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Urinary Iodine in Egyptian Patients with Thalassemia Major: A Focus on its Potential Contributing Impact on Consequent Hypothyroidism

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Abstract

Background: Primary hypothyroidism is one of the most frequent endocrine complications observed in patients suffering from thalassemia where iodine deficiency is a major contributing factor. We aim at assessing the iodine status in multitransfused pediatrics with thalassemia major, compliant to iron chelation, to evaluate its contributing role to the occurring hypothyroidism for the potential future replacement therapy.

Procedure: Sixty young thalassemia patients (31 males, 29 females; aged 14.4 ± 3.83 years) were randomly selected from the hematology clinic of the Children's Hospital, Cairo University, added to 36 age and sex matched control subjects.

Results: The study revealed a highly significant difference in urinary iodine, FT3, FT4 and TSH between the thalassemic group and their controls (P<0.001). Twenty seven patients (45%) had overt hypothyroidism (low T4 and elevated TSH >10 uIU/ml), and 34/36 had normal urinary iodine level. Severe iodine deficiency was manifested in 9/60 patients (15%), moderate deficiency in 27/60 patients (45%) and mild deficiency in 24/60 patients (40%). A negative correlation was found between urinary iodine and both serum ferritin and TSH (r=-0.413 at P<0.001, r=-0.881 at P<0.001; respectively).

Conclusion: To this end, iodine deficiency is at least partially responsible for the high prevalence of thalassemia–induced hypothyroidism among Egyptian young patients.

Keywords: Thalassemia; Hypothyroidism; Iodine deficiency

Introduction

Thalassemia disorders are a heterogeneous group of inherited anemias characterized by defects in the synthesis of one or more globin chain subunits of the hemoglobin [1,2]. Beta-thalassemia Major is a common hereditary hemoglobinopathy which is a direct cause of microcytichypochromic anemia and extramedullary ineffective erythropoiesis [2,3]. The mainstay of treatment is blood transfusion to maintain adequate levels of hemoglobin. However, multiple blood transfusions can lead to iron overload resulting in endocrine dysfunction [3-5]. Iron deposits saturate transferrin in the reticuloendothelial system, then enters the parenchyma, causing important oxidative damage, mostly to the heart, thyroid, gonad, pituitary, liver and endocrine glands [6]. Endocrinal complications have become one of the most common complications in Beta-thalassemia Major patients after the dramatic increase in their life span.

One of the resulting endocrinopathy is hypothyroidism which includes primary hypothyroidism, subclinical hypothyroidism as well as secondary hypothyroidism. Subclinical hypothyroidism is characterized by modest elevation of TSH level and free T4 level remains in normal range. Primary hypothyroidism is characterized by an elevated TSH level and decreased (low) T4. Secondary or central hypothyroidism is characterized by decreased T4 and low TSH [7-11].

Hypothyroidism may be either central, due to deposition in the pituitary or the hypothalamus and is usually associated with other endocrinopathies, or primary by deposition in the thyroid gland or even other organs [12].

For severely affected patients, gradual replacement with L-thyroxine is recommended. In mild hypothyroidism the decision to treat depends on each individual case. Preclinical hypothyroidism requires only careful follow up [13].

Iodine is as a constituent of the hormones thyroxin and triiodothyronine, both are secreted by the thyroid gland and affect growth, development, and the metabolic rate of the body [14].

We hypothesize that the resulting hypothyroidism in thalassemia patients in Egypt, may be, at least in part, due to a common nutritional defect, namely iodine deficiency, which must be excluded before treating the diagnosed cases with L-thyroxine. Correction of iodine deficiency, if present, would be more physiological and avoids the administration of L-thyroxine and its required dose adjustment in an

anemic patient with a hyperdynamic circulation and may be cardiac complications.

The aim of the present study was to assess the levels of thyroid hormones and urinary iodine in children with thalassemia major to detect cases of hypothyroidism and moreover, to investigate the contributing effect of iodine deficiency in hypothyroidism associated with thalassemia.

Patients and Methods

Sixty young thalassemia patients (31 males, 29 females; with a mean age of 14.4 ± 3.83 years), followed up at the hematology clinic of the New Children Hospital, Cairo University, were randomly selected to participate in this study together with thirty six age and sex matched control subjects.

All patients were subjected to detailed history taking and thorough clinical examination with special emphasis on growth parameters according to the Egyptian Growth curves and signs of hypothyroidism, nutritional deficiency and puberty according to Marshall and Tanner [15] classification. Patients were considered compliant when serum ferritin was below 1500 ng/dl.

