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Follow-up study of thyroid function in polytransfused thalassemic patients

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The aim of our investigation was to evaluate thyroid function by a follow-up study in 45 polytransfused thalassemic patients, since endocrine abnormalities are frequent consequences of iron overload in thalassemia major. Significant changes of thyroid function have been revealed in the time elapsing the observation, despite unchanged haematological parameters; at the end of the present study five patients were affected by overt hypothyroidism and 15 patients by subclinical hypothyroidism. Ultrasound thyroid volume in 13 randomly selected patients was greatly reduced, while thyroid Magnetic Resonance Imaging (MRI) was not able to detect tissue alterations. Inversely, liver MRI was markedly reduced in 14 patients and negatively related to ferritine levels ($P<0.01$). We conclude that polytransfused thalassemics are frequently affected by thyroid disfunction; haepatic haemosiderosis due to iron overload seems influence hormonal peripheral metabolism, although the patients display a moderate compliance with iron chelation therapy. Therefore, periodic thyroid investigation should be carried out in thalassemic subjects in order to detect patients who need hormone replacement therapy.

Keywords: thyroid function; thalassemia major; ultrasound, magnetic resonance imaging

Introduction

Thyroid is one of the endocrine parenchyma most affected by the iron body overload in polytransfused thalassemic patients (Landau *et al.*, 1978; Madeddu *et al.*, 1978; Costin *et al.*, 1979; De Sanctis *et al.*, 1990; De Sanctis *et al.*, 1992) and in the last years various degrees of thyroid hypofunction have been reported (Cavallo *et al.*, 1981; Sabato *et al.*, 1983; Livadas *et al.*, 1984; Phenekos *et al.*, 1984; Bisbocci *et al.*, 1987; Martino *et al.*, 1990). However the patients studied by the different authors are not often comparable for age, duration of chelation therapy and treatment compliance. Thus so far the main matter to be clarified in thalassemics concerns the evolutive trend of thyroid function with the elapsing of time and its relationship to chelation therapy. This prompted us to carry out a follow up study on thyroid function in 45 polytransfused thalassemic patients tested in 1988 and again in 1993.

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Results

When we grouped thalassemic patients according to sex and/or age as well, no significant differences in hormonal-metabolic values were found. Thus, we grouped individual data and we expressed each hormonal-metabolic parameter as mean value. Hormonal mean levels are shown in Table 1. In 1988 individual values revealed that out of the 45 thalassemic subjects studied, one male patient (2.2%) exhibited an overt hypothyroidism (TSH basal value: 55 mUI/l, T3: 0.6 ng/ml, T4: 27 ng/ml) and one female patients (2.2%) displayed an increase of TSH release to TRH consistent with subclinical hypothyroidism condition, according to the criteria reported in material and methods section.

No significant changes in haematological-metabolic parameters were revealed in the time elapsing between the two tests (Table 2), while significant changes in thyroid function have been observed. Indeed out of the 44 patients tested again in 1993, four (9%, two females and two males) developed an overt hypothyroidism and 14 patients (31.8%, eight females and six males) displayed a subclinical hypothyroidism with a significant decrease of FT3 levels (14 Thalassemics in '93: 3.3 ± 0.7 pg/ml *vs* Controls: 5.3 ± 0.3, $P<0.01$), resulting in a lower FT3/FT4 molar ratio with respect to previous investigation of the same patients (14 Thalassemics in '93: 0.38 ± 0.1 *vs* '88: 07 ± 0.27) and with respect to controls (14 Thalassemics in '93: 0.38 ± 0.1 *vs* Controls 0.8 ± 0.16, $P<0.01$). Thus, at the end of the present study five patients (11.1%) were affected by overt hypothyroidism and 15 (33.3%) by subclinical hypothyroidism.

