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Effect of Iron Overload and Lipid Peroxidation on Hyroid Function in Iraqi Patients with ß- Thalassemia

Najat AR. Hassan[1], Raad J. Musa[2], Sadik A. Abdullah[3].

[1]Professor in Dept. of Chemistry and Biochemistry, College of Medicine,
Al-Nahrain University
Baghdad, Iraq

[2] Professor in Dept. of Pathology and Forensic Medicine, College of Medicine,
Al-Nahrain University, Baghdad, Iraq

[3]Dept. of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University
Baghdad, Iraq

ABSTRACT—

Background: *Iron overload due to blood multi transfusion can lead to thyroid dysfunction in beta-thalassemia major patients with increased cell damage due to imbalance of the redox system .An iron chelation is essential to prevent the deposition of iron in tissues and glands such as thyroid.*

Objectives: *to investigate the reflections of blood transfusion induced iron overload and lipid peroxidation on the status of thyroid functions in Iraqi children and young adults with beta-thalassemia major.*

Methods: *Blood samples were taken from 60 males & females with Beta thalassemic major (BTM) with age ranged (3-32) years. An automated hematology analyzer (Sysmex) was used in the measurements of blood Hb concentration and red cell indices. malondialdehyde(MDA), serum iron and total iron binding capacity (TIBC) concentration were determined spectrophotometrically . The triiodotyronine (T_3) ,Free T_3,thyroxine (T_4),Free T_4 ,thyroid stimulating hormone (TSH) and Serum Ferritin concentrations were assayed by an ELISA method .*

Results: *There were low mean concentration of Hb in BTM and a significant increase in serum ferritin and MDA in comparison with the normal corresponding values for the control . Hypothyroidism was found in 12 (20%) out of 60 patients with BT major, (12%) were having subclinical hypothyroidism and five (8%) suffer from primary hypothyroidism with a positive a correlation of serum ferritin concentration with both MDA and number of monthly transfused blood units.*

Conclusion: *Transfusion associated iron overload play an effect on lipid peroxidation and the thyroid hormones (T_3, T_4) metabolism which leads to a disturbance in thyroid hormones secretion as consequences of thyroid dysfunction.*

Keywords— beta-thalassemia, serum ferritin, MDA, hypothyroidism.

1. INTRODUCTION

The thalassemias are heterogeneous group of genetic disorders of hemoglobin synthesis, all of which result from a reduced rate of the production of one or more of the globin chains of hemoglobin (1- 2).

The ß-thalassemias are the most important types of thalassemia because they usually produce severe anemia in their homozygous states and so common that they occurs widely in areas ranging from the Mediterranean and parts of North and West Africa through the Middle East and Indian subcontinent to South-East Asia, southern Russia and China (2-3).

Most individuals who are homozygous or compound heterozygous for beta thalassemia have thalassemia major, a severe, life threatening anemia that requires regular blood transfusion for survival. Some patients, however, are less severely affected with a milder non transfusion dependent disorder referred to as thalassemia minor (4-5).

The thyroid gland produces two related hormones, thyroxine (T_4) and tri- iodothyronine (T_3). Acting through nuclear receptors, these hormones play a critical role in cell differentiation during development and help maintain thermogenic and metabolic homeostasis in adults.Thyroid stimulating hormone (TSH), secreted by the thyrotrope cells of the anterior pituitary, plays a pivotal role in the control of the thyroid axis and serves as the most useful physiologic marker of thyroid hormone action (6).

Thyroid dysfunction with variable severity has been reported in 13–60% of patients with thalassemia. Shamshirsaz et al. (2003) reported that even in the studies in which the prevalence of overt hypothyroidism as a complication of thalassemia major is relatively low, milder forms of thyroid dysfunction are much more common(7).Patients with hypothyroidism have a decrease in red blood cell mass and a normochromic, normocytic hypoproliferative anemia (8). Free iron is biochemically dangerous because it catalyzes the conversion of hydrogen peroxide into reactive oxygen species. Thalassemic patients usually need regular blood transfusions and so they are subjected to viral infection as well as peroxidative tissue injury by the secondary iron overload (9).Therefore, desferrioxamine (desferal) is considered the first- line drug to catch iron from the circulation and protect organs damage (10).So, this study was conducted to reveal the effects of blood transfusion and iron status on lipid peroxidation and thyroid functions in Iraqi children and young adults with beta-thalassemia **.**

2. SUBJECTS AND METHODS

This study was carried out in Thalassemia Treatment Center in Ibn Albaladi Hospital in Baghdad. Sixty patients were diagnosed as β-thalassemia , 35 were males while 25 were females with an age range of 3-32 years.Twenty patients were diagnosed as β -thalassemia minor and another twenty healthy persons with matching age were chosen as control. Blood specimens have been collected prior to regular blood transfusion and sera were separated and kept frozen till the analysis day. Patients suffering from additional hemoglobinopathies such as sickle cell anemia or chronic diseases such as diabetic mellitus , heart or kidney diseases were excluded from this study, The minimum period between recent and last transfusion is 14 days and the maximum period was 60 days.All of the studied subjects were distributed into three subgroups according to age(Group A: 3-14 years, Group B: 15-21, Group C: > 21years).

Determination of Red cell indices:

Full blood count and Hb concentration were measured by an automated hematology analyzer (Sysmex, USA).The principle depends on using fluorescent flow cytometry and cell counting methods to reliably detect abnormal samples and reduce false positive results (11).

