Study of the effect of iron overload on the function of endocrine glands in male thalassemia patients

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Abstract:

Background: Iron overload is an important issue in the state of thalassemic patients due to the harmful effect of high concentration of iron deposited in different tissues in human body including endocrine glands. In the present work, an attempt is carried out to estimate the effect of iron overload in thalassemic patients on the function of endocrine glands through the estimation of their ability to secrete adequate amounts of certain hormones. **Materials and Methods:** Seventy eight male children with beta-thalassemia, in the age-group of 4–11 years, were enrolled for this research. These children were being treated with frequent transfusions and long-term iron chelation therapy. Thirty age and sex matched children without thalassemia constituted the control group. Ferritin and different hormones were estimated by ELISA technique. **Results:** The results showed a mild reduction in the function of endocrine glands through the decrease in the level of some hormones. These changes due mainly to the hypoxia and precipitation of iron in certain glands and overlapping with the synthesis or secretion of the hormones. **Conclusion:** There is a different hormonal disturbances in beta thalassemia patients. Reduction of total body iron store is an important goal of the treatment of thalassemia and measuring the hormones concentration is necessary for the follow up of the thalassemic patients especially during puberty.

Key words:

Glands, hormones, iron overload, thalassemia

Introduction

Thalassemia is a serious problem in Iraq due mainly to the nonavailability of equipment and drugs during different periods of turmoil and war. Of 1064 couples recruited from the Public Health Laboratory in Basra, southern Iraq, about 5% had the beta-thalassemia trait, and carriers of major beta-globin disorders comprised 11.48% . This indicates a real public health problem and calls for an effective management plan, including public health education programs, to facilitate early diagnosis and treatment.[1] In the Najaf governorate (with a population of about 1.2 million people in 2009), till the samples collection time (September 2009), there are records of 331 patients receiving treatment in the Thalassemia Unit of Al-Zahra'a Teaching Hospital for Obstetrics and Pediatrics. Thalassemia has been studied in different areas of Iraq by different researchers.[1–5] The biochemical parameters of Iraqi thalassemia patients have been studied in a previous research carried out by our group.[6] However, the changes in the endocrine system of these patients have not yet been studied. These patients are dependent on blood transfusions to maintain the levels of hemoglobin and packed cell volume in their blood, but siderosis is a major complication of treatment. Repeated transfusions lead to accumulation of iron in different tissues, including the tissues of the endocrine glands. Different endocrine problems seen in thalassemia

major are considered to be the result of iron deposition in the endocrine glands. These disorders have been proven to be the result of hemosiderosis of secretory cells such as the gonadotroph cells of the pituitary gland.[7] 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 described.

Despite therapy with deferoxamine to treat iron overload, the risk of secondary endocrine dysfunction remains high. Hypogonadism was one of the most frequent endocrine complications seen in one study.[8] Impaired glucose tolerance, short stature, hypocalcemia, and subclinical and overt hypothyroidism are also frequent.[9]

The aim of the present study was to identify the prevalence of endocrine disturbances in Iraqi thalassemia patients who received repeated blood transfusions as part of their treatment regimn. The hormones estimated in this study included cortisol; growth hormone (GH); T3, T4, and thyroid stimulating hormone (TSH); testosterone; prolactin (PRL); luteinizing hormone (LH); and follicle stimulating hormone (FSH). A second objective of this study was to examine the correlation between hormone levels and the parameters of iron status, including serum iron, ferritin, total iron binding capacity (TIBC), hemoglobin (Hb), and transferrin iron saturation percentage (TISP).

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Materials and Methods

Subjects

During the period from January 2009 till September 2009, 78 male children with thalassemia, in the age-group of 4–11 years, were enrolled for this research from the Al-Zahra'a Teaching Hospital for Obstetrics and Pediatrics. These children were all homozygous for beta-thalassemia and were being treated with frequent transfusions and long-term iron chelation therapy. Thirty age and sex matched children without thalassemia constituted the control group.

Biochemical measurements

Blood was aspirated from the subjects in the morning (9–11 AM) and separated into two aliquots, with one sample being stored in EDTA tubes for hematological estimations and the other in plain tubes for estimation of the other parameters in sera.