It is worthy to remark that the group of patients developing subclinical hypothyroidism during the follow-up study, displayed in both periods of investigation an increase of ALT transaminase activity with respect to the remaining patients ($88{:}62.8 \pm 13.7$ UI/l *vs* 30.5 ± 6.16, $P<0.01$; '93: 45 ± 9.2 UI/l *vs* 26 ± 3.6, $P=0.02$) and in 1993 an increase of ferritine levels (3282 ± 585 ng/ml *vs* 2096 ± 266, $P=0.03$) which appeared positively related to AST and ALT concentrations ($r=0.7$, $P<0.01$; $r=0.8$, $P<0.01$, respectively). In 1988 as well as in 1993 thyroglobulin and microsomal antibodies although augmented were below the positivity limit in all patients (Table 1).

Any thalassemic subject presented thyroid enlargement by palpation at both times of investigation. Thyroid volume randomly evaluated by ultrasound in 8 euthyroid thalassemic patients (five females and three males), in three with subclinical hypothyroidism (two

Table 1 Hormonal-metabolic parameters (X ± SEM) evaluated in thalassemic patients in 1988, in 1993 and in 25 control subjects

	bv TSH (mUI/l)	*mxv TSH*	*εTSH*	*T3* (ng/ml)	*T4* (ng/ml)	*FT3* (pg/ml)	*FT4* (pg/ml)	*rT3* (ng/dl)	*TBG* (ug/ml)	*HTg* (ng/ml)	*AAT* (U/ml)	*AAM* (U/ml)
C (n.25)	1.66±0.4	13±1.9	632.7±30.1	1.9±0.06	82.7±5.2	5.3±0.3	9.2±0.3	19.7±0.1	22.3±0.1	18.2±1.8	31±1.8	3.9±0.42
Th '88 (n.44)	2.4±0.3	12.3±1.1	688±66*	1.4±0.07	76±4.8	4.6±0.2	8.6±0.4	18±0.2	20±0.2	20±2.8	42±2.5*	5.9±0.5†
Th '93 (n.40)	2.1±0.2	19.1±1.3†	1234±107*	1.5±0.08	72.4±2.5	4.1±0.6	8.3±0.5	16.9±0.3	19.2±0.1	34.2±4.8†	48.1±2.7*	5.2±0.3†

bv TSH: basal value TSH; mxv TSH: maximal value achieved by TSH upon TRH; ε TSH; integrated area of TSH response to TRH; rT3: reverse T3; TBG: thyroid binding globulin; HTg: thyroglobulin; AAT: Thyroglobulin antibodies; AAM: microsomal antibodies. †$P<0.01$; *$P<0.001$.

Table 2 Haematological-metabolic parameters (X ± SEM) evaluated in thalassemic patients in 1988 and in 1993

	Hb (gr/dl)	*UT* (year)	*Fe* (ng/ml)	*DS* (mg/kg/day)	*I. Ex.* (mg/day)	*Ch.In.*	*Co.In.*	*AST* (UI/l)	*ALT* (UI/l)
Th '88 (n.45)	10.3±0.1	24.4±0.8	2651±261	28.9±1.3	1.3±0.9	1.4±0.2	0.65±0.02	22.1±2.3	30.1±3.7
Th '93 (n.45)	10.1±0.1	22.2±0.7	2501±282	30.7±1.1	15.4±1.6	1.24±0.1	0.68±0.02	16.7±1.6	32.2±4.2

Hb: haemoglobin pretransfusional levels; UT: blood transfused units; Fe: serum ferritine levels; DS: desferrioxamine administered; I.Ex.: daily urinary iron excretion; Ch.In: Chelaton Index; Co.In.: compliance index.

females and one male) and in two with overt hypothyroidism (one female and one male), was greatly reduced with respect to 15 healthy controls (seven females and eight males) (Th: 5.5 ± 4.2 ml *vs* C: 15.2 ± 2.3, $P<0.001$) and negatively related to TSH maximal value ($r = -0.66$; $P<0.02$) (Figure 1) as well as to integrated area of TSH response to TRH ($r = -0.68$; $P<0.02$). It is interesting to note that patients displaying overt hypothyroidism and two of those developing subclinical hypothyroidism showed thyroid volume values clearly below to the lower limit of euthyroid thalassemic range values (Figure 2). The glandular size did not reveal any correlation with the ferritine values of the same patients. For instance it is interesting to observe that in one patient with elevated ferritine levels (3240 ng/ml) the thyroid volume was still maintained (10.8 ml).

