increases affecting the function of the gonads at puberty, the main cause may be iron overload. Many previous researches have shown a significant difference in serum ferritin levels between thalassemia patients with endocrine complications and those without endocrinopathies (Abdulzahra et al., 2011). Also, Shamshirsaz et al., (2003) reported that there was a significant difference between mean serum ferritin in thalassemic patients with endocrine complications and thalassemic patients without endocrinopathies.

These disorders have been proven to be the result of hemosiderosis of secretory cells such as the gonadotroph cells of the pituitary gland (Abdelrazik and Ghanem, 2007; Shamshirsaz et al., 2003).

Many researchers have suggested that higher serum ferritin values (>2000 ng/ml) are likely to be an indicator of iron overload state, as has been shown in different disorders (Abdelrazik, 2007; Ikram et al., 2004) which is the case in the patients enrolled in this study (mean serum ferritin was 4485.9±2820.9 ng/ml). However, in most clinical laboratories serum ferritin protein levels >400 ng/ml might define iron overload but, in fact, such interpretation requires confirmation by the finding of a high percentage of saturation with iron of the iron-binding capacity (transferrin) (Herbert et al., 1997).

The anterior pituitary is particularly sensitive to the iron overload (Skordis, 2011). And in thalassemia, iron overload can affect both pituitary and gonadal function equally and simultaneously or either gland can be affected predominantly and before the other (De Sanctis et al., 1988).

Other investigators demonstrated through (magnetic resonance imaging) pituitary gland atrophy in β-thalassemic patients with hemochromatosis (Soliman et al., 2000) and the signal intensity reduction in the anterior lobe of the pituitary gland correlated with serum ferritin level and the severity of pituitary dysfunction (Sparacìa et al., 2000). Furthermore, even a modest amount of iron deposition within the gland could interfere with its function (De Sanctis, 2002).

Patients with transfusional iron overload began to develop pituitary iron overload in the first decade of life; however, significant iron deposition were observed beginning in the second decade. Heavy pituitary iron deposition were predictive of hypogonadotropic hypogonadism in these patients (Noetzli et al., 2011) this explains our results where the occurrence of pituitary and gonadal dysfunction is more in older age patients than younger age patients. Also, in this study the serum levels of testosterone, progesterone and E2 were significantly lower among thalassemic patients than controls (p<0.05) indicating pituitary-gonadal dysfunction.

The present study found a significant negative correlation between serum ferritin levels and FSH in accordance with Papadimas et al., (1996). These results confirmed the effect of iron overload on the activity of the pituitary secretion of FSH.

REFERENCES


