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Assessment of Liver, Thyroid Gland and Growth Hormone Functions in Beta Thalassemia Major

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Abstract

Labile plasma iron and tissue iron overload are major complications of thalassemia disease that increase mortality rate. The iron that is exceeding the capacity of transferrin and ferritin is the leading cause of cell oxidation of many organs such as liver, heart, endocrine systems, etc. This study is designed to investigate the status of liver, thyroid gland and the growth hormone in beta thalassemia patients. In a cross-sectional study, 65 samples of beta thalassemia major were taken who were on a regular chelation therapy and blood transfusion and were to be compared with reference values. The results of the study estimated that 98.46% of the cases had high serum ferritin level, 12.3% high ALT, 27.7% high AST, 86.15% high ALP, 69.23% high total serum bilirubin, and 36.92% high TSH level. The results revealed that more than half of the patients had Growth Hormone Deficiency (GHD). The serum ferritin was found to be correlated with ALT and AST enzymes ($p< 0.01$. Furthermore, alkaline phosphatase and serum bilirubin can be good markers for monitoring bile duct obstruction resulted from hemolysis and blood transfusion.

Keywords: Beta thalassemia major, growth hormone, thyroid hormones, and beta thalassemia.

تقدير وظائف الكبد، الغدة الدرقية، و هورمون النمو عند مرضى بيتا الثالاسيميا الرئيسى

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الخلاصه

ان الحديد الحرفى الدم و المتراكم فى الانسجة من العوامل الرئيسية المسببة للكثير من الاعراض الجانبية عند مرضى الثالاسيميا مزيدا بدورهما احتمالية الوفاة. عندما تكون نسبة الحديد اعلى من القابلية الاستعابية للترانسفيرين و الفيريتين فأن تلك النسبة الزائدة من الحديد تكون حرة و تسبب تأكسد الخلايا فى جميع أعضاء الجسم كالكبد، القلب، الغدة الدرقية و الغدد الصماء. تم جمع ٦٥ عينة فى دراسة مقطعية من شريحة مرضى البيتا الثالاسيميا الرئيسى. أظهرت النتائج بأن ٩٨.٤٦٪ من الحالات لديم مستوى عالى من الفيريتين، ١٢،٣٪ ALT عالى، ٢٧،٧٪ AST عالى، ٨٦،١٥٪ ALP عالى، ٦٩،٢٣٪ TSB عالى، و ٣٦.٩٢٪ TSH عالى. اضافة الى ذلك أظهرت النتائج أن أكثر من نصف المرضى لديهم نقص في هرمون النمو. كذلك تم

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ايجاد علاقة بين نسبة الفيريتين الموجود فى الدم و نسبة الانزيمات ALT وASTمستوى معنوية ($p< 0.01$).
هذه العلاقة تبين بأن فيريتين الدم يمكن استخدامه كمؤشرلكمية الحديد المخزون فى الكبد. ALP و
TSBتبين أيضا بأنهما مؤشران جيدان انسداد القناة الصفراوية الناتج عن تحلل و نقل الدم.

1. Introduction

In beta thalassemia major, the levels of labile plasma iron become high due to the high degradation rate of RBC and blood transfusion [1]. If plasma iron surpasses the binding capacity of transferrin, it can permeate into the organs especially the liver. Hence increasing the tissue iron overload and radical toxicity [2]. Normally plasma iron bounds with transferrin, passes into spleen and liver through transferrin receptor to be stored as ferritin [3,4]. But when the releasing iron exceeds the capacity of either transferrin or ferritin, due to the two previous reasons, it accumulates inside the tissues potentially damaging organs such as in liver, heart, endocrine glands, pancreas, etc [5].

The excess iron has a significant role in catalysing more free radicals which are responsible for necrosis of many organs among many thalassemia patients [6]. There are many studies that have referred to the importance of reducing iron level by chelation therapy. An imbalance makes the reactive oxygen cause cytotoxicity [7]. Currently serum ferritin is routinely measured to assess the status of iron overload and to regulate the chelation therapy because serum ferritin rises proportionately in most of the patients of thalassemia major [8]. Altogether labile plasma iron, tissue iron overload, and serum ferritin are good markers for showing the potential of radical toxicity as they increase during iron overburden [5].