The liver MRI, markedly reduced in 14 randomly selected thalassemic patients with respect to controls (Th:45.6 ± 7.4 *vs* C: 145 ± 20, $P<0.01$) and particularly in the patients with overt hypothyroidism (20.4 ± 5.2) (Figure 3), appeared negatively related to ferritine

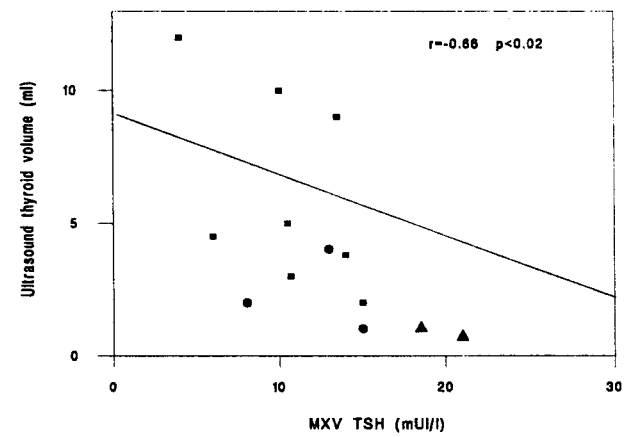

Figure 1 Correlation between ultrasound thyroid volume and TSH maximal value achieved upon TRH in thalassemic patients with overt hypothyroidism (▲), with subclinical hypothyroidism (●) and with normal thyroid function (■)

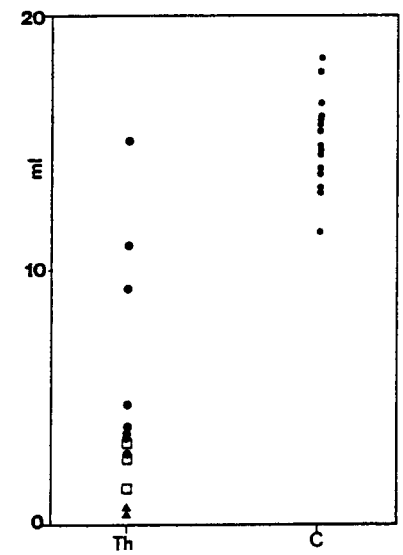

Figure 2 Thyroid volume evaluated by ultrasound in thalassemic patients (Th) with overt hypothyroidism (▲ $n=2$), with subclinical hypothyroidism (□ $n=3$), with normal thyroid function (● $n=8$) and in controls (C) (● $n=15$)

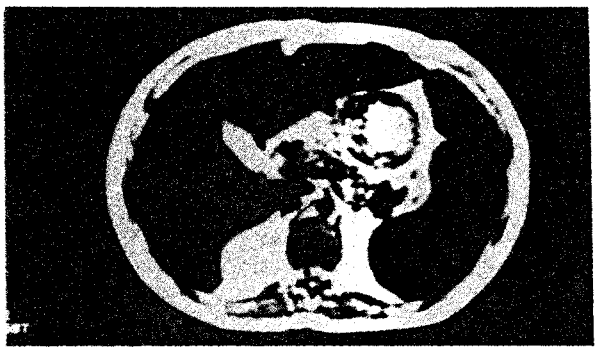

Figure 3 Marked reduction of liver and spleen MRI in a female 16 years old thalassemic patient with overt hypothyroidism

levels ($r = -0.82$, $P < 0.01$) as well as to AST concentrations ($r = -0.78$, $P < 0.01$). In thalassemic subjects thyroid MRI, limitately to the technique applied, did not detect significant differences with respect to controls.

