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Original article

The frequency of hypothyroidism and its relationship with HCV positivity in patients with thalassemia major in southern Iran

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Summary. *Introduction:* Hypothyroidism is one the most complication due to iron overload in patients with β-thalassemia major (TM). On the other hand these patients are prone to Hepatitis C virus (HCV) infection that can cause thyroid dysfunction by itself or as the side effect of treatment with interferon (INF) or IFN plus ribavirin. The aim of this study is to evaluate the association of hypothyroidism with HCV positivity and serum ferritin levels in patients with TM. *Methods:* In this cross-sectional study, 201 randomly selected patients with TM who were registered at the Thalassemia Clinic of a tertiary hospital in Shiraz, southern Iran were investigated. Thyroid function tests and serologic screening assays for HCV seropositivity (HCV Ab and HCV-RNA) were conducted for all patients. *Results:* Frequency of hypothyroidism was 22.9% including 19.9% subclinical hypothyroidism, 2% primary overt hypothyroidism and 1% central hypothyroidism. Eighty six patients (42.8%) were HCV Ab positive and 60 patients (29.9%) were HCV RNA positive. No significant relationship was found between hypothyroidism and HCV positivity or receiving IFN-α (P>0.05). Hypothyroidism showed a borderline significant association with high serum ferritin levels in TM patients (P=0.055). *Conclusion:* Our results showed no significant association between hypothyroidism and HCV infection in TM patients. It seems that the main mechanism of hypothyroidism in our patients is iron overload; however, for better evaluation a larger multicenter study is recommended. Also due to the importance of consequences of HCV infection, more careful pre-transfusional screening of blood should be considered in TM patients. (www.actabiomedica.it)

Key words: hypothyroidism, hepatitis C, iron overload , thalassemia major

Introduction

Red blood cell transfusion is the main treatment of β-thalassemia major (TM) patients but it leads to excessive iron stores and causes endocrine complications, such as thyroid dysfunctions (1-6). Prognosis and longevity in TM patients have improved in the last 20 years due to recent medical advances in transfusion and iron chelation therapy (1, 7). A chronic hepatitis, secondary to hepatitis B and C virus infections, due to the frequent blood transfusions, frequently occur in TM patients (8).

In patients with chronic hepatitis C, thyroid dysfunctions can occur with an autoimmunity mechanism. Thyroid autoantibodies such as anti-thyroperoxidase (TPO) and anti-thyroglobulin (TGA) are detected in chronic hepatitis C patients even without treatment with IFN-α and ribavirin. Innate immune system in response to viral infection can induce endogenous IFN-α and β in thyroid gland. Endogenous and exogenous IFN can cause production of thyroid antibodies by NK cells memory T cell activation (9).

Combination therapy with pegylated interferon (PEG-IFN) and ribavirin remains the choice of treatment for hepatitis C. In addition, in patients treated with IFN-α and ribavirin, thyroid dysfunctions ,including hypothyroidism, hyperthyroidism and thyroiditis, can occur. [10-12] PEG-IFN plus ribavirin can cause hypophysitis in chronic hepatitis C patients, which can cause a central hypothyroidism (9, 10). In a review article, an autoimmune thyroiditis has been reported in up to 20% of the patients during IFN treatment (11). The IFN-α molecule and ribavirin have immunomodulating properties and may act on thyroid gland through a direct toxic effect and/or autoimmune mechanism (induction of TSH receptor autoantibodies, antithyroid autoantibodies, thyroid cell apoptosis, cell mediated immunity, expression of major histocompatibility complex and cytokine production regulation).IFN-α in cultured human thyroid cells inhibited iodine organification and T4 release, and can aggravate the preexisting autoimmunity in chronic hepatitis C patients (9, 10, 12, 13).

Furthermore, the occurrence of central hypothyroidism as a possible effect of hepatitis C virus and/or adverse effect of IFN-α and ribavirin treatment is not rare (14).

