Evaluation of some elements and biochemical parameters levels in the serum of β-Thalassemia major.

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Abstract

Thalassemia is one of the most dangerous disease. It is an inherited of hemoglobin production , in which there is partial or complete failure of the synthesis of goblin chain .The samples were collected from patients whom consulted the "Thalassemia Center" in the Babylon Hospital – Babylon Governorate – Iraq for the care and treatment thalassemia patients during the period of January 2011 to May 2011 . 50(30male and 20 female) subjects with beta thalassemia major were included .Serum levels of Sodium, potassium ,Calcium ,Iron, Total Iron Binding Capacity , and Ferritin were measured .The finding were compared with 40 (25male and 15 female) matched healthy controls. A significant increase in levels of potassium, Calcium ,and Iron ($p<0.005$) and ferittin ($p<0.001$). Non significant increase in the sodium and total iron binding capacity levels were found in the patients when compared with controls .It has been concluded that thalassemic patients presented multiple abnormalities due to repeated transfusion , iron overload ,hepatitis ,cardiac and renal dysfunction.

Keywords

β-Thalassemia major, Sodium ,Iron overload , Ferittin

الخلاصة

الثلاسيميا هي احد الامراض الخطرة الوراثية لتخليق الهيموكلوبين ، والذي يؤدي الى فشل جزئي أو كلي في تخليق سلسلة الكلوبين.تم جمع النماذج من المرضى المراجعين لمركز الثلاسيميا في مستشفى بابل –محافظة بابل- العراق- لغرض العناية والعلاج وللفترة الممتدة من كانون الأول 2011ولغاية مايس 3`20 . 50(30 ذكر و20 انثى) من المصابين بالثلاسيميا العظمى شملوا بالدراسة .تم قياس الصوديوم ، البوتاسيوم ، الكالسيوم ، الحديد ،السعة الكلية لارتباط الحديد والفرتين . النتائج تمت مقارنتها مع 40 (25ذكر و15 أناث) من الاصحاء السيطرة. كانت زيادة معنوية في كل من البوتاسيوم ، الكالسيوم والحديد قيمة pاقل 0.005 والفرتين p أقل 0.001 لم تلاحظ زيادة معنوية في قيم كل من الصوديوم والسعة الكلية لارتباط الحديد للمرضى عند مقارنتها بالأصحاء السيطرة . تم الاستنتاج بأن مرضى الثلاسيميا موضوع الدراسة المقدمة تحدث لديهم تغيرات متعددة غير طبيعية ناتجة بسبب نقل الدم المتعدد زيادة الحديد التهاب الكبد إضافة الى فشل القلب والكلى.

الكلمات الدالة : بيتا ثلاسيميا العظمى ، الصوديوم ، التركيز الحديد الزائد ،الفرتين .

Introduction:

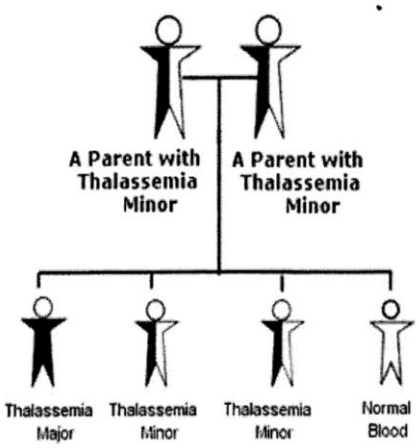
β-Thalassemia major was first described in 1925 by Thomas Cooley and Lee(Raghuveer 2009). Thalassemia occurs throughout the world and is one of the major public health ,such as the Mediterranean countries, Middle East, North Africa, and Asia(Olivieri1999).

The thalassemia syndrome are a group of inherited disorders characterized by microcytie 'hypochromic red blood cells (Weatherall1965) .

In thalassemia minor ,the hemoglobin gene are inherited during conception , one from the mother (egg) and one from the father

People with a thalassemia trait in one gene are known as carries or are said thalassemia minor .(Najdecki1998)

The severe form of thalassemia, thalassemia major, occurs when a child inherits two muted gene , one from each parent . Children born with thalassemia major usually develop the symptoms of severe anemia within the first year of life. (Zurlo1989)



They lack the ability to produce normal adult hemoglobin (red blood cell). Iron is a component of hemoglobin , essential to the body , but once red blood cells breaks down the excess iron cannot be removed , resulting in the absorption of too much iron in the body .(Aldouri1990)

It is necessary that the excess iron be removed because it store in the vital organs of the body ,such as the heart and liver . Fortunately ,a drug called Desferrioxamine (Desferal) is designed to remove the excess iron from the thalassemia patients . (Vichinsky2005)