Diagnosis of thyroid dysfunction was based on the following criteria; Primary hypothyroidism is diagnosed when FT4 is <12 pmol/L), and TSH is >5 mIU/ml. Subclinical hypothyroidism is diagnosed when T4 is normal, and TSH is >5 mIU/ml. Central hypothyroidism is diagnosed when FT4 is <12 pmol/l and TSH is low or normal.

Laboratory assessments

The recruited patients were examined for overt and subclinical hypothyroidism by measuring TSH (thyroid stimulating hormone), T3 (triiodothyronine) and T4 (thyroxin) which were measured using commercial kit from Diagnostic products Corporation (DPC), Los Angeles, CA 90045-5597, USA.

Patients are classified according to their serum levels of FT4 and TSH. Overt hypothyroidism is diagnosed when FT4 is low and increased TSH levels >10 micro U/ml), while compensated hypothyroidism includes normal FT4 and TSH range from 5-10 micro U/ml, and abnormal TRH test. Moreover, patients with subclinical hypothyroidism are diagnosed when FT4 is normal, basal TSH 0-5 micro U/ml, abnormal TRH test. However, subclinical hypothyroidism was not confirmed by TRH test in this study.

The estimation of FT4 and FT3 were done using Coat-A-Count which is a solid phase radioimmunoassay, where in I-125 labeled FT4 and FT3 analogue competes for a fixed time with free T4 and FT3 in the patient sample for sites on T4 and T3 specific antibody. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody bound fraction of the radio labeled free T4 and T3.Counting the tubes in a gamma counter. Sample levels are measured from the calibration curve.

Coat-A-Count TSH IRMA is a solid-phase immunometric assay based on monoclonal and polyclonal anti-TSH antibodies: one ri25 labeled anti TSH polyclonal in liquid phase, and monoclonal anti-TSH antibodies immobilized to the wall of a polystyrene tube. In the procedure, TSH is captured between monoclonal anti-TSH antibodies immobilized on the inside surface of the polystyrene tube and the radio labeled polyclonal anti-TSH tracer. The TSH concentration is directly proportional to the radioactivity present in the tube after the wash step. The radioactivity is counted using a gamma counter, after which the concentration of TSH in the patient sample is obtained by comparing the patient counts per minute with those obtained for the set of calibrators provided. Serum ferritin was measured by immunoradiometric assay.

Urinary Iodine test: According to ICCIDD, WHO, UNICEF, 2001 [16]. The urinary iodine concentration is the most useful test for assessing iodine nutrition in populations. Samples are easy to obtain and over 90% of dietary iodine eventually appears in the urine. About 1-2 ml urine were collected, placed in tube and stopper tightly. Each tube was heated, containing 0.15 ml of urine, and 1.0 ml ammonium persulfate solution, for one hour in the block at 100°C, after cooling at room temperature, 0.5 ml arsenious acid solution was added to each tube, and mixed on a vortex. Urinary iodine deficiency was classified according to urinary iodine level as follows: severely deficient when Median Urinary Iodine Concentration (mcg/L) was below 20, moderate from 20-49, mildly deficient when 50-99, optimal when 100-199, more than adequate 200-299 and possibly excessive when higher than 299 (mcg/L) .

Statistical methods

Data were statistically described in terms of mean ± standard deviation of the mean (± SDM) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using Student *unpaired t* test. A probability value (p value) less than 0.05 was considered statistically significant. Correlation between various variables was done using Pearson moment correlation equation. The statistics was performed using SPSS program, version 12.

Results

In the current study 60 thalassemia patients were enrolled, including 31 males, 29 females; with a mean age of 14.4 ± 3.83 years. Their stage of hypothyroidism was classified according to their level of FT4 and TSH. As illustrated in Figure 1, 27/60 (45%) patients had overt hypothyroidism (both low free T4 and elevated TSH >10 uIU/ml), 22/60 (36%) had compensated hypothyroidism (normal FT4, TSH 5-10 micro U/ml). The rest constituted 11 patients with euthyroid functions. Thirty six age and sex matched control subjects were also enrolled in investigation and served as comparative group.

The diagnostic features of thalassemia patients were shown in Table 1 as follows; FT3 was low in 49/60 patients (81.7%), FT4 was low in 41/60 patients (68.3%) and TSH was elevated in 49/60 patients (81.7%). Twenty seven patients (45%) had overt hypothyroidism (low FT4 and elevated TSH >10 uIU/ml), while all control subjects were euthyroid and only 2 had mild iodine deficiency according to the level of urinary iodine.