Chronic liver disease can cause abnormalities in liver function tests, e.g. TBG elevation, which can cause elevation of T3 and T4. However, free T4 and TSH are usually normal. Serum T3 can be normal, increased or decreased, diminished conversion of T4 to T3 can cause low serum T3. Despite the remarkable changes of thyroid values, most patients have normal TSH and FT4 levels (15).

The aim of this study was to determine the association of the frequency of hypothyroidism with HCV positivity, antiviral therapy with IFN-α plus ribavirin, and with serum ferritin levels in TM patients.

Methods

This study was a cross sectional survey that was carried out on 201 patients older than 10 years at Thalassemia Center of a tertiary hospital in Shiraz, the capital of Fars province in the south of Iran from Feb 2014 to Feb 2015. The study was conducted at the Hematology Research Center of Shiraz University of Medical Sciences and was approved by the Ethics Committee of Shiraz University of Medical Sciences (registered with grant no 3450). Written consent form was obtained from the patients or their legal parents.

Study participants

The participants in this study were TM patients, aged 11-42 years who had regular follow-up schedule and regular blood transfusions with a frequency of 2-4 weeks.

Systematic random sampling was used to determine our sample group from all 700 registered patients at Thalassemia Center of tertiary hospital in Shiraz. The inclusion criteria were: TM patients older than 10 years, under regular follow up and regular blood transfusion; patients treated with chelating agents including deferoxamine, deferiprone or deferasirox and patients living in an iodine sufficient area (16, 17). Patients wth thalassemia Intermedia, cirrhosis, heart failure, renal failure, history of previous treatment with L-thyroxine were excluded from the study. Individuals taking medications affecting the thyroid function test (such as: steroids, anticonvulsants, propranolol, amiodarone, salicylates, furosemide, lipid lowering agent) and pregnant women were also excluded from the study.

After applying the above exclusion criteria, 201 subjects were eligible for the study.

An extensive medical history including data on age at first transfusion, duration and type of iron chelation therapy, compliance with treatment, and associated complications were obtained.

Physical examination included anthropometry (weight, height, BMI), vital signs (blood pressure, heart rate) and pubertal assessment.

Body mass index (BMI) was calculated as the body weight divided by the height squared (Kg/m^2).

A subject was considered overweight when the BMI was between 25 and 29.9 and obese when the BMI was above 30.

Biochemistry

Blood samples were drawn in the morning after an overnight fast to measure the serum concentrations of glucose, FreeT3, FreeT4, TSH, TotalT4, Total T3, urea, creatinine, electrolytes ,calcium,phosphate and total proteins.

In order to exclude severe liver injury and dysfunction, serum concentrations of alanine aminotransferase (ALT), gamma glutamyl transferase (γGT), alkaline phosphatase (ALP), total and direct bilirubin, albumin, prothrombin time (PT) and international normalization ratio (INR) were measured. Serologic screening assays for hepatitis C virus seropositivity (HCVab and HCV-RNA) were also obtained.

All biochemical and serologic tests were carried out in accordance with the routine procedures of the central laboratory.

Iron stores were assessed by an indirect method. Serum ferritin was measured by electrochemiluminescence immunoassay. Reference range values were 30-350 μg/l in males and 15-150 μg/l in females

Thyroid hormones were measured by the automated Cobas electrochemiluminescence (ECLIA) technique, using commercial kits from Roche Diagnostics (Mannheim, Germany) using Elecsys 2010 analyzer and molecular analytics E170.

The intra-assay and inter-assay coefficients of variation (CV) of our assays were between 1.6% and 3.5%, respectively.