Previously it was shown that liver transaminase enzymes can be correlated with ferritin level especially when serum ferritin exceeds 1000 ng/L [8]. Further evidence on hepatic damage, a histological examination of the liver showed that most of the thalassemia patients who have iron overload has also swallowed hepatic mitochondria detected by electronic microscope [9]. Bile duct obstruction is another complication of beta-thalassemia major. The high rate of hemolysis causes an over production of bilirubin, which is a precursor for gallstones formation especially among the older ones [10]. It is referred to as the gallstones that are promoted by precipitation of highly produced unconjugated bilirubin and calcium bilirubinate inside the gallbladder lumen [11].

Thyroid dysfunction is another complication caused by iron overload occurring as a result of hemolysis and blood transfusion [12]. It has been reported that thalassemia patients experience faster permanent thyroid dysfunction when ferritin level is above 1800 mg/L or when subjected to late chelation therapy [13]. The correlation between hypothyroidism chemical markers and serum ferritin level is still controversial. Some studies have found that out but others did not find any such correlation [14,15]. As hypothyroidism is the outcome of impairments that could occur in hypothalamus, pituitary, thyroid gland axis. The following biochemical markers are imperative for knowing the location of impairment (primary, secondary, or tertiary); serum thyroid releasing hormone (TRH), serum thyroid stimulating hormone (TSH) and serum bound thyroxines T4 and T3 and free thyroxines FT4 and FT3 [16].

Growth hormone impairment is another characteristic of thalassemia in which the pituitary gland is significantly intoxicated by iron overload [17]. There are many studies that experienced growth retardation in 53% of beta thalassemia major. The growth retardation is characterized by reduction in growth hormone - insulin like growth factor-1 axis [18,19]. Some data analyses also revealed that IGF-1 secretion is not responding and maintained low despite GH administration, knowing that IGF-1 is majorly regulated by growth hormone besides thyroxin and slightly by sex steroid [20, 21]. In addition to Soliman, et al. (2015) referred to that IGF-1 level can be affected and improved by the administration of some supplements such as vitamin D and zinc [22].

It is noteworthy to refer that there is a mutual contribution of both thyroid and growth hormones in affecting the growth rate that some described it as a complex inter-relationship [23,24]. There are some studies that have observed a lower storage level of growth hormone (GH) in male rats when subjected to provoked hypothyroidism [25].

This study is aimed at assessing the liver function by measuring enzymes that are sensitive to hepatocyte impairment, along with the evaluation of thyroid gland function and growth hormone among beta thalassemia major.

2. Experimental Part

2.1Study Method

A cross sectional study was carried out on 65 cases of beta thalassemia major 43 males and 22 females, ages ranged between 15 to 34 with mean value 21.74 ± 4.5. All of the patients were on a regular chelation therapy and blood transfusion. All participants were given an explanation of the study and a prior consent was also obtained. The study excluded autoimmune diseases and alcohol drinkers. Blood samples were taken during October to December 2020 at 8 to 9 AM at the Thalassemia Center/Sulaimani city. All experiments that followed were in accordance with Helsinki Declaration of 1975, as revised in 2000. The blood samples were let 15 minutes to clot, centrifuged at 1200x for 15 minutes, and then measured by auto analyser cobas 4000, measuring the liver enzymes ALT, AST, and ALP, total serum bilirubin (TSB), serum ferritin, serum transferrin, total iron binding capacity, serum iron, growth hormone (GH), insulin like growth factor-1 (IGF-1), thyroid stimulating hormone (TSH), total thyroxine(T4) and free thyroxine(FT4), total triiodothyronine (T3) and free triiodothyronine (FT3).

2.2 Data Analysis

The collected data was compared with the references values regarding to gender. The data was submitted to analysis variance (ANOVA), estimating mean ± standard deviation and Pearson's correlation coefficient with significance level $p < 0.01$. The data was sorted and statistically analysed by SPSS 16.

3. Results

The samples were sorted into male and female groups in order to estimate the levels of each biochemical marker; high, low, and normal with regarding age of the patients especially for alkaline phosphatase (ALP) and insulin like growth factor 1 (IGF-1).

The data analysis showed a significant number of the patients 97.7% of the males and 100% of females have high serum ferritin level, with the average value of 1447 and 1298 ng/mL for male and female respectively. 26.1% of total sample had ferritin above 1800 ng/mL that proved of having cumulative effects Tables 3.1 and 3.2.