As depicted in Table 2, the comparison of urinary iodine, FT3, FT4 and TSH between the thalassemic- and the control group, revealed a highly significant difference (P<0.001). In thalassemia patients the levels of urinary iodine, FT3 and FT4 were significantly lower that the control group (P<0.001). Moreover, the TSH was significantly elevated compared to their control counterparts (P<0.001).

Severe urinary iodine deficiency was present in 9/60 patients (15%), moderate deficiency in 27/60 patients (45%), mild deficiency in 24/60

patients (40%) and 2/36 control had mild iodine deficiency. Thyroid function tests results varied among these groups of iodine deficiency (Figure 2 and Table 3).

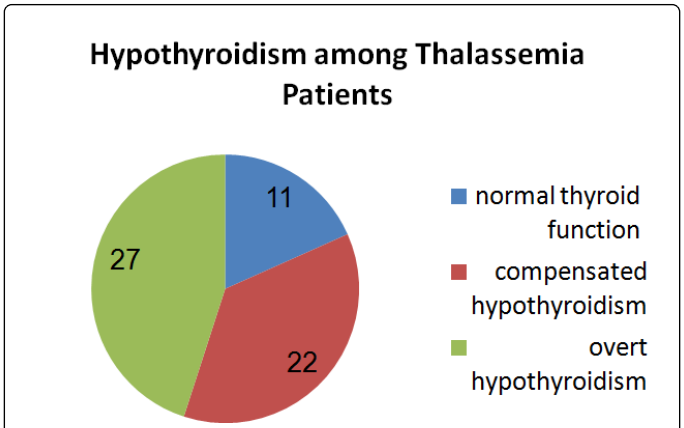

Figure 1: Classification of thalassemia-induced hypothyroidism in the studied group

Parameters	Thalassemia-patients (n= 60)	Control-counterparts (n= 36)
Age	15 ± 4	15 ± 4
Gender Male/ female	31/ 29	19/ 17
High TSH	49/60 (81.7%)	0/36
Low FT3	49/60 (81.7%)	4/36
Low FT4	41/60 (68.3%)	4/36

Table 1: Frequency of abnormal levels of TSH, free T3 and free T4 in Thalassemia-patients

	Group		P value
	Thalassemia Cases (N=60)	Controls (N=36)	
Urinary Iodine(mcg/dl)	43.62 ± 14.766	120.81 ± 33.844	<0.001*
FT3(pg/ml)	1.397 ± 0.2668	2.403 ± 0.6984	<0.001*
FT4(ng/ml)	0.808 ± 0.1239	1.358 ± 0.2999	<0.001*
TSH(uIU/L)	14.173 ± 9.8638	2.797 ± 1.1103	<0.001*
Ferritin(ng/ml)	1670.30 ± 823.717	157 ± 96.2	<0.001*

Table 2: Comparison of thyroid function and urinary iodine between Thalassemia patients and control group

Patients on oral chelators represent 57/60 (95%), while only 3 patients (5%) were on subcutaneous chelation and the mean serum ferritin was 1670.3 ± 1823.72 ng/ml. As regards compliance to chelation, 23/60 (38.3%) were compliant to chelation and 37/60(61.7%) patients were not compliant.

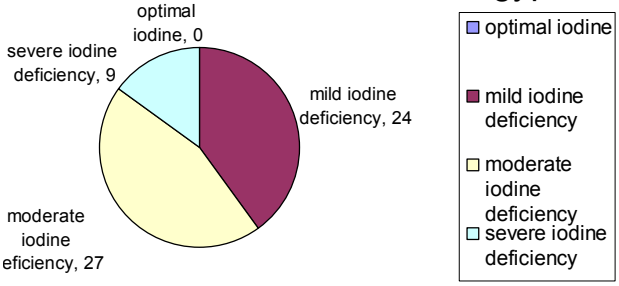

Figure 2: Urinary iodine deficiency in Thalassemia patients.

On comparing the thalassemia patients' group according to their state of compliance to chelation, FT3, FT4,TSH, urinary iodine and serum ferritin were highly significant in the compliant group versus the non compliant one (P<0.001) (Table 3).

Table 4 presents the percentage of occurrence of clinical signs of hypothyroidism. Moreover, TSH and urinary iodine level varied significantly in the presence of different clinical signs (Table 5).

Table 6 presents the negative correlation was found between urinary iodine and both serum ferritin and TSH (r = -0.413, P<0.001, r = -0.881, P<0.001). A positive correlation was found between TSH and both serum ferritin and age (r=0.355, P<0.001, r=0.491, P<0.001).