Hemoglobin electrophoresis of all patients showed decreased hemoglobin A (Hb A) and increased hemoglobin F (Hb F), with variable level of hemoglobin A_2 (Hb A_2).

Assays
Iron status

Hemoglobin level was estimated colorimetrically according to Drabkin's method, using the Spinreact® kit (Spain). Serum iron was estimated by the colorimetric ferrozine method using the Biomaghreb® kit (Morocco). TIBC was estimated colorimetrically using the Randox® kit (UK). The method is as follows: An excess of iron is added to the serum iron to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation, the iron in the supernatant is determined. The ferritin quantitative test (BioCheck®, USA) is based on a solid-phase enzyme-linked immunosorbent assay (ELISA). TISP was calculated by dividing the serum iron concentration by the TIBC.[10] The transferrin concentration was calculated from the serum iron and the transferrin saturation percentage, using the following formula: Serum iron (µmol/l)/transferrin (g/l) × 3.98. This formula is based on the maximal binding of 2 mol Fe^{3+} per mol of transferrin and a molecular weight of 79570 for transferrin.[11]

Hormonal assay

All hormones were estimated in serum by the ELISA technique using ready-for-use kits supplied by Monobind Inc., USA. The procedure of the estimation was according to the manufacturer's instructions (mentioned in each kit's leaflet).

Statistical analysis

The results were expressed as mean ± standard deviation. The pooled *t*-test was used for examining the significance of differences between the healthy and control groups. The correlation coefficient (r) was calculated to examine the correlation between the parameters.

Results

The results of iron status determination in the two groups are shown in Table 1. As expected, there is significant difference between thalassemia patients and healthy subjects in all parameters of iron status.

The results of the hormone assays in the two groups are shown in Table 2. The number (and percentage) of patients with lower and higher levels of serum hormones than the cutoff values are also mentioned. The results show that there is a significant decrease ($P < 0.05$) in the serum levels of cortisol, GH, and LH. However, no significant difference ($P > 0.05$) is noticed in the rest of the measured hormones. We found that in patients with thalassemia low levels of hormones were more common than raised levels. The most pronounced decrease was noticed in LH and GH, low serum levels being seen in 33 (42.3%) and 28 (35.9%) of the thalassemia patients, respectively.

There was correlation between serum ferritin and GH (r = –0.58), serum iron and GH (r = –0.62), TIBC and cortisol (r = 0.47), serum ferritin and LH (r = –0.48), serum iron and LH (r = –0.53), and serum iron and FSH (r = –0.52). The rest of the correlation coefficient values were not significant and are not cited here.

Discussion

Some hematological and biochemical characteristics of our thalassemia patients, along with that of healthy controls, are listed in Table 1. Iron indices, with the exception of TIBC and transferrin protein concentration, were markedly increased in thalassemia patients, and the mean concentration of serum ferritin was more than eight times higher than normal [Table 1]. In states of iron overload or excess, the iron composition of ferritin increases and this may be the most important cause for the elevation of serum ferritin. A high serum ferritin accompanied by a high percentage of saturation of a normal serum transferrin usually indicates iron overload;[12] this was clearly apparant clinically and biochemically in our patients. Serum ferritin protein levels >400 ng/ml define iron overload in most clinical laboratories but, in fact, such interpretation requires confirmation by the finding of a high percentage of saturation with iron of the iron-binding capacity (transferrin).[13] Other researches have suggested that higher serum ferritin values (>2000 mg/dl) are much more likely to be an indicator of iron overload state, as has been shown in different disorders, including in thalassemia patients with hemochromatosis.[14] Hence, the Iraqi patients in this study are less likely to have themochromatosis as a syndrome but instead they suffer from iron overload [Table 1] and subsequent mild iron deposition n in tissues as shown previously.[15] Many previous researches[16] have shown a significant difference ($P = 0.01$) in the mean serum ferritin levels between thalassemia patients with endocrine complications and thalassemia patients without endocrinopathies. This indicates the harmful effect of increased iron storage and deposition on the endocrine glands.