Assessment of the Thyroid functionand iron status:

The thyroid status of thalassemic patients and controls were assessed by measuring the TSH, T_3, FT_3, FT_4 and T_4 as well as serum ferritin by using Enzyme-Linked Immunosorbent Assay kit (bioactiva, Germany) . In this assay an enzyme conjugated with an antibody reacts with a colorless substrate to generate a colored reaction product. The serum ferritin is also assessing by this technology (12).the serum Reverse T_3 (rT_3) concentration was calculated from the following equation (13).

$$FT_4 = TT_4 \times rT_3$$

The serum iron concentration and TIBC were measured using the iron colorimetric kit (Randox, France) The intensity of the colored complex formed was read at 595 nm (Young et al., 2001(14). The percentage of transferrin saturation (TS %) was calculated from the following equation (15):-

$$\text{The TS \%} = \text{S.Iron (mmole/L)} / \text{TIBC (mmole/L)} \times 100$$

Assessment of Serum Malondialdehyde (MDA).This is based on a principle that in presence of trichloroacetic acid (TCA) and heat, MDA reacts with thiobarbituric acid (TBA) to produce pink colored end products that absorb light at

530-540 nm .The intensity of the color measured at 532nm corresponds to the level of lipid peroxidation in the sample (16).

Statistical analysis

The statistical analysis was performed using the Statistical Package for the Social Sciences version 17 (SPSS Inc, Chicago, IL, USA). The student's *t*-test for continuous variables was used to compare the differences between patient groups. A p-value of < 0.05 was considered to be statistically significant.

3. RESULTS

Results in table (1) showed low level of Hb in BT major group in addition to highly elevated S.ferritin and MDA concentration in BT major group in comparison with BT minor and controls. Within BT major group, according to gender distribution males have significant increase of S.ferritin concentration than females.

Figure (1) showed the Spearman Rank correlation between frequency of blood transfusion and serum ferritin in β-thalassemia major group($r = 0.47$, $p<0.001$).

The results in table (2) show the gender distribution of the means of thyroid hormones concentration in the studied groups, in BT major group concentration of T_4 has low level, concentration of TSH has high level such as in the total BT patients group in compared with BT minor and control group.

Figure (2) show the Spearman Rank correlation between serum MDA conc. and serum ferritin concentration in β-thalassemia major group ($r = 0.47$, $p<0.001$).

The results in table (3) show the comparison of the effect of age on the means of thyroid functions in different β-thalassemia major age groups (Group A: 3-14 years, Group B: 15-21, Group C: > 21years) Subgroup C show high increasing in concentration of MDA, TSH, S.ferritin and low concentration of T_4 in compared to A subgroup, but in compared to B subgroup has same finding except the T_4 parameter. Figure (3) revealed the Bar graph of the mean of T_4 concentration in BT major group which is lower than the concentration of T_4 in BT minor and control groups.

Figure (4) revealed the Bar graph of the high concentration mean of TSH in BT major group versus BT minor and control groups. The results in table (4) Comparison of hypothyroidism in β-thalassemia major age subgroup C (> 21years) with mean of the Serum Ferritin and MDA concentration, (12%) were having subclinical hypothyroidism and five (8%) suffer from primary hypothyroidism. The primary hypothyroidism patients have higher level of S.ferritin than the subclinical hypothyroidism patients.

Figure (5) revealed Bar graph of disease and gender distribution. Among BT major patients, 4 male &1 female has primary hypothyroidism.4 male & 3 female has subclinical hypothyroidism. Furthermore 12% and 8% of thalassemic patients suffer overt or subclinical hypothyroidism, respectively.

4. DISCUSSION

In beta thalassemia (BT) major, the Hb mean conc. of both of males and females were highly significantly decreased above the respective gender mean Hb values of the control group ($p<0.01$) and those of BT minor group ($p<0.01$).Yet, statistical analyses revealed no significant difference between male and female Hb mean conc. in BT major group. Similar findings were recorded by Irshaid and Mansi (17).

Karamifar 2006(18) reported a significant increase($p<0.001$) in the mean serum ferritin level in patients with multiple blood transfusions as compared to values in patients without transfusions .Moreover, he showed an incidence of hypothyroidism in patients with (22%) and without(21%) blood transfusion .The number of blood units and the amount of the accumulated iron justify that older BT major patients accumulate an increasing amounts of iron during their life more than the younger patients. The probable explanation for this observation may be: - First, ferritin is a protein which reflects the inflammatory status of a subject and it is well known that in thalassemic patients, especially older ones, HCV-related chronic hepatitis is frequently associated (19). Second, the grade of iron load is determined by visual evaluation and a standardized definition is difficult because it depends on the thickness of the intracellular iron granules). As hepatic fibrosis progresses and inflammatory cells decrease the liver gradually loses its capacity to synthesize and release ferritin, and in the final stage of cirrhosis the ferritin level is generally low (20-21).

We reported a highly significant increase in the mean MDA concentration in BT major group ($p < 0.01$) as compared to the mean values of the control group and those of the BT minor group. In line with our findings, Ra'id M. Hannun. Al-Salih, et al. 2011(22) reported a significant elevation ($p < 0.05$) in MDA level for BT major group before blood transfusion as compared those after blood transfusion. Serum ferritin concentration, urea and creatinine (these two was checked but the numerical values was not reported) levels in these patients were found to be increased significantly ($p \leq 0.05$) when compared to the disease free controls (23).

In 2005, Al-Mudalal (24) and co-researchers reported that red cell superoxide dismutase activity was greatly increased in homozygous beta-thalassemia, and inversely correlated with severity of anemia with no significant correlation between SOD activity, Catalase activity and the last time of blood transfusion

The increased MDA level in thalassemic patient confirm this assumption that these radicals play a major role in red blood cell RBCs destruction and hemolysis in reacting with labile polyunsaturated fatty acid in RBC membrane and possibly in membranes of other major organs. The extent of lipid peroxidation (LPO) denotes the amount of ROS generated, which have not been scavenged by the defense mechanism. LPO may not be related to the primary tissue injury, but may amplify the original injury (25-26).