Iron overload is the major cause of morbidity for patients on chronic transfusions .Nontransfused patients with thalassemia intermediary have increased intestinal absorption dietary iron as well and can have significant complications from iron overload as well.(Kontoghiorghes 2006)

Major morbidity and mortality can result from iron overload , liver dysfunction and failure ,pan-endocrine failure , heart failure and fatal cardiac arrhythmias , which constitute the main concerns in poorly chelated patients.(Raghuveer2009)

Patients and sampling:

Samples were collected from patients whom consulted the "Thalassemia Center" in the Babylon Hospital – Babylon Governorate – Iraq for the care and treatment thalassemia patients.50 transfusion dependent beta-thalassemia (30male and 20 female) subjects were selected in the present study during the period of January 2011 to May 2011. Their ages ranged between 5 to 21 years with mean age of 12.5±5yrs. All patients were evaluated with complete medical history, some examination as (Hb, PCV ,RBC) were carried investigation to all patients. Forty(25male and 15 female) healthy men and women donor were recruited to serve as controls.

Blood samples:

Five milliliters of blood were collected in morning from fasting subjects. The serum was obtained by leaving the blood samples to clot, centrifuged at 3000 rpm for 10 minutes .After centrifugation , aliquots of serum was stored at -20C^{o} until use for biochemical tests.

2.1-Determination of Sodium and potassium:

The concentration of sodium and potassium in serum was measured by the flame photometer type AFP100.

2.2.1-Determination of serum Calcium :

2.2.2-The Calcium was measured by the (Randox Kit). Calcium with O-cresolphtaleine complex compound ,at alkaline PH ,yield a red colored complex ,which intensity is proportional to the calcium concentration.

2.3.1Determination of total Iron :

The determination of total in the serum was achieved by colorimetric method with the (Randox UK) kit.

2-3-2Determination of total iron binding capacity:

The total iron binding capacity was determined by the methods as described in the ref(Pesce1987).

2-3-3Determination of serum Ferritin:

Serum Ferritin concentration was assayed using a commercial kit (Biosystems, Spain).The concentration of serum Ferritin in ng/ml was determined by the following formula .

Serum ferritin (ng/ml)=(A_2-A_1)sample/(A_2-A_1)standard x C

Where A_1 =first absorbance,A_2=second absorbance and C=standard concentration.

Statistical analysis

Statistical analysis was applied using SPSS 17 program computer. The result are expressed as number, range , percent, confidence interval C.I. 95‰ and whenever possible as mean ±SD (SE) of number of observation.

The data was analyzed by Z test were used .The differences were considered significant when $p \leq 0.05$.

Results and Discussion

Results of this study point to several significant finding .The hematological parameter of thalassemic patients showed much variation than reference values (table 1).The value of RBC for controls (4.65±0.25)million/ml for male and (4.55±0.14)million/ml for female and thalassemic patients (3.53±0.98) million/ml and(3.59± 0.86)million/ml respectively the p value was ($p<0.005$).

The patients were deprived of red blood cells due to their early degradation as a result of abnormal globins molecule(-Bushra2013, Che2013).

Most of the patients had moderate to severe anemia .This results in low hemoglobin in male (8.68±2.5)g/dl and female (8.44±2.46)g/dl while for controls (13.26±0.26) and (12.69±042)g/dl the p value was ($p<0.005$).

The packed cell volume PCV for the male patients was (28.7±8.05) and female patients was (28.35±7.54) while for the controls was (43.66±1.44) and (42.25±1.92) the p value ($p<0.005$).

Table 1-Hematological parameters in controls and thalassemia.

Variable		n	Mean ± SD	Range		SE	95%C.I.		*P* value
				Min	Max		lower	upper	
RBC (10^{12}/L) Patients	♂	30	3.43±0.95	2.2	5.40	0.18	3.26	3.93	p≤0.05
	♀	20	3.54±0.81	2.7	4.5	0.17	3.14	3.93	
RBC (10^{12}/L) controls	♂	15	4.25±0.23	4.12	5.13	.06	4.52	4.71	p≤0.05
	♀	12	4.52±0.14	4.3	4.45	0.04	4.41	4.62	
Hb for Patients (g/dl)	♂	30	8.68±2.5	6.0	15.5	0.46	7.72	9.64	p≤0.05
	♀	20	8.42±2.26	5.57	12.60	0.54	7.22	9.58	
Hb for controls (g/dl)	♂	20	13.15±0.5	12	14	0.13	12.85	13.53	p≤0.005
	♀	12	12.39±0.52	12.20	13.31	0.12	12.43	12.85	
PCV for Patients	♂	30	28.65 ±8.05	17.0	49	1.47	25.64	31.73	p≤0.005
	♀	20	28.15±6.54	16.0	41	1.48	24.52	31.41	
PCV for controls	♂	15	43.55±1.64	41.0	46.3	0.37	43.86	44.46	p≤0.005
	♀	12	42.76±1.52	40	46	0.41	41.32	43.19	