Figure 3 illustrates a scatter diagram for TSH vs. urinary Iodine, reflecting a negative correlation (r = - 0.881 at P< 0.001).

Discussion

Thyroid dysfunction is known to occur frequently in Beta-thalassaemia Major and conflicting results have been reported considering the prevalence of thyroid dysfunction in Beta-thalassemia patients, due to variable severities in different cohorts and incomplete description of their long-term natural history. The discrepancy may also be attributed to the different treatment protocols considering transfusion rate and chelation therapy [17]. Hypothyroidism is common in patients who are anaemic and/or poorly chelated. For severely affected patients gradual replacement with L-thyroxine is recommended [18], while preclinical hypothyroidism requires only careful follow up. Among our studied group, no gender difference was observed in relation to occurrence of hypothyroidism resembling previous results of Pappa and Tzoumari [19] and Rasheed and Ahmad [20], denoting that development of hypothyroidism is not sex- related.

In our study, overt hypothyroidism was found in 45% of the cases. Clinical hypothyroidism has been reported in 5.3% of a thalassemic group and subclinical hypothyroidism in 2.6% in a study of Khan et al. [21], while 15% of subclinical hypothyroidism cases were accounted by Zervas et al. [22]. A prevalence of 30%, 13.4%, 19.4% and 8.74% have been previously reported by different studies [3,23-25].

	Urinary Iodine Deficiency Level (mcg/L)			
	Mild def. N = 24	**Moderate def. N = 27**	**Severe def. N = 9**	**Total N = 60**
FT3 (pg/ml)	1.563 ± 0.22	1.378 ± 0.20	1.011 ± 0.03	1.397 ± 0.26
FT4 (ng/ml)	0.879 ± 0.03	0.789 ± 0.10	0.678 ± 0.067	0.808 ± 0.12
TSH (uU/L)	6.479 ± 2.34	15.333 ± 6.48	31.211 ± 7.50	14.173 ± 9.86
Ferritin (ng/ml)	868.24 ± 416.34	2234.33 ± 342.09	2117.00 ± 724.78	1670.31 ± 823.71

Table 3: Average levels of FT3, FT4, TSH in relation to Urinary Iodine Deficiency

Clinical signs of hypothyroidism	Percentage of occurrence
Anorexia, laziness and lack of concentration	46.7
Delayed puberty changes	48.3
Delayed growth parameters	55
Constipation	30
Symptoms of other nutritional deficiencies	28.5
Bradycardia	26.7

Table 4: Clinical signs of hypothyroidism varied among the studied cases

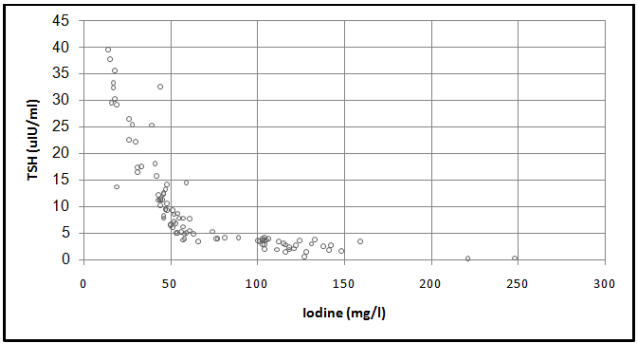

Figure 3: Scatter diagram for TSH vs. Urinary Iodine, reflecting r = - 0.881 at P<0.001.

De Sanctis, [18] has observed that a good compliance to chelation therapy in iron overloaded patients may improve the thyroid function. In his series, 52% of thalassaemic patients with preclinical hypothyroidism their thyroid functions were restored to normal following intensive subcutaneous chelation therapy [17].

P value		Urinary Iodine (mg/l) Mean ± SD	*P value	TSH (uIU/ml) Mean ± SD	Symptoms and Signs
CNS symptoms	Present (28)	16.7 ± 9.28	0.006	38 ± 14.28	0.01*
	Absent (32)	12.2 ± 10.32		48 ± 14.432	
Constipation	Present (18)	20 ± 10.18	0.007	32.5 ± 14.518	<0.001*
	Absent (42)	11.7 ± 8.742		48 ± 12.42	
Symptoms of other nutritional deficiency	Present (17)	20 ± 12.17	0.01	34 ± 18.17	<0.001*
	Absent (43)	14 ± 8.43		47 ± 12.43	
Bradycardia	Present (16)	23.6 ± 10.16	<0.001	28.3 ± 14.416	<0.001*
	Absent (44)	10.7 ± 7.44		49 ± 10.44	
Growth parameter	≤ 3rd percentile (33)	17.6 ± 1035	<0.001	37.5 ± 15.35	<0.001*
	>3rd percentile (18)	9.1 ± 7.25		52 ± 9.525	