This is a cross-sectional study of thyroid function tests (T_4, T_3, free T_3 and FT_4 and thyrotropin, TSH) that was carried out in 60 transfusion-dependent ß-thalassemic patients. The mean serum T_4 level was significantly lower ($p<0.01$) in BT major as compared to the controls and BT minor ($p<0.01$) mean values. Only the mean of TSH level was significantly increased in the BT major group, with no significant change in the means of serum reverse T_3, Free T_3 and Free T_4 concentration in all BT studied groups.

Pirinççioğlu et al. 2011(27) recorded that all thyroid parameters in BT major groups were in the normal ranges compared with the controls except three of them which had high TSH levels. Serum ferritin level in these patients was significantly higher than in controls .This implies that hypothyroidism could be even seen in the first decade of life in patients with beta-thalassemia major in spite of improved hematological cares.

In this study, the mean serum T_4 concentration in the BT major age subgroup C (>21 years) was significantly lower and the mean conc. of TSH of age group C was significantly increased when compared to those of younger age groups. Hypothyroidism was found in only twelve patients (20%) of the total 60 patients who suffer from β-thalassemia major and Euthyroidism is found in all BT major groups. All patients were clinically Euthyroid, but some symptoms (for example lethargy and growth failure) may not have been identified as separate from those of the primary disease none had goiter.

Primary hypothyroidism occurs in a significant proportion of thalassemia major patients in the absence of obvious clinical signs of hypothyroidism but normal concentration of T4 (Criteria for the diagnosis of subclinical hypothyroidism (SH) was an elevated basal TSH concentration (>5 TSH μIU/ml) or an increase of the TSH levels during the test more than 20 μIU/ml from the basal value or additional low levels of FT4 and FT3 respectively (28-29). In this study, of 12 patients with hypothyroidism (20%) seven patients (12%) were having subclinical or compensated hypothyroidism. Whereas, five of BT major patients (8%) suffer from primary or overt hypothyroidism. Both types of hypothyroidism are predominant in males with BT major disease. These results were comparable to those reported by other investigators (30).The incidence was high possibly because of inadequate dosing, poor compliance, or relative poor thyroid protection from DFO-specific properties. DFO contrary to oral chelators is a large positively charged, lipophobic molecule with low membrane permeation abilities and thereby low cell iron extraction capacity (31). In the study of De-Sanctis, 1998 (32) hypothyroidism was detected in 5.7% of patients. The difference in incidence of hypothyroidism noted is probably related to differences in transfusion. In some retrospective studies the high incidence of the primary hypothyroidism was shown to decrease over time according to compliance, but in most cases there was a progression from subclinical to overt hypothyroidism (33).

In the present study the mean serum ferritin level of thalassemic patients with primary thyroid impairment (3721± 29.21 ng/ml) was significantly higher than that of thalassemic patients with normal thyroid function (2395.41 ng/ml, $p<0.05$). This result contradicts the finding of other researchers who reported no significant difference in mean serum ferritin between hypothyroid patients and other researchers (34-35-36).

We also recorded a tendency for serum mean TSH values to increase with increasing age, and there was obvious tendency for T_4 values to decreasing with age. But there was no obvious T_3 related age change. Hashemi.et al. 2011 (37) reported higher incidence of subclinical hypothyroidism in patients less than 10 years of age. Malik et al., 2010 (38) reported a ratio of 2.1% primary hypothyroidism in BT major patients aged 2-17 years (39). Thyroid hormones may also

play a critical role in brain development in infants and in modulating brain metabolic activity in adults as shown by structural changes related to myelin, studied by brain imaging techniques. Without optimal thyroid function, mood disturbance, cognitive impairment and other psychiatric symptoms can emerge (40).

5. CONCLUSIONS

This study revealed that hypothyroidism is prevalent in 20 % of patients with beta thalassemia major with a positive correlation between iron overload and endocrine status of the patients. Thalassemia major patients with hypothyroidism, showed significantly higher rates of blood transfusion, serum ferritin and MDA levels than thalassemia major patients with normal endocrine status. Thyroid gland disorders are associated with disturbance of hematological parameters such as reduced Hb, as well as PCV, MCH and MCHC(not shown here). Anemia of chronic diseases such as β-thalassemia major leads to disturbance in thyroid hormones secretion. Transfusion associated iron overload play an effect on lipid peroxidation and the thyroid hormones (T_3, T_4) metabolism. Hypothyroid patients have usually serum ferritin values higher than those of normal controls.

6. ACKNOWLEDGMENTS

I would express my sincere appreciation to all medical staff at Ibn albalady Hospital for their support and help in data collection and support during the laboratory analysis.

Table (1): Comparison Of The Means Of Iron Indices, Malondialdehyde (MDA) And Hemoglobin (Hb) Concentration According To Gender Distribution (M=Male, F=Female) In Different Thalassemic Groups.