Table 3.1 - Descriptive analyses of serum chemical tests for male group

Tests	Reference value		Abnormal Cases	Mean ± Std. dev.
	Normal range	Age		
Serum Ferritin	24 – 336 µg/L	Adult	42 (97.7%) H	1447 ± 938.9
TS%*	20% – 50%	Adult	32 (74.4%) H	66.3 ± 22
ALT	< 45 IU/L	Adult	4 (9.3%) H	24.03 ± 14.29
AST	< 35 IU/L	Adult	22 (51.2%) H	37.7 ± 18.58
ALP	82 – 331 IUL	15 – 17 year	35 (81.4%) H	290.65 ± 168
	55 – 149 IU/L	7- 19 year		
	40 – 129 IU/L	≥19 year		

TSB	0.2 – 1.4 mg/dL	Adult	31 (72.1%) H	2.07 ± 1.45

* Male group n = 43, TS%, Transferrin saturation; H, high; L, low

Transferrin saturation is another alternative parameter that reveals the status of labile plasma iron and iron overload, especially when serum ferritin level in not significant. It shows free iron in the blood. The results showed 74.4% of male and 54.5% of female patients had transferrin saturation above normal range. The average values were 66.3 and 61.7 for males and females respectively. Despite serum ferritin and transferrin factor gave strong indication during beta thalassemia major but their correlations were not relevant.

The liver function was examined testing alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total serum bilirubin (TSB). The results showed that 9.3% of the male patients had high ALT with the average value 24.03±14.29IU/L.18.2% of the female patients showed high ALT value 23.89±11.97 IU/L. AST level was also high for 51.2% of the male group 37.7 ± 18.58 IU/L and 36.4% of female group with mean value 33.17 ±12.89 IU/L. The data analyses revealed that in both male and female groups, the patients who had high ALT also had high AST level assigned to the hepatic damage.

Table 3.2 - Descriptive analyses of serum chemical tests for female group.

Tests	**Reference value**		**Abnormal Cases**	**Mean ± Std. dev.**
	Normal range	**Age**		
Serum Ferritin	20 – 307 μg/L	Adult	22 (100%) H	1298 ± 682
TS%*	15% – 50%	Adult	12 (54.5%) H	61.7 ± 29.5
ALT	< 35 IU/L	Adult	4 (18.2%) H	23.89 ± 11.97
AST	< 31 IU/L	Adult	8 (36.4%) H	33.17 ± 12.89
ALP	50 – 117 IUL	15 – 17 year	21 (95.4%) H	373.14 ± 160
	35 – 104 IU/L	17- 19 year		
	40 – 129 IU/L	≥19 year		
TSB	0.2 – 1.4 mg/dL	Adult	13 (59.1%) H	1.79 ± 0.93

* Female group n = 22

81.4% of male and 95.4% of female patients showed high ALP enzyme activity 290.65±168IU/L and 373.14±160 IU/L respectively. Also 72.1% of males and 59.1% of females tested high total serum bilirubin 2.07±1.45 mg/dL for males and 1.79±0.93 mg/dL for females.

Pearson correlation plot revealed that ALT and AST levels are moderately related with serum ferritin level (r = 0.559 and 0.508) respectively with $p < 0.01$. This correlation between serum ferritin and liver enzymes ALT and AST justifies the statement that ferritin is an indicator for evaluating the liver status Figure 3.1. Regarding ALP and total serum bilirubin, they did not show any correlations with serum ferritin level (not shown).