Table 1: The parameters of iron status in thalassemia patients as compared with healthy control

Biochemical parameter	Thalassemia (M ± SD)	Control (M ± SD)	Significance
Serum ferritin (µg/l)	773 ± 111	87 ± 28	Significant
Hemoglobin (g/l)	77 ± 19	128 ± 24	Significant
Serum iron (µmol/l)	48.8 ± 14.7	21.1 ± 6.1	Significant
Serum TIBC (µmol/l)	48.4 ± 9.3	54.8 ± 7.8	Significant
TISP (%)	91.8 ± 21.2	30.4 ± 11.1	Significant
Transferrin concentration (mg/l)	129 ± 23	148 ± 37	Significant

TIBC = Total iron binding capacity; TISP = Transferrin iron saturation percentage

Table 2: Serum level of some hormones in the thalassemia and control groups

Hormone	Control	Thalassemia	Significance	No. of patient (low conc in %)*	No. of patients (high conc. in %)**
Cortisol (µg/dl)	12.7 ± 6.11	8.83 ± 3.17	S	22 (28.2°6)	6 (7.7)
FSH (mIU/ml)	3.12 ± 2.68	4.36 ± 4.03	NS	15 (19.2)	2 (2.6)
GH (ng/ml)	2.48 ± 1.79	0.85 ± 0.66	S	28 (35.9)	1 (1.3)
LH (mIU/ml)	3.61 ± 1.44	1.50 ± 2.35	S	33 (42.3)	0 (0)
Prolactin (ng/ml)	6.66 ± 3.13	5.83 ± 3.42	NS	8 (10.3)	1 (1.3)
Testosterone (ng/ml)	3.3 ± 1.7	3.8 ± 2.9	NS	4 (5.1)	0 (0)
T3 (ng/ml)	0.84 ± 0.44	0.96 ± 0.37	NS	6 (7.7)	0 (0)
T4 (µg/dl)	8.23 ± 3.36	11.3 ± 2.92	NS	7 (9.0)	0 (0)
TSH (µIU/ml)	3.62 ± 2.34	3.47 ± 1.68	NS	2 (2.6)	2 (2.6)

*S = Significant ($P < .05$); **NS = Nonsignificant ($P > 0.05$); FSH = Follicle stimulating hormone; GH = Growth hormone; LH = Luteinizing hormone; TSH = Thyroid stimulating hormone

In transfusion-dependent thalassemia major, increased iron deposition in the pituitary gland has a cytotoxic effect, leading mainly to hypogonadotropic hypogonadism. Early detection and quantification of iron in the pituitary gland are of particular importance for successful treatment. Thalassemia leads to variable pituitary iron overload and, thus, hypophyseal damage. This endocrine disturbance is becoming less frequent nowadays with early and intensive chelation therapy.[17] The prevalence of various endocrine complications shows a difference between centers, particularly for GH deficiency, hypoparathyroidism, and hypothyroidism.[18] In the present study, the prevalence of GH deficiency in thalassemia patients was 35.9% as compared with a prevalence of 7.9% in males in a previous study[18] and up to 25% in another study.[19]

The low level of GH in thalassemia patients as compared to healthy subjects [Table 2] is of importance. Most of the thalassemia patients were significantly shorter than the healthy subjects in our study, indicating the need for vigilant monitoring of patients with thalassemia in order to treat endocrine dysfunction at an appropriate age.[20]

The prevalence of endocrine complications shows a difference between centers, particularly for GH deficiency.[18] Reduced GH secretion in thalassemia patients are related to a neurosecretory dysfunction as a result of iron overload rather than to liver damage.[21]

Serum LH showed a significant difference ($P < 0.05$) between the two groups, with 42% of patients having levels lower than the cutoff value [Table 2]. This result indicates the significant alteration in the activity of gonadotrophs in the anterior pituitary gland where secretion of LH hormone takes place, affecting the normal growth and function of the gonads at puberty. Similar results were obtained in another work,[22] where the basal LH values were lower in thalassemia patients than in controls and did not increase significantly even after luteinizing hormone–releasing hormone (LHRH) administration. Hypogonadism is one of the most frequent endocrine complications in thalassemia patients.[8] Despite recent therapeutic advances in the management of beta-thalassemia major, the risk of secondary endocrine dysfunction remains high.