	Control N=20		**Thalassemic patients N=80**		**BT Major N=60**		**BT Minor N=20**	
	M=10	F=10	M=45	F=35	M=35	F=25	M=10	F=10
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Serum Iron (mmole/l)	24.17 ± 5.46	19.26 ± 5.29	23.17 ± 7.40	19.94 ± 6.87	20.61 ± 0.96	20.56 ± 1.2	19.42 ± 1.16	20.38 ± 0.97
Transferrin (%)	40.03 ± 7.74	31.57 ± 7.78	45.90 ± 18.10	37.98 ± 18.24	36.01 ± 3.44	36.97 ± 3.87	37.34 ± 4.01	37.16 ± 3.91
Total Iron Binding Capacity (mmole/l)	61.74 ± 6.55	61.22 ± 6.34	53.19 ± 10,54	54.08 ± 10.70	59.86 ± 1.09	61.34 ± 1.27	62.09 ± 1.32	62.06 ± 1.24
Serum Ferritin (ng/ml)	122.43 ± 27.75	123.05 ± 27.35	1801.55 ^{a**} ± 24.05	1602.84 ^{a**} ± 23.74	3392.42^{a,b**} $^{* c*}$, ± 24.83	2807.2 ^{a,b***} ± 26.63	184.5 ± 24.19	164.3 ± 25.39
Serum MDA (mmole/l)	4.37 ± 3.00	3.72 ± 3.01	6.73 ^{a*} ± 2.99	6.59 ^{a*} ± 3.04	9.39 ^{a,b**} ± 2.95	8.99 ^{a,b**} ± 3.03	4.30 ± 3.02	3.81 ± 2.91
Hb Concentration (g/dl)	13.97 ± 0.42	11.79 ± 0.38	11.08 ^{a*} ± 0.19	9.27 ^{a*} ± 0.15	8.61^{a**,b*} ± 0.14	8.07 ^{a**,b*} ± 0.12	12.04 ± 0.20	10.81 ± 0.22

Student t.test: ^{a} Between groups comparison of male or female thalassemic subgroup versus corresponding gender value in the control group: *p<0.05 **p<0.01,*** p<0.001,

[b] Comparison of male or female value of BT major versus corresponding value in the BT minor group: * p<0.5,** p<0.01,*** p<0.001

[c] Within each subgroup comparison between male and female mean value
:*p<0.01

Table (2): Gender Distribution (M=Male, F=Female) Of The Means Of Thyroid Hormones Concentration In The Studied Groups.

	Control N=20		**Thalassemic patients N=80**		**BT Major N=60**		**BT Minor N=20**	
	M=10	F=10	M=45	F=35	M=35	F=25	M=10	F=10
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Triiodothyronine (nmole/l)	1.54 ± 0.43	1.72 ± 0.39	1.55 ± 0.44	1.47 ± 0.42	1.53 ± 0.37	1.47 ± 0.40	1.58 ± 0.38	1.48 ± 0.41
Thyroxin (nmole/l)	104.56 ± 31.72	95.10 ± 35.90	86.22 ± 33.58	83.58 ± 34.64	76.67[a]*,[b]** ± 32.73	72.78[a]*,[b]** ± 32.87	96.57 ± 34.56	94.58 ± 33.19
Thyroid stimulating hormone (µIU/ml)	2.94 ± 1.23	3.43 ± 1.17	5.30 [a]* ± 5.22	5.67 [a]* ± 5.29	7.86 [a],[b]** ± 1.62	7.08 [a],[b]** ± 4.94	2.89 ± 3.84	3.04 ± 2.46
Reverse Triiodothyronine (nmole/l)	1.57 ± 1.83	1.96 ± 2.94	1.66 ± 2.56	1.52 ± 1.73	1.83 ± 2.36	1.65 ± 2.71	1.05 ± 1.97	1.21 ± 2.11
Free Triiodothyronine (pg/ml)	5.06 ± 1.32	5.82 ± 1.24	4.11 ± 1.13	4.98 ± 1.14	4.69 ± 1.19	4.51 ± 1.23	5.60 ± 1.16	5.13 ± 1.29
Free thyroxine (ng/ml)	1.16 ± 0.20	1.21 ± 0.19	1.23 ± 0.26	1.15 ± 0.26	1.23 ± 0.24	1.15 ± 0.22	1.25 ± 0.26	1.16 ± 0.21

Student t.test: [a] Between groups comparison of male or female *β-thalassemia major, β-thalassemia minor* versus corresponding gender value in the control group: ** p<0.01,*p<0.05

[b] Comparison of male or female value of *β-thalassemia major versus corresponding gender in the β-thalassemia minor* group: ** p<0.01

Table (3): Comparison of the effect of age on the means of thyroid functions in different β-thalassemia major age groups (Group A: 3-14 years, Group B: 15-21, Group C: > 21years).

	Group A n=34	**Group B n=12**	**Group C n=14**
Triiodothyronine (nmole/l)	1.44±0,46	1.51±0.49	1.65±0.49
Thyroxin (nmole/l)	81.43 ±17.35	76.74±15.43	65.97 [a]*±13.54
Thyroid stimulating hormone (µIU/ml)	4.32 ±5.08	4.98 ±2.40	14.53 [a], [b]**±1.13
Reverse Triiodothyronine (nmole/l)	1.78±0.47	1.67±0.61	1.68±0.96
Free Triiodothyronine (pg/ml)	4.61±0.44	4.57±0.53	4.69±0.62
Free thyroxine (ng/ml)	1.19±0.26	1.29±0.28	1.13±0.29
Serum MDA mmole/L	8.11±1.97	9.18 [c]*±1.92	10.31[a]**, [b]*±1.99
Serum Ferritin (ng/ml)	2500±28.03	3250 [c]*±24.05	3678 [a]**, [b]*±23.46

Student t.test: [a] BT major age group C patients versus the age group A: * $p<0.05$, ** $p<0.01$

[b] BT major age group C patients versus the age group B:* $p<0.05$, ** $p<0.01$

[c] Age group B patients versus the age group A:* $p<0.05$

Table (4): Comparison of hypothyroidism in β-thalassemia major age subgroup C (> *21years)* with mean of the serum ferritin and malondialdehyde (MDA) concentration.