Discussion

According to recent reports (De Sanctis *et al.*, 1990, 1992), thalassemic patients living in Mediterranean countries still have a low compliance with iron chelation therapy. Our data show that thalassemics with elapsing of time may develop thyroid disorders with various degrees of severity, although a moderate compliance with iron chelation therapy. Indeed out of the 44 patients tested again in 1993, four developed overt hypothyroidism and 14 displayed an augmented TSH response to TRH administration consistent with a subclinical hypothyroidism condition. Thus the incidences of overt and subclinical hypothyroidism were greatly increased at the end of our investigation (11.1% and 33.3% respectively). Serum ferritine concentration up to value of 4000 ng/ml seems to be directly correlated only to the haepatic iron deposition: 1 mg/l of serum ferritine is equivalent to 1 mg/g dry weight of liver iron, although such an equation should not be applied to a single value, and above all in the presence of liver disease (Worwood *et al.*, 1980; Cazzola *et al.*, 1983). Thus individual serum ferritine concentration is unable to provide an estimate of iron accumulation in the various organs because iron distribution is certainly not homogeneous (Cohen *et al.*, 1981). On the basis of the above reported observations it is not surprising that ferritine circulating levels appeared unrelated to the reduced thyroid volume revealed by ultrasounds in all patients investigated. We may speculate that iron store with elapsing of time inducing *per se* in thyroid tissue the syntesis of collagen fibers, leads to autonomous fibrotic regressive changes, making glandular parenchima unable to respond to any stimulatory hormonal influence and explaining consequently why we did not observe thyroid hypertrophy in any patient (Sabato *et al.*, 1983; Weintraub *et al.*, 1988). Ferritine levels appeared positively related to the augmented AST and ALT values in subjects developing subclinical hypothyroidism in 1993, indicating very likely that an impaired liver function was associated with the onset of the hypothyroidism condition. It is interesting to note that the same subgroup of patients with unchanged rT3 levels, displayed FT3 and FT3/FT4 molar ratio markedly decreased with respect to that observed 5 years earlier. We suggest a possible haepatic influence *per se* on thyroid function and/or on hormonal peripheral metabolism, as previously shown in patients with other liver diseases (Long *et al.*, 1980). For instance, the extrathyroidal conversion rate of T4 to T3 has been found lowered in liver disfunction (Chopa *et al.*, 1987). Thus we may conclude that in thalassemic patients developing subclinical hypothyroidism a reduced activity of haepatic iodothyronine 5′ monodeiodinase could lead to the lowering of FT3 levels (Chopa *et al.*, 1987). The above observations are supported by the liver MRI which is markedly reduced in all thalassemic patients studied with respect to controls, negatively related to ferritine levels as well as to AST concentrations. Thyroid MRI values in thalassemic patients were comparable to controls. We believe however that the technique applied is not able to detect the amount of iron stored in the strongly regressed thyroid tissue.

The levels of thyroglobulin and microsomal antibodies below the positivity limit in both periods of investigation indicate that in our patients autoimmune mechanisms seem not involved in the impairment of thyroid function, as previously reported (Phenekos *et al.*, 1984; Martino *et al.*, 1990).

Finally, on the basis of the above observations, we may conclude that: – an impairment of thyroid function with different degrees of severity, without goiter, frequently occurs in thalassemic patients in both sexes; – the absence of thyroid antibodies seems to exclude autoimmune mechanisms; – an impaired liver function leads to a probable extraglandular component of thyroid disease; – a moderate compliance with chelation therapy does not seem to protect patients from thyroid dysfunction; and – a periodical thyroid evaluation represents a useful tool to set up a therapeutic treatment in order to prevent overt hypothyroidism.

Materials and methods

Patients

45 polytransfused thalassemic patients (8–35 years old; 24 females and 21 males) were studied in 1988 and 5 years later. 25 healthy subjects (14 females and 11 males) of the same age range were used as controls.