Table 5: Level of TSH and urinary iodine in relation to different clinical parameters among patients

The high percentage depicted in our study may be attributed to non compliance to chelation and high ferritin levels. High ferritin levels and non compliance to chelation were found in 61.7% of our cases. Comparing patients with a high ferritin level with those with a fair one [less than 1500], there was a highly significant difference in TSH, FT3 and FT4. Similar observations were also noticed by other investigators as Cavallo et al. [26] and Filosa et al. [3], while Zevras et al. [27] and Gathwala et al. [28] found that no significant difference in thyroid functions between the well and poorly chelated patients.

In 1990, Porcelli and colleagues [29] found that manifestations of hypothyroidism are vague and were detected in a high percentage of thalassemic patients [78.3%] and may be due to many overlapping factors as anemia and iron overload. Constipation, mental changes and bradycardia were found in 36.2% of patients, while a previously lower result of 13.7% has been previously reported [30].

According to the Egyptian growth curves, 55% of our patients showed delayed growth parameters, while a prevalence rate of 29% was previously reported by Fawaz and Mohamed, [31]. This difference is related to the selection of cases in the former study, where the patients were chosen compliant to chelation and on regular blood transfusion. Interestingly, iodine levels were significantly lower in children below the 3rd percentile.

	Urinary Iodine	TSH
Serum Ferritin	r = - 0.413, P<0.001	r = 0.355, P<0.001
Age		r =0.491, P<0.001
TSH	r = - 0.881, P< 0.001	

Table 6: Correlational Analysis between serum Ferritin, age, TSH and each of Urinary iodine and TSH.

In our study, 95% of cases were on oral chelators and only 5% were on subcutaneous (sc) Deferroxamine and the mean serum ferritin was 1670.3 ± 1823.72 ng/ml. Mariotti et al. [32] found no significant difference in the incidence of hypothyroidism and type of chelation, resembling the results of Gamberini et al. [33] who denoted that the incidence of hypothyroidism is not different in patients on oral or sc chelators on a long term treatment. However, in our study, the group of sc chelation of 3 cases only were not hypothyroid, however, the number is too small to be able to draw a conclusion.

A positive correlation was observed between TSH and both serum ferritin and age, a result consistent with Filosa and his colleagues; [3] who reported worsening of thyroid functions with age.

In this study, according to the standards of urinary iodine levels chosen set by the WHO/Unicef/ICCIDD [1994] [34], all investigated patient cases had some degree of iodine deficiency. This raises the possibility of defective intake, absorption or metabolism of iodine. Iodine deficiency may be caused by overall malnutrition, due to general anorexia resulting from chronic anemia. Also, dietary restrictions in cases of thalassemia, such as restriction of iron rich food, salt restriction due to cardiac problems, cause further aggravation of the condition. Another possibility of this decrease maybe due it's binding to the tissues and or the thyroid gland being unable to utilize it, due to its siderosis. This needs to be clarified by testing the intestinal absorption of iodine, a thyroid scan or by measuring the gland's iodine content.

Severe urinary iodine deficiency was found in 15% of thalassemia patients. The negative correlation between urinary iodine level and both TSH and ferritin indicates that both factors play a role in thalassemia associated hypothyroidism. Despite the coexistence of Beta-thalassemia with deficits of several micronutrients, global under nutrition as a principle cause of growth abnormalities has not been adequately studied, as stated by Tienboon.

Iodine replacement cannot be ignored as a treatment modality or adjuvant. Correction of iodine deficiency, if present by iodine supplementation, would be more physiological and avoids the administration of L-thyroxine and dose adjustment in an anemic patient with a hyperdynamic circulation and maybe cardiac complications. A clinical trial with careful adjustment of the iodine dose is necessary to confirm that.

Conclusion

We recommend measurement of thyroid function and urinary iodine level in all young thalassemia patients to detect any deficiency. Moreover, we recommend a trial of iodine replacement prior to thyroid hormone replacement therapy at least in cases of mild hypothyroidism with careful monitoring of the thyroid status.

Limitation of the Study

The small sample size used is the major limitation of the current study. Therefore, it is considered a pilot study and further studies on a larger cohorts are warranted.

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