Hypothyroidism	Patients		Patients				Serum ferritin (ng/ml)	Serum MDA mmole/l
	Total		Male		Female			
	No	%	No	%	No	%	Mean ± SEM	Mean ± SEM
Primary	5	8.33	4	6.66	1	1.66	3721 [a]* ± 29.21	9.79 ± 1.91
Subclinical	7	11.67	4	6.66	3	5	2395 ± 30.07	9.33 ± 1.94
Total	12	20	8	13.33	4	6.66		

Student t.test: [a] Primary versus sub clinical hypothyroidism:*p<0.05

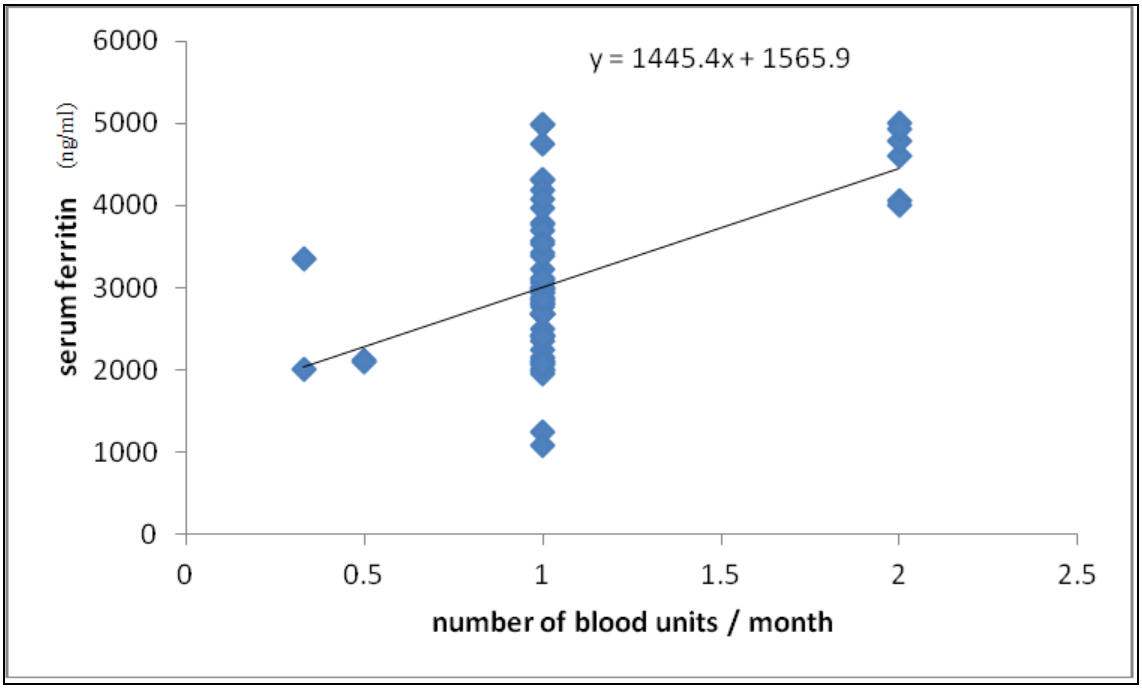

Figure (1): Spearman Rank correlation between frequency of blood transfusion and serum ferritin in β-thalassemia major group(r = 0.47, p<0.001)

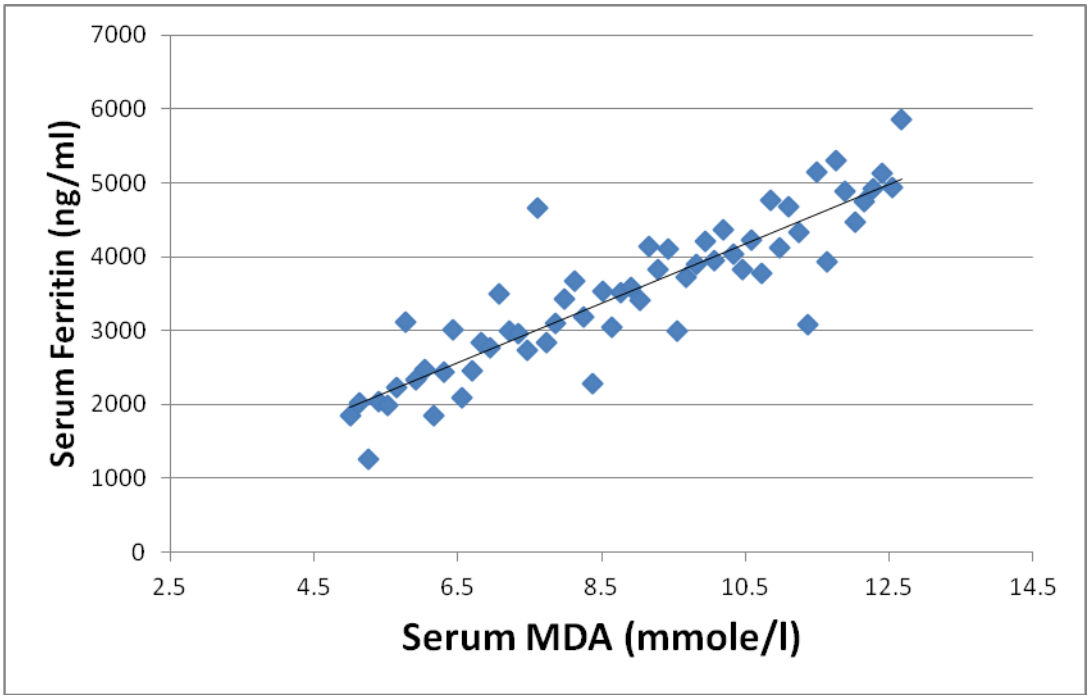

Figure (2*):* Spearman Rank correlation between serum malondialdehyde (MDA) concentration and serum ferritin concentration in β-thalassemia major group ($r = 0.47$, $p<0.001$).