The results of serum sodium levels was summarized in table2-fig1. No significant difference was found between the mean serum Na in thalassemic and controls. For the male patients (154±23)mEq/l and mean of female (151±20)mEq/l and for controls (142±4)mEq/l and (142±2) mEq/l respectively the p value was ($p>0.05$).Although the Na level in thalassemic patients reveal higher level compared to the controls it may be due to the sodium butyrate that activate bone marrow.

Table 2-The pattern of Serum sodium levels in thalassemic and controls.

Variable Na^+ (mEq/L)	n	Mean ± SD	Range		SE	95%C.I.		*p* value
			Min	Max		lower	upper	
Patients male	30	154±23	134	182	4	146	163	
Controls male	15	142±4	141	149	8	140	144	$p>0.05$
Patients Female	20	151±20	126	183	4.5	141	160	$p>0.05$
Controls Female	15	142±2	135	145	0.9	140	143	
Total Patients Controls	50 30	153±21.91 142±3.11	130 136	211 149	3.1 0.56	146 141	159 143	$p>0.05$

The potassium levels(table3-fig2) showed that the mean K level in the thalassemic was significantly higher increase than in the controls ($p<0.05$),whereas an increased K level occurs in patients , with red blood cell hemolysis, which may occur in stored blood that is transfused to the patients since K tends to leak from RBC to the plasma in the stored blood(Prakash2013).

Table 3-The pattern of Serum potassium levels in thalassemic and controls.

Variable K^+(mEq/L)	n	Mean± SD	Range		SE	95%C.I.		*p* value
			Min	Max		lower	upper	
Patients male	30	5.58±0.94	3.90	7.60	0.17	5.22	5.93	*p*<0.005
Controls male	25	4.31±0.45	3.60	5.00	0.11	4.05	4.56	
Patients Female	20	5.89±0.51	5.50	6.80	0.11	5.65	6.12	*p*<0.005
Controls Female	15	4.23±0.28	3.90	4.80	0.07	4.07	4.39	
Total Patients Controls	50 40	5.70±0.80 4.27±0.37	3.90 3.60	7.50 5.00	0.11 0.06	5.47 4.13	5.93 4.41	*p*<0.005

The mean serum Ca level of patients was significant (p<0.005) when compared with controls(table4-fig3).The value in all patients were higher than in controls (fig3) Hypercalcemia in thalassemic patients remain unclear. Hypercalcemia in these patients may be seem to be related to hyperparathyroidism which is a well-known syndrome associated with thalassemia major.

Table 4-The pattern of Serum Calcium levels in thalassemic and controls.

Variable Ca++(mg/dL)	n	Mean ±SD	Range		SE	95%C.I.		*p* value
			Min	Max		lower	upper	
Patients male	30	12.40±3.1	5.90	21.8	0.54	11.28	13.53	
Controls male	25	9.53±0.53	9.10	10.30	0.13	9.23	9.83	*P*<0.005
Patients Female	20	13.23±2.50	5.29	16.20	0.56	12.06	14.40	*p*<0.005
Controls Female	15	9.32±0.56	8.50	10.10	0.18	8.91	9.72	
Total Patients Controls	50 40	12.73±2.82 9.45±0.45	5.9 8.50	16.20 10.30	0.39 0.10	11.93 9.22	13.54 9.67	*p*<0.005

From(table5-fig4)a significant increase (p<0.005) in serum iron was observed in beta- thalassemia major when compared with controls .

Excess iron is toxic to many tissues, including the liver, endocrine organ and heart, leading to a series of complications which cause morbidity and mortality in these patients(Ellis et al2010) .
The iron overload lead to formation of ROS such as superoxide anions

($^{\cdot}O_2^-$),hydroxide radical($^{\cdot}OH$) and hydrogen peroxide (H_2O_2) which induces oxidative stress in thalassemia major patients via Fenton reaction (2012, Fatemeh2011).

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^-$

Table 5-The pattern of Serum Iron levels in thalassemic and controls.