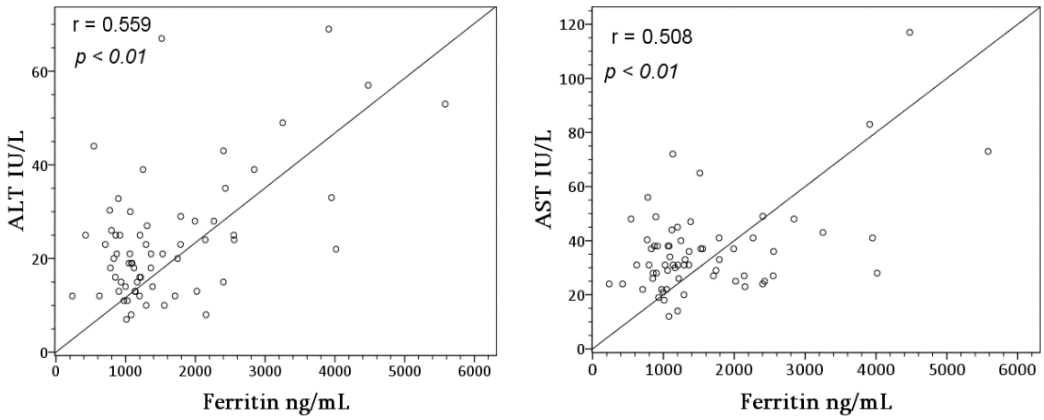

Figure 3.1-Pearson correlation coefficient of ALT and AST enzymes against serum ferritin

Table 3.3 - Descriptive analyses of the hormone tests for male group

Tests	Reference value		High or low cases	Mean ± Std. dev.
	Normal range	Age / year		
GH	0.4 – 10 ng/mL	Adults	22 (51.2%) L	1 ± 1.15
IGF-1	134 – 836 ng/mL	15 – 18	36 (83.7%) L	106.9 ± 50.8
	202 – 433 ng/mL	19 – 25		141.6 ± 122
	135 – 449 ng/mL	25 - 85		101.1 ± 71.23
TSH	0.3 – 4.2 mIU/L	> 13	17 (39.5%) H	4.33 ± 2.85
FT3	3.1 – 6.8 pmol/L	Adult	0	6.5 ± 1.07
FT4	12 – 22 pmol/L	1 – 87	1 (2.3%) L	16.8 ± 3.5
T3	80 – 210 ng/L	1 – 20	0	151 ± 23.9
	70 – 204 ng/L	20 – 50		132 ± 54.6
T4	4.6 – 11 μg/L	15 – 60	0	8.75 ± 2.1

Regarding thyroid gland function, TSH levels were high in 39.5% males and 31.8% females 4.33±2.85 mIU/L and 4.13±3.09 mIU/L respectively. While T3 and T4 did not show any abnormality and were almost within the reference range. Only two patients, one female and one male, had low FT4 level Tables 3.3 and 3.4.

Table 3.4-Descriptive analyses of the hormone tests for female group

Tests	Reference value		High or low cases	Mean ± Std. dev.
	Normal range	Age / year		
GH	1 – 14 ng/mL	Adult	12 (54.4%) L	1.45 ± 1.7
IGF-1	152 – 660 ng/mL	15 – 18	19 (86.4%) L	145 ± 77.6

	231 – 550 ng/mL	19 – 25		127.5 ± 46.9
	135 – 449 ng/mL	25 – 85		67.11 ± 30.1
TSH	0.3 – 4.2 mIU/L	> 13	7 (31.8%) H	4.13 ± 3.09
FT3	3.1 – 6.8 pmol/L	Adult	0	6.2 ± 1.14
FT4	11 – 22 pmol/L	1 – 87	1 (4.5%) L	18.33 ± 4.27
T3	80 – 210 ng/L	1 – 20	0	137.8 ± 20.6
	70 – 204 ng/L	20 – 50		137.1 ± 30.4
T4	5.5 – 11 μg/L	15 – 60	0	9.02 ± 1.56

The GH – IGF-1 axis tests, revealed low levels of GH for 51.2% males and 54.4% females, whereas IGF-1 levels were low for 83.7% male and 86.4% females. It was estimated within the two groups that 18 males and 11 females had low GH and low IGF-1 as well which is accepted as non-primary growth hormone deficiency. There were not any correlations of serum ferritin with any of the parameter used for examination of thyroid gland and growth hormone.

4. Discussion

Thalassaemia is an inherited blood disorder that leads to haemolytic anaemia with different rates, depending on the severity of the mutation [26]. It is imperative to check the status of organs because beta thalassemia major has cumulative effects and causes many problems to the body such as growth delay, liver cirrhosis, thyroid dysfunction, enlarged spleen, etc [27].