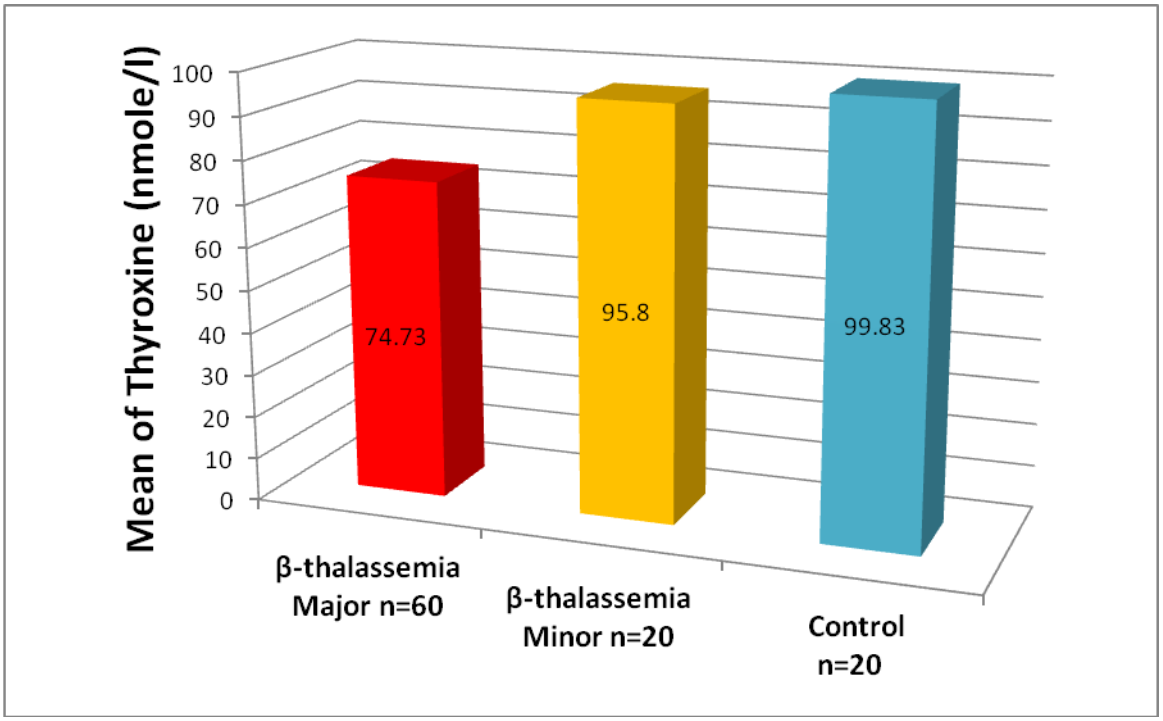

Figure (3*):* Bar graph of the mean concentration of thyroxine in β-thalassemia major, β-thalassemia minor and control groups.

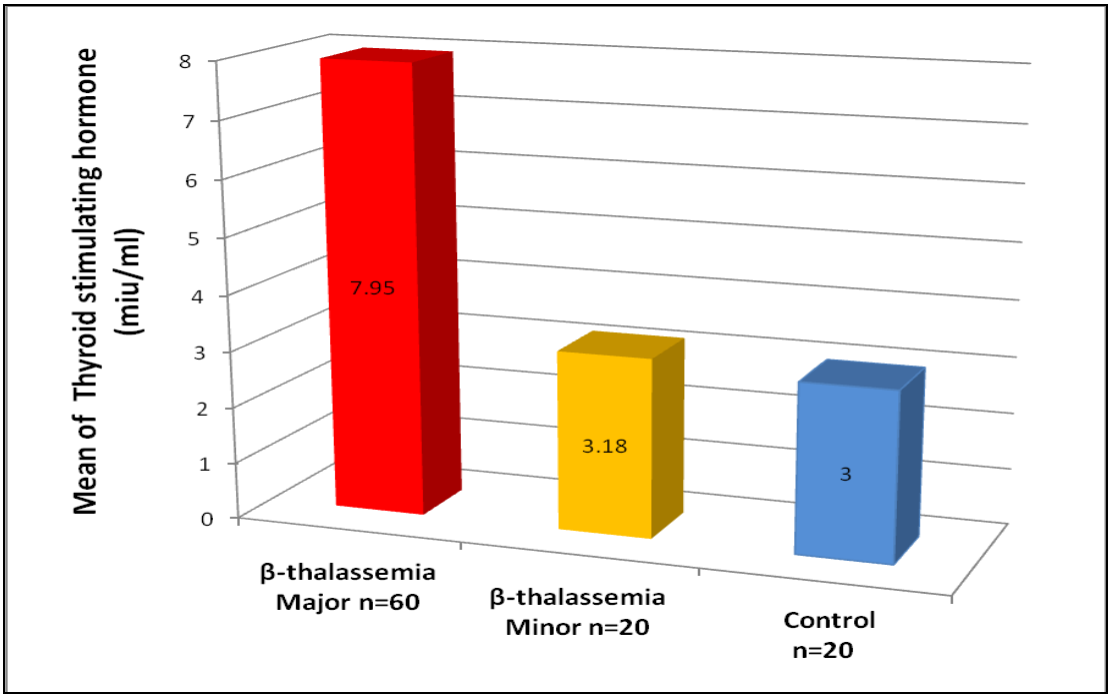

Figure (4)*:* Bar graph of the mean of thyroid stimulating hormone concentration in β-thalassemia major, β-thalassemia minor and control groups.