Although 19.2% of the thalassemia patients in our study have FSH value lower than the cutoff value [Table 2], the difference between the two groups is not significant ($P > 0.05$). This result agrees with many other studies[23,24] that have also shown no significant difference in FSH levels between thalassemia patients and normal subjects. However, there are some studies[22] that have found a significant decrease in FSH and LH values in the thalassemia group as compared to a control group.[25]

In a study carried out in one of Iraq's neighboring countries, Iran, the prevalence of hypogonadotropic hypogonadism in thalassemia patients was 76.2%; the patients in this study were short and had growth retardation. Compared with normal controls, patients with homozygous beta-thalassemia major had lower serum LH, FSH, and testosterone levels.[26] The reason for this different results from our findings is that many thalassemia major patients in the Iranian study had very high serum ferritin levels (3503 ± 201 ng/ml), much higher than the mean seen in our Iraqi sample (773 ± 111 ng/ml). These differences may be due to differences in the type and location of mutation defects, race differences, and sampling variations. Iraqi thalassemia patients differ from those in surrounding countries,[2] especially with regard to transferrin saturation and ferritin level.[27] Iron-overloaded thalassemia patients with severe organ damage are likely to have irreversible damage to their hypothalamo-pituitary axis, while those with less severe iron overload are likely to have potentially reversible hypogonadotropic hypogonadism.[28] Most of the previous studies have concluded that there is a risk of failure of puberty in thalassemia patients, which calls for newer protocols of treatment, correct blood transfusion, and proper chelation therapy.[7]

Serum levels of testosterone in our study [Table 2] were lower in patients than in controls but the difference was not statistically significant, which agrees with findings from previous studies.[25,29] In one earlier study[30] all patients had normal basal serum cortisol, T4, and prolactin (PRL) concentrations, which was also noticed in our work. This appears to be a common finding in thalassemia with iron overload.

In our study, the basal PRL values did not differ significantly between the two groups, even though 10% of the patients had low PRL levels. These results are in accordance with other previous researches.[22] No statistically significant differences were found in FSH, LH, PRL, GH, and cortisol secretion between patients and normal subjects.[23]

The results of serum T3, T4, and TSH assay showed no significant difference between healthy subjects and thalassemia patients [Table 2]. Only a small percentage of thalassemia patients have serum levels lower than that seen in healthy controls. Although the patients were euthyroid, some showed disturbances in serum

T3 or T4. These results are in accordance with other works.[31,32] Table 2 shows that only about 8% of patients have low serum T3 or T4 levels. Studies from other countries have reported various rates of thyroid impairment, e.g., 19.4%, 4%, and 3.2% .[18,33,34] Al-Hader *et al.*[35] have stated that impaired thyroid function is present in a considerable proportion of transfusion-dependent beta-thalassemia patients with associated iron overload. However, no statistically significant correlation was found between serum ferritin levels and thyroid function in our study, though some previous studies have reported otherwise.[23,34]

The correlation coefficient values, in general, show low correlation between hormone levels and iron indices (with a few exceptions).

This indicates that the effect of iron overload is dependent upon the duration of the disease and the frequency of blood transfusion and that the disease progression is slow.[36] The age of the patients in the present study is relatively low and, therefore, the number of blood transfusions is also low. We had no records of the number of blood units transfused for each patient. However, the results of many researches[19,36,37] showed that definite disturbances in serum hormone levels occur in thalassemia patients at the time of puberty due to changes in the iron indices.

Conclusion

It appears that there is no major impairment of endocrine function in the basal state in Iraqi thalassemia patients aged 4–12 years with moderate to severe degrees of iron loading. This study is limited by its relatively small sample size ($n = 78$).

The monitoring of iron status is of importance to prevent possible damage to vital organs. The functional fluctuations found in some hormone levels can be explained as a consequence of coexisting hemosiderosis. Since several endocrine glands may be affected in patients with 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.

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