During haemolysis and blood transfusion ferritin and transferrin saturation become high in order to compensate the high level of labile plasma iron and to reduce the radical toxicity in and outside the cells [28,29]. More than 70% of body iron is stored in the liver in the form of ferritin with certain amount of ferritin is secreted into the plasma. The concentration of plasma ferritin is positively correlated with the size of body iron store [30]. Therefore, to limit the risk of liver damage and protect other organs from the oxidative stress, it is vital for thalassemia patients to periodically monitor the level of ferritin and manage the excess iron when needed by chelation therapy [31]. The reason of continuously monitoring ferritin is that if the iron level exceeds ferritin capacity, free iron spills over into the hepatic parenchyma cells causing hepatic necrosis with the risk of late development of fibrosis and cirrhosis [32]. As hepatic iron overload cannot be completely avoided, it is imperative to measure ALT and AST enzymes periodically to assess the scale of hepatic damage as damaged hepatocytes release more ALT and AST enzymes into the blood [33].

It was observed that a higher rate of total cases 46.5% had high AST level more than the cases that have high ALT 12.3%. This difference could be due to the more specificity of ALT to the hepatic tissues whereas AST is not specific and is available inside many tissues and red blood cell. Therefore, it can be assigned that AST moderately increases under two circumstances: one as a result of high haemolysis rate and secondly due to hepatic damage in thalassemia patients.

Bilirubin is another product of erythrocyte haemolysis which is metabolized in the liver, to be stored in the gallbladder. Most of beta thalassemia major shows a high level of serum bilirubin [34]. Increased levels of both bilirubin and ALP clarify high risk of bile duct narrowing and gallstone formation. Overproduced bilirubin that cannot be excreted by the body, will eventually accumulate in the gallbladder as calcium bilirubinate. Hence, leading to

gallstones obstructing the bile duct [35]. Obstruction of bile duct for long period of time also leads to high serum ALP level, as ALP is highly available along the bile tree and stimulated by the trapped bile acid [36]. Altogether, high levels of ALP and serum bilirubin are chemical markers for prognosis of cholestasis in most thalassemia patients.

Short stature is a prevalent feature of beta thalassemia patients due to impairment in the GH IGF-1 axis [18,37]. In the current study, it turned out that 18 out of 23 male patients who had abnormal GH levels also had low IGF-1 levels. Same thing is true for 11 out of 12 female patients. It can be assumed that the iron overburden can affect the organs separately and with different severity. The iron overload affected the hypothalamus and/or pituitary in patients with low GH and IGF-1and the liver for those with only low IGF-1 level separately [38]. Patients with low GH and IGF-1 levels can be categorized as non-primary growth hormone deficiency. However due to the lack of GHRH test, it cannot be verified whether the low levels of GH and IGF-1 are caused by hypothalamus or pituitary impairment [39]. It is a fact that ALT and IGF-1 can be used to monitor the liver status. Previous studies relied on liver function and IGF-1 tests for concluding liver healing when returning IGF-1 to high level and ALT to low level while studying the improving role of chelation therapy. The current study, on the other hand, does not observe any negative correlation between ALT and IGF-1 [40,41].

Impairment of thyroid hormone axis can be related to the primary, secondary, or tertiary hypothyroidism depending on the accumulation site of the excess iron whether in hypothalamus, pituitary, or thyroid glands [14,15]. According to data analysis 39.5% males and 31.8% females showed high TSH levels and normal T4 and T3 levels. Hence, it can be referred that the iron accumulation having damaging effect on thyroid gland is at the early stage (subclinical hypothyroidism) before T4 and T3 levels could change [42].

There are also some conflicting reports on T4 and T3 results. A study reported that no significant differences have been estimated in T3 between the control and case groups, but it did for T4 [43]. Whereas another study referred to primary hypothyroidism as the prevalent case among thalassemia patients in which TSH is high and T4 and T3 are low [44]. Finally, there were not any relevant correlations between thyroid functional tests and serum ferritin.

5. Conclusion

It is relevant though that serum ferritin is an important indicator of iron overload. It was deduced that iron overload has a great contribution in the elevation of liver enzymes. The shown correlation coefficient between ferritin, ALT and AST enzymes proved that the iron toxicity can significantly damage hepatocyte. Alkaline phosphatase and serum bilirubin are also proved to be good markers for prognosis of gallstones as long as their elevations depend on the rate of haemolysis and blood transfusion, especially among older thalassemia patients. Some patients in both groups showed lower levels of GH and IGF-1 so it can be concluded that hypothalamus and/or pituitary glands have been damaged.

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