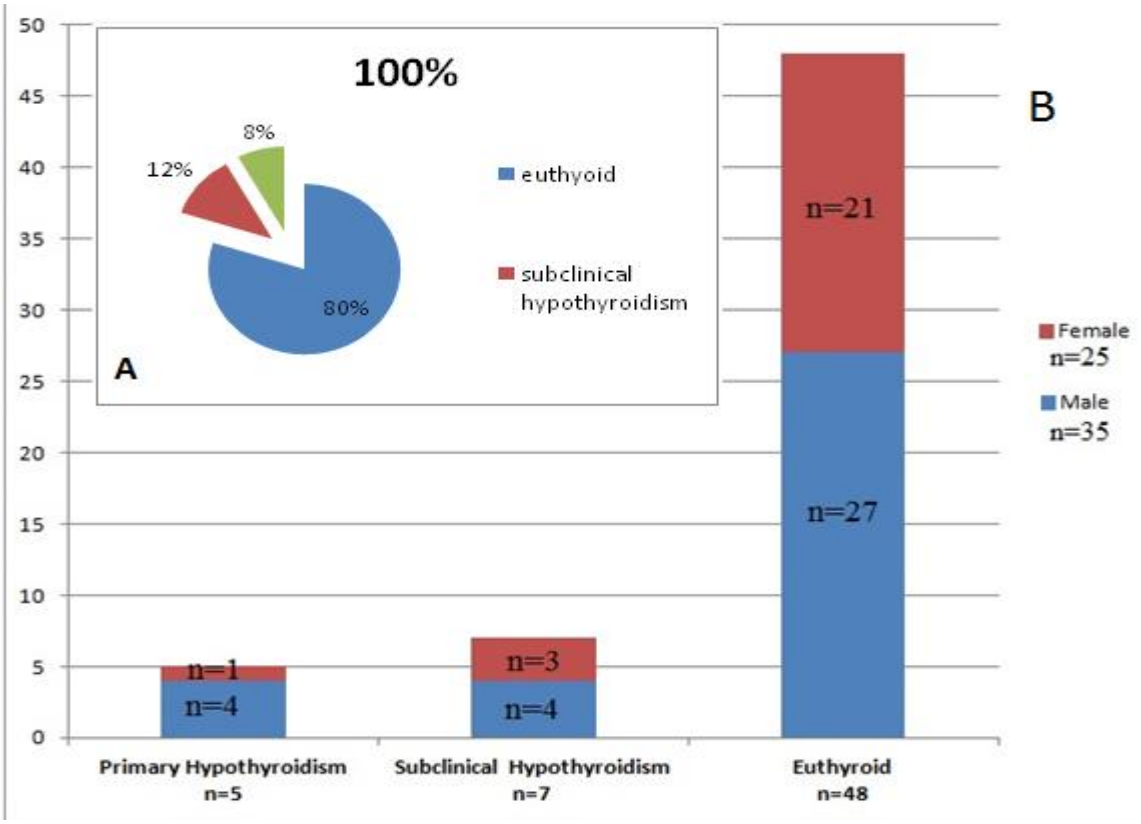

Figure (5)*:* Bar graph of (A) the percentage prevalence of hypothyroidism, (B) Gender distribution among β-thalassemia major patients of > 12 years of age according to types of hypothyroidism.