Definition of hypothyroidism

Hypothyroidism was categorized in three categories:

A) Primary overt hypothyroidism: elevated TSH level (> 10 mIU/L) associated with low levels of thyroid hormones

B) Subclinical primary hypothyroidism: elevated TSH level (>4.2 mIU/L - <10 mIU/L) with normal thyroid hormones

C) Secondary hypothyroidism: normal-low TSH level and normal-low T4 and FT4

Statistical analysis

Data were analyzed by SPSS software version 17. Normality of data was checked by Kolmogorov Smirnov test. Descriptive data were shown as mean, standard deviation, or median, range and percentage. Comparison of qualitative data was carried out using Chi-square test. Quantitative data were compared between the two groups of patients using student t test or Mann Whitney test. P value less than 0.05 was considered statistically significant.

Results

Demographic and clinical characteristics of the TM patients are summarized in Table 1. Mean age of the patients was 22.9±5.1 and ranged from 11 to 42 years, 49.3 % of them were females and 50.7% were males.

According to the patient's medical history some of these patients have had multiple endocrinopathies such as: hypoparathyroidism 27.4% [37 patients], hypogonadism 56.9% [66 patients], diabetes mellitus type I 18.2% [31 patients], and diabetes mellitus type II 7% [12 patients].

Overall 86 out of 201 patients (42.8%) were HCV Ab positive and 60 patients (29.9%) had HCV RNA positivity; of whom 56 patients received IFN-α plus ribavirin.

Overall frequency of hypothyroidism was 22.9% (46 patients) in the studied population. Including 19.9% (40 patients) with subclinical hypothyroidism, 2% (4 patients) with primary overt hypothyroidism and 1% (2 patients) with central hypothyroidism .

In Table 2, two groups of patients with and without hypothyroidism were compared based on sex, age, serum ferritin levels, HCV positivity and receiving IFN-α. Frequency of patients with HCV positivity or receiving IFN-α was not significantly different in euthyroid and hypothyroid patients. Serum ferritin levels showed a borderline significant association with hypothyroidism (median (range): 2936 (928-9500) ng/ml in hypothyroidism vs 2148 (106-19043) in euthyroid patients, P=0.055).

Table 1. Characteristics of patients with beta thalassemia major

Parameter	Value
Age (year) Mean±SD	22.9±5.1
Age at diagnosis of thalassemia (month) Median (range)	6 (2-9)
Age of HCV positive patients(year) Mean±SD	25±5
Age at diagnosis of HCV hepatitis(year) Mean±SD 18±7	
BMI (kg/m²) Mean± SD	19.5±3.3
Serum ferritin levels (µg/L) Median (range)	2256 (106-19043)
Desferoxamine (number,%)	180 (90)
Deferiprone (number,%)	80 (40)
Deferasirox (number,%)	20 (10)
Hypoparathyroidism (number,%)	37 (27.4)
Hypogonadism (number,%)	66 (56.9)
Diabetes mellitus type I (number,%)	31 (18.2)
Diabetes mellitus type II (number,%) SD: standard deviation	12 (7)

Table 2. Comparison of euthyroid β-thalassemia major patients and patients with hypothyroidism regarding sex, age and serum ferritin levels as well as HCV positivity and receiving IFN-α

	Euthyroid N=155	Hypothyroid N=46	P value
HCV RNA PCR Positive	48 (31%)	12(26.1%)	0.586
HCV Ab ELISA Positive	68 (43.9%)	18 (39.1%)	0.614
Receiving IFN-α	44 (28.6%)	12 (26.1%)	0.853
Serum ferritin (ng/ml)	2148 (106-19043)	2936 (928-9500)	0.055
Sex (Male)	74 (47.7%)	28 (60.9%)	0.131
Age (year)	23.1±5.5	22.7±3.8	0.631

All data are presented as number (percent) except age (mean ± SD) and serum ferritin levels (median and range)