Variable Ironμmol/L	n	Mean ±SD	Range		SE	95%C.I.		*p* value
			Min	Max		lower	upper	
Patients male	30	29.44±6.03	15	37.8	1.10	27.19	31.69	*p*<0.005
Controls male	25	20.59±4.31	12.9	27.2	1.11	18.20	22.98	
Patients Female	20	26.93±5.62	16.90	35.0	1.25	24.30	29.56	*p*<0.005
Controls Female	15	18.90±1.60	15.90	21.30	0.41	18.01	19.78	
Total Patients Controls	 50 40	 28.44±5.94 19.74±3.31	 15.00 12.90	 36.20 27.20	 0.84 0.60	 26.75 18.50	 30.12 20.98	*p*<0.005

(Table6-fig5) showed a non significant value between thalassemic patients when compared with controls (p>0.05).The level of total binding iron capacity of patients was(56.50 ±19.79) μmol/L and the value of controls was (56.30±6.12) μmol/L. Our results was in agreement with other researchers.(Sonali et. al. 2013)

Table 6-The pattern of Serum Total Binding Iron Capacity levels in thalassemic and controls.

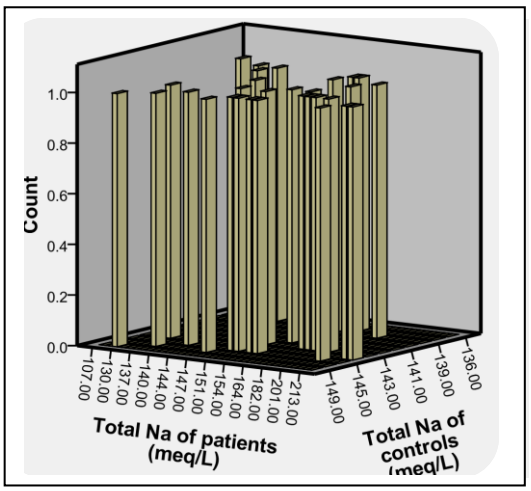
Variable T.B.I.C. μmol/L	n	Mean ±SD	Range		SE	95%C.I		*p* value
			Min	Max		lower	upper	
Patients male	25	54.59±20.66	25	90	4.3	46.01	60.26	*p*>0.05
Controls male	25	57.35±6.33	48	67	1.4	54.43	60.26	
Patients Female	20	58.59±18.88	30	88	4.57	48.88	68.30	*p*>0.05
Controls Female	15	54.69±5.82	48	67	1.61	51.17	58.21	
Total Patients Controls	 45 40	 56.50±19.79 56.30±6.12	 26 47	 88 67	 3.1 1.0	 50.18 54.13	 62.81 58.47	*p*>0.05

We studied the concentrations of serum Ferritin as shown in following table. Our finding showed that serum Ferritin level of β- thalassemia subjects was more than ten times the controls and also the standard deviation of study patients was much higher than normal healthy controls. In the thalassemic patients had increased serum ferritin levels, are known (Nadeem2004).

Serum ferrritin (table7-fig6) as an index of iron overload .A significant increase in serum ferrittin concentration(p<0.005) in β-thalassemia major patients compared to controls (2154±1321ng/ml vs. 155±56ng/ml respectively). Our findings are in agreement with the other researchers .(Sonali et. al. 2013)

Table7-The pattern of serum ferritin in thalassemic and controls .

Ferritin (ngm/ml)	N	Mean ±SD	Range		SE	95%C.I		p value
			Min	Max		lower	upper	
Patients male	30	2226±1316	459	5600	226	1773	2678	p<0.001
Controls male	25	193±46	55	234	11.9	167	218	
Patients Female	20	2160±1437	520	6100	293	1553	2767	p<0.001
Controls female	15	107±22	75	140	6	93	121	
Total Patients Controls	 50 40	 2154±1321 155±56	 459 55	 6100 234	 173 10.9	 1806 132	 2501 177	p<0.001