7. REFERENCES

[1] Higgs D.R. -Gene Regulation In Hematopoiesis: New Lessons from Thalassemia. Hematology: pp1 -13. 2004
[2] Hoffbrand A.V., Catovsky D. and Tuddenham E. Postgraduate Haematology 3rd ed. Blackwell Publishing, Oxford, , pp 85-103. 2005
[3] Lichtman M.A., Beutler E., Kipps T.J., and Williams W.J., Williams Manual of Hematology 6thed. McGraw-Hill, New York, , pp 91-98. 2003
[4] Thein S.L., Hesketh C, Wallace R.B. and Weatherall D.J. - The molecular basis of thalassemia major and thalassemia intermedia in Asian Indians : application to prenatal British journal of haematology, 70: pp 225-231. 1988,
[5] Thong M,Tan J.A,Tan K.L. and Yap S.F.-charctrization of β-Globin Gene Mutation in Malaysian children :a strategy for The Control of β-Thalassemia in Developing country.Journal of Tropical Pediatrics, , 51: pp326-333. 2005
[6] Wondisford F.E.Magner J.A.and Weintraub B.D. chemistry and biosynthesis of thyrotropin. The thyroid, 7th ed. Philadliphia: Lippincott-Raven, , 190: pp 207. 1996
[7] Shamshirsaz A. A., M. R. Bekheirnia, M. Kamgar, N. Pourzahedgilani, N. Bouzari, M. Habibzadeh, R. Hashemi1, A. A. Shamshirsaz, S. Aghakhani, H. Homayoun and B. Larijani. Metabolic and endocrinologic complications in beta-thalassemia major: a multicenter study in Tehran. BMC Endocrine Disorders, 3: pp 1-6. 2003
[8] Magro S., Puzzanio P., Consarino C., Galati MC, Morgione S., Porcelli D., Grimaldi S., Tancre D., Arcuri V., De Santis V: Hypothyroidism in patients with thalassemia syndromes. Acta Haemato, , 84: pp 72-6. 1990
[9] Green ST, Ng JP, Chan-Lam D: Insulin dependent diabetes mellitus, myasthenia gravis, pernicious anemia, autoimmune thyroiditis and autoimmune adrenalitis in a single patient. Scott Med J 33: pp 213, 1988.
[10] Rund D, Rachmilewitz E., Beta–thalassemia. N Engl J Med, 353: pp 1135 - 1146. , 2005
[11] Hohenwallner W., Wiesinger K., Wimmer E. The reticulocytes: automatic counting, indication and interpretation. Sysmex J Int.;2: pp 120-135. 1992
[12] Richard A. Goldsby. Thomas, Antigen-Antibody interactions, principles and applications, Kuby immunology; Chapter 6, pages 147-150. , 2007
[13] David McAuley, Pharm.D. , Basic Skills in Interpreting Laboratory Data, 2012.
[14] Young ds, effect of drugs and disease on clinical lab 4th edition, 2001.
[15] Deighton N, Hider RC: Intracellular low molecular weight iron. Biochem Soc, Trans, 17: pp 490. 1989,
[16] Fong, K.L., McCay, P.B., and Poyer, J.L.; J. Biol. Chem., evidanc that peroxidation of lysosome membrane in intiated by hydroxyl free radicals production during flavine enzyme activity, , 248: pp 7792. 1973
[17] Irshaid F. and Mansi K. Status of Thyroid Function and Iron Overload in Adolescents and Young Adults with Beta-Thalassemia Major Treated with Deferoxamine in Jordan. International Journal of Biological and Life Sciences 7:1: pp 47-52. 2011
[18] Karamifar H, Karimi M, Amirhakimi GH, Badiei M. Endocrine function inthalassemia intermedia. IJBS; 2: pp 236-240. 2006
[19] Brechot C. Hepatitis virus C infection. In: New Trends in Therapy for Hemoglobinopathies and Thalassemias. Paris, , pp 19-22, 1994.
[20] Bassett ML, Halliday JW, Powell LW. Value of hepatic iron measurements in early hemochromatosis and determination of the critical iron level associated with fibrosis. Hepatology; 6: pp 24-9. 1986
[21] Bacon BR. Causes of iron overload. N Engl J Med; 326: pp 126-7. 1992.
[22] Ra'id M. Hannun. Al-Salih , Mohammed A. Awda Ali M. Ali AL-Azeirjawy, Oxidant and Antioxidant Status in Serum of Patients with beta-Thalassemia After and Before Blood Transfusion. Journal Of College Of Education: pp 302-326. , 2011
[23] Subuh S. Al-Mudalal, Raad J. Musa, Najat Al-Duliami. Antioxidant Status In Thalassemic Patients, Iraqi J Med Sci, Vol. 4: pp 38-44. 2005;
[24] Naithani, R. , Chandra, J. , Bhattacharjee, J. , Verma, P. , and Narayan, Sh., Pediatr. Blood Cancer, , 46: pp 780. 2006
[25] Owayes M. Al-Hassany. Lipid Peroxidation, Antioxidant Status and some Blood Components in β-Thalassemia. Jou. Raf. Sci., ,Vol. 21, pp. 24-32. 2010
[26] Pirinççioğlu A. Gözü,Turgay Deniz,; Deniz Gökalp, Nurcan Beyazıt, Kenan Haspolat, and Murat Söker. Assessment of Thyroid Function in Children Aged 1-13 Years with Beta-Thalassemia Major. Iranian Journal of Pediatrics; 21: pp 77-82. , 2011
[27] Kallistheni Farmaki, Hypothyroidism in Thalassemia, Hypothyroidism - Influences and Treatments, Dr. Drahomira Springer (Ed.), ISSN: 978-953-51-0021-8. 2012,

[28] Mehrvar A , Azarkeivan A , Saberi Nejad, Mehrvar N, Faranoosh M, Vosoogh P. Prevalence of hypothyroidism and hypoparathyroidism in Iran. Blood J 5(1): pp 53-59. 2008

[29] De Sanctis V, De Sanctis E, Ricchieri P, Gubellini E, Gilli G, Gamberini MR, Mild subclinical hypothyroidism in thalassaemia major: prevalence, multigated radionuclide test, clinical and laboratory long-term follow-up study, Pediatr Endocrinol, , Vol.6, pp. 174-80. 2008.

[30] De Sanctis V, Tangerini A, testa MR, et al. Final Height and endodcrine function in thalassemia intermedia. Endocrinol Metab. 11: pp 965-971, 1998.

[31] Sabato AR, De Sanctis V, Atti G, et al. Primary hypothyroidism and the low T3 syndrome. Arch Dis Child,;58:pp120-127. 1983.

[32] Alireza Abdullah et al.: Metabolic and endocrinologic complications in beta-thalassemia major: a multicenter study in Tehran. BMC Endocrinol Disord.,3: pp 4, 2003.

[33] Hashemi.A1 MD, Ordooei.M2 MD, Golestan.M3 MD, Akhavan Ghalibaf. M4 MD, Mahmoudabadi. F4 BSc, Arefinia. M4 BSc, Amanat. M4 BSc, Purshamsi.F4 BSc, Afkhami.M4 MD, Hypothyroidism and Serum Ferritin Level in Patients with Major ß Thalassemia, 2011.

[34] Malik SA, Syed S, Ahmed N. Frequency of hypothyroidism in patients of beta-thalassemia. Pak I Med Assoc; 60: pp 17-29. 2010

[35] Klein I, Danzi S., Thyroid disease and the heart. Circulation, 2007, vol. 116, pp. 1725.

[36] Bernal J., Action of thyroid hormone in brain, Journal of Endocrinological Investigation, , Vol. 25, pp268–288. 2002.