Discussion

In this study, the frequency of hypothyroidism in patients with TM was determined. Also the relationship of hypothyroidism with HCV infection and iron overload were evaluated. Overall frequency of hypothyroidism was 22.9% including 19.9% subclinical hypothyroidism, 2% primary overt hypothyroidism and 1% central hypothyroidism. Our results support the fact that primary hypothyroidism is the most common form of thyroid dysfunction observed in these patients and it is resulted mainly because of abnormalities of the thyroid gland which leads to insufficient production of thyroid hormones (4). Iron overload secondary to multiple blood transfusions is the main cause of such complications hence proper and effective iron chelation therapy is essential for inducing a reduction of iron overload in various endocrine glands (1, 3, 5, 6). Similar to our study, hypothyroidism has been reported from 4% to 21.6% of TM patients with different severity, (11, 14, 18, 19). Zervas et al. reported a frequency of 4% for overt hypothyroidism, and 12.5% for subclinical hypothyroidism (3).

In the past, the reported prevalence of primary hypothyroidism in Iranian TM patients living in Shiraz was about 6% (personal observations) but in the present study the prevalence was lower (2%). On the contrary, the prevalence of subclinical primary hypothyroidism seems to be higher than before(19.9%). It is probably due to the better management (blood transfusion and chelation therapy) available in the last decade in patients followed in our Center.

Endocrine complications are mainly attributed to iron overload (1, 4, 5) and are uncommon in optimally treated patients (6). In our patients, serum ferritin levels showed a borderline significant association

with hypothyroisim; however, if we used more accurate methods such as T2MRI of heart and liver, the results were more precise and reliable. These diagnostic methods can be more helpful in early detection of iron deposition in the endocrine glands compared to serum ferritin levels (18, 19). Also if we had a larger sample size the difference probably will be more significant.

Overall 88 TM patients (42.8%) were HCV Ab positive and 60 patients (29.9%) were

HCV RNA positive. Fifty-six TM patients (28%) were receiving IFN-α and ribavirin.

It is well known that hepatitis C can induce autoimmunity as extra hepatic manifestation and hypothyroidism is more common in patients with chronic hepatitis C even in the absence of INF treatment.

This may be due to an autoimmune process that impairs thyroid hormone, however, a direct relation between HCV infection and thyroid diseases has been also suggested.

IFN has important immunomodulatory properties due to which it can induce autoimmune phenomena like autoimmune thyroiditis with hypo - or hyperthyroidism (20). Autoimmune thyroiditis has been reported in up to 20% of patients during IFN-based therapies (9). Little information are available in literature about the development of central hypothyroidism in these patients.

Zantut-Wittmann et al. evaluated 308 HCV patients treated with standard INF-α and/or PEG-IFN associated with ribavirin. FT4 and TSH levels were measured before, during and after treatment. Before treatment, 18 patients (5.8 %) presented central hypothyroidism (CH) and twelve patients developed CH during the treatment. Among the 29 patients (9.4 %) with CH, 11 patients were treated with IFN-α, six used PEG-IFN and 12 patients used two or more therapeutic schedules (14).

The prevalence of CH estimated in general population is about 1 in 80,000 to 1 in 120,000. In our study we found two cases of CH out of 201 TM patients which is higher compared to general population (21). This could be due to the population selected in our study, which included TM patients who are prone to develop thyroid dysfunction, secondary to iron deposition in the pituitary and thyroid (5, 6, 8). Furthermore, the assessment of thyroid function in our TM patients was evaluated just after treatment and, despite CH being more prevalent than normal population, there was no significant correlation between TM patients receiving INF and development of CH, and also between HCV positivity and CH.

Therefore, we believe that more studies are needed to evaluate this possible effect of HCV and IFN-α in TM patients (22-25).

In conclusion, our results showed no significant relationship between hypothyroidism and HCV infection in TM patients. It seems that the main mechanism of hypothyroidism in our patients is iron overload; however, for better evaluation a larger multicenter study is recommended. On the other hand, due to the importance of high mortality and morbidity related to HCV infection, it is recommended that more careful pre-transfusional screening of blood for anti-HCV should be introduced in our blood banks and better assessment for thyroid dysfunction is needed in HCV positive TM patients especially in those who are receiving IFN.

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