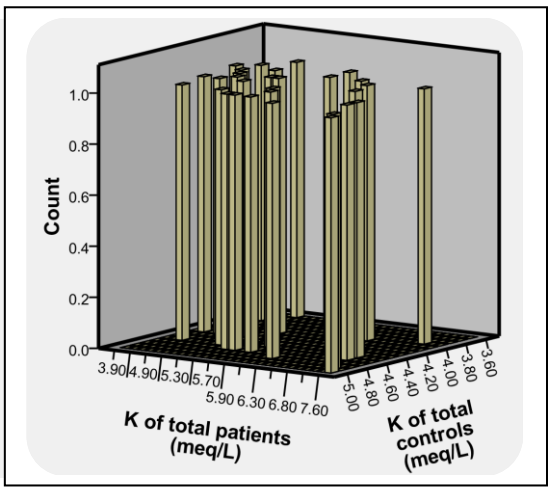
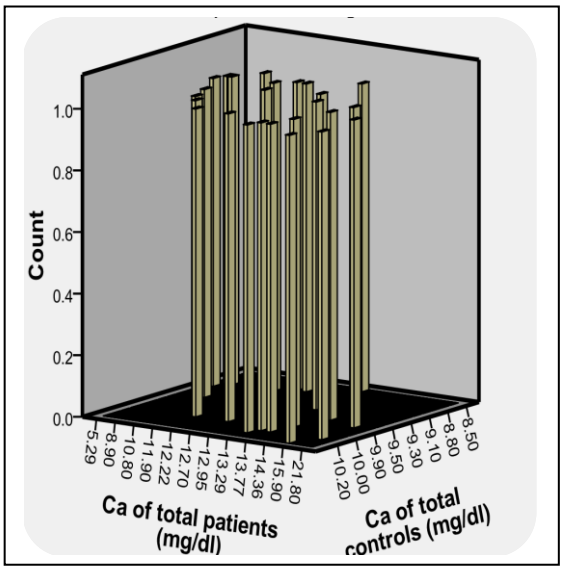


Fig1-The total sodium values of patients vs. controls. **Fig2-The total potassium of patients vs. controls.**



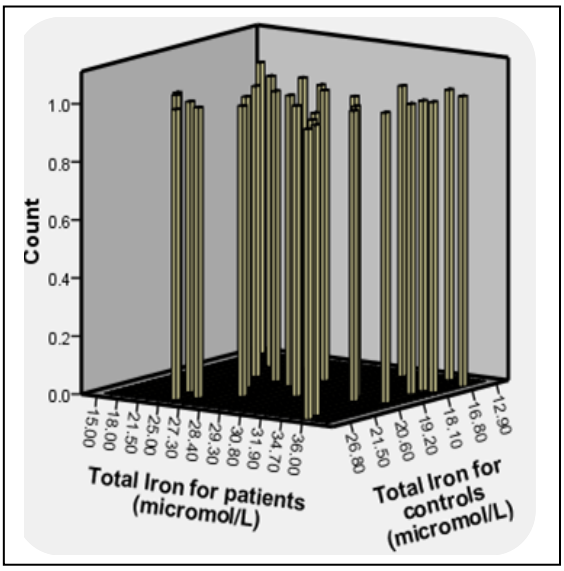
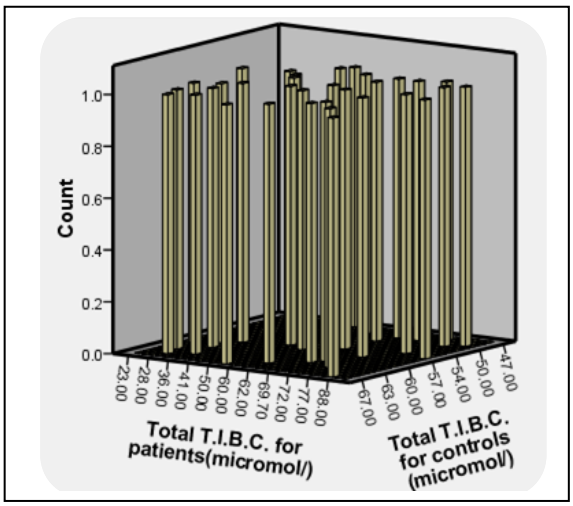


Fig3-The total calcium values patients vs. controls. **Fig4-The total iron values of patients vs. controls**



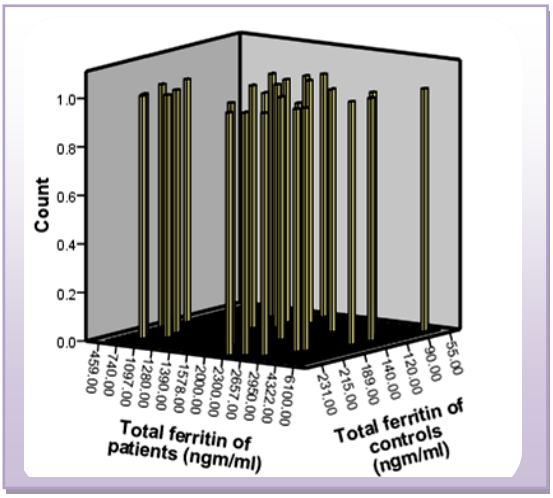


Fig5-The total TBIC values of patients vs. controls. **Fig6- The total ferritin of patients vs controls .**

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