Haematological-metabolic parameters

Before both times of investigation the following parameters have been checked in each patient and here expressed as mean value of almost three determinations: haemoglobin pretransfusional levels, ferritine serum levels, blood transfused units/year, desferrioxamine administered by subcutaneous infusions with portable microinfusers (mg/kg/day), the ratio iron chelated/transfusional iron introduced as chelation index, the number of desferrioxamine infusions in a year as chelation compliance, daily urinary iron excretion, aspartate and alanine transaminase activities (AST and ALT, respectively) (Modell *et al.*, 1984). Haemoglobin levels were measured by automatic counter (Coulter Counter S-Plus Jr, Hialed, Florida-USA). Ferritine serum levels were assayed by immunoenzymatic method (Enzymum test, Boehringer Mannheim, Italy). Urinary iron excretion was evaluated by colorimetric method (Enzycolor-Sibar, Poli Diagnostici, Milano-Italy). AST and ALT transaminase activities were measured by spectrophotometric method (Refloton system, Boehringer Mannheim, Italy).

Hormonal-metabolic parameters

Total and free thyroid hormones (TT4, TT3, FT3, FT4), reverse T3 (rT3), thyroglobulin (Tg), thyroxine binding globulin (TBG), thyroglobulin and microsomal antibodies (AAT and AAT), thyrotropin (TSH) in basal conditions and 30, 60, 90, 120 min after a standard dose of TRH (200 ug i.v.) were measured. The TSH release to TRH administration was evaluated as maximal level achieved (mxv) as well as integrated area of response (ε). In the presence of thyroid hormonal levels still maintained in the normal range, a maximal value of TSH upon TRH more than 1 SD over control mean, was considered as index of subclinical hypothyroidism condition (Bartalena *et al.*, 1991; Ross *et al.*, 1991). Serum TSH and Tg were measured by IRMA sensitive commercial kits (Byk-Sangect, Germany). Serum TT4, TT3, rT3 and TBG were assayed by RIA commercial kits (Baxter, Cambridge USA; Byk-Sangect, Germany; Radim, Roma-Italy). Serum FT3 and FT4 were measured by RIA commercial kits with preliminary chromatographic separation (Tecnogenetics, Milano-Italy). AAT and AAM were assayed by RIA coated tube (Biodata-Italy and Sorin Biomedica-Italy). Values >100 U/ml were considered as positive results for AAT and values >10 U/ml were considered as positive results for AAM. The intraassay and interassay C.V. were $<10\%$ in all previous reported determinations.

Ultrasound evaluation

In 1993 ultrasound evaluations of thyroid volume were carried out on 13 (five females and eight males) randomly selected patients and on 15 (seven females and eight males) healthy controls of the same age range, according to criteria previously reported (Chauoine *et al.*, 1991). The volume of each lobe was calculated separately using the formula of an ovoid (depth × length × width × $\pi/6$). The total thyroid volume represents the values of both lobes.

Magnetic resonance imaging

In order to obtain more sensitive values of tissue iron accumulation, recent physical methods of investigation such as computerized thomography and liquid diagnostic X-ray spectrometry have been purposed. Magnetic Resonance Imaging (MRI) have been also applied to detect alterations in the magnetic relaxation times induced by iron overload (Brasch *et al.*, 1984; Stark *et al.*, 1985; Kaltwasser *et al.*, 1990; Borgna Pignatti *et al.*, 1991). This tecnique widely applied to study liver tissue, has been still used in a few cases to detect iron accumulation in thyroid tissue (Noma *et al.*, 1987, 1988). In 1993 liver and thyroid MRI were carried out on 14 (six females and eight males) randomly selected patients and in 10 (four females and six males) healthy controls of the same age range by an 0.5 T superconducting magnet using SE T1 and T2 weighted and IR sequences. Quantitative analysis was made by calculating signal intensity and relaxation times coming from almost eight different points of observation in liver and thyroid parenchyma respectively.

Statistical analysis

Statistical analyses were carried out by Student's t-Test and Pearson's correlation.

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