Studies of Biochemical Changes in Serum of Patients with Different Types of Thalassemia

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Abstract

Background: Still more then 85 years after the fascinating initial description of peculiar bone changes and other signs and symptoms of the disorder, Thalassemia represent the most common single gene disorder causing a major public health problem.

Aim & objective:

1. To determine the changes in iron status, minerals, trace elements and albumin in subjects with thalassemia
2. Study the distribution of patients according to blood groups& Rh factors

Materials &methods: Cross-sectional study conducted for the period from 1/9-1/12/2002 in the Center for Anemia of Mediterranean Origin in Ibn –Albalady hospital, 157 patients were randomly selected using convenient sampling and patients attending the center for blood transfusion. Blood samples were taken from all the studied patients, the serum separated and stored at -20° C until used.

Results: The mean age on diagnosis was 10.6 years, thalassemia major was found in 77.1%, serum calcium & selenium, iron & iron binding capacity were significantly correlated with each others among studied sample, patients with blood group O were the commonest among patients then comes blood group B and A.

Conclusion: Based on findings of the present study, it can be concluded that thalassemia causes multiple abnormalities in biochemical parameters in blood of the patients.

Keywords: Biochemical changes, thalassemia

Introduction

Thalassaemia had been considered the most common genetic disorder worldwide. It occurs in a particularly high frequency in a broad belt extending from the Mediterranean basin through the Middle East, Indian subcontinent, Burma, and South East Asia [1]. Iraq is one of the countries in which 6-10% of the population have hemoglobinopathy of which thalassemia is a major part [2].

The estimated genetic frequencies range from five to 10 percent in some areas [3]. The thalassemia syndrome is a group of metabolic inherited disorders [4] characterized by microcytic hypochromic red blood cells. The homozygous state, thalassemia major results in a severe anemia and often death before puberty. The heterozygous state, thalassemia minor is less severe and may be asymptomatic with little or no anemia [5]. Antioxidants are a complex and diverse group of molecules that protect key biologic sites from oxidative damage [6]

Materials & Methods

Cross-sectional study conducted for the period from 1\9-1\12\\2002 in the Center for Anemia of Mediterranean Origin in Ibn-Albalady hospital, 157 patients randomly selected using convenient sampling and patients attending the center for blood transfusion. Blood samples for hemoglobin estimation and estimation of various levels of mineral, trace elements and albumin were taken from the entire studied sample, haemolyzed samples discarded. The blood left at room temperature for 10 minutes for clotting, centrifuged at 3000 rpm for 10 minutes, then serum separated and stored at-20 until used.

Methods:

Chemicals and reagents

All chemical and standard solutions used in this work were the highest analytical grade, and used without purification.

Measurement:

1-Serum trace elements were measured by flame atomic absorption spectrophotometer (Schimadzu AA 646)

2-Determination of Zinc and Copper

1000-ppm stock solution was diluted with deionized water to give the following concentrations of the working standard (0.0,0.4,0.8,1.2,1.6 and 2 ppm)of zinc and copper. Frozen samples allowed to thaw and come to room temperature then mixed gently.

Samples were diluted 1:10 with 6% butanol as diluents. This method achieved 30% increase in sensitivity compared to use of water only[7]

This effect is due to decrease viscosity and difference in droplet formation, and this technique is widely used .zinc and copper ratio was determined.

3-Determination of Iron Serum Iron measured using Olson and Hamlin method as follow[8]: Five hundred micro litters of 20% trichloroacetic acid were added to 500 micro litters of serum and heated at 90° C for 15 minutes, cooled, centrifuged and the Iron level in the supernatant determined by flame atomic absorption spectrophotometer at 248.3n m.

4-Determination of Total Iron binding capacity, It is the approximate estimate of serum transferring, 1 ml of serum was ended to saturated Iron solution

mix them, let stand for 5 minutest then add 170 mg of magnesium hydroxycarbonate, wait 20 minutes, shaking and persistently, centrifuge for 10 minutes, pipette 1 ml as supernatant and measure Iron, treating it as serum[8].

5-Serum Selenium, Selenium in serum was measured bay atomic absorption (flameless) [9]

6-Serum Albumin, Albumin in serum was measured by Bromocresol Green (BCG) method[10] depending on the procedure of Iraqi Sera and vaccines Institution Kit. The measurement of serum albumin is based on its quantitative binding to the indicator biomocresol Green (3, 3',5,5',tetrabromo,m,cresolsulbhonphthalin). The albumin/BCG/complex absorbs maximally at 578 nm.

7-Hemoglobin determination. The cyanomethaemoglobin using Drabkin test applied. Five mls of Drabkin solution was added to 0.02 ml whole blood, and allow the tube to stand for 10 minutes, the absorbance is measured, against the blank in the photoelectric calorimeter at 540 NM, find the concentration of hemoglobin from the calibration curve with the following working standard of hemoglobin (12 gm\100 ML, 100 ml, 7.5 gm\100 ml and 5 gm\100 ml) mix volume of standard solution of hemoglobin solution with two volume of normal saline [11]

Statistical analysis:

Frequency tables used statistical tests were done using correlation tests P values≤ 0.05 were considered significant.

Results

Table (1) shows that there 63.1% males, and 36.9% females in the sample, there were 46.5% patients below the age of 10 years, while there were 56.1% of patients below the age of one year on diagnosis, 77.1% of patient had thalassemia major, only 0.6 % had thalassemia minor, while Alpha thalassemia was present in 2.5 % of patients

Table (2) showed that 26.8% of patients had blood group A, 31.8% blood group B, 32.5% blood group O and only 8.9% were with blood group AB. Rh positive factor represent 91.7% among the sample

Table (3) demonstrate that the mean hemoglobin level was 8.4 g\100 ml

Serum copper, selenium, TIBC were statistically increased in blood of thalassemic patients (P< 0.001), While, blood level of zinc, calcium, magnesium, iron, albumin were statistically decreased (P<0.05) **table (4).**

There was significant correlation between blood level of copper and zinc and between selenium and calcium level in blood of patients **table (5).** There was no significant difference between Zn/Cu ratio among males and females **table 6**

Table (1): Distribution of patients (157) according to different variables

Variables	No	%	Cumulative %
Age of patient/year			
<10.0	73	46.5	46.5
10-19.9	67	42.7	89.2
20-29.9	17	10.8	100.0
Mean±SD 10.8±10.0			
(Range) 0.75-28.0			
Age on diagnosis/year			
<1.0	88	56.1	56.1
1-2.99	41	26.1	82.2
3-4.99	14	8.9	91.1
5-9.99	11	7.0	98.1
10-19.99	2	1.3	99.4
15-19.99	1	0.6	100.0
Mean 1.6			
Mode 0.5			
Mean±SD 1.6±1.7			
(Range) 0.08-17.0			
Sex			
Male	99	63.1	63.1
Female	58	36.9	100.0
Diagnosis			
Thalassemia			
Major	121	77.1	77.1
Intermediate	31	19.7	96.8
Minor	1	0.6	97.5
Alpha thalassemia	4	2.5	100.0

Table (2): Distribution of the sample according to their Blood group& Rh Factors

Variables	No.	%	Cumulative %
Blood groups			
Group A	42	26.8	26.8
Group B	50	31.8	58.6
Group AB	14	8.9	67.5
Group O	51	32.5	100.0
Total	157	100.0	
Rh factors			
Positive	144	91.7	91.7
Negative	13	8.3	100.0
Total	157	100.0	

Table (3): Distribution of the Sample according to anemia and age groups

Age Groups\years	Frequency	%	Mean Hb	SD	Minimum	Maximum
<5	26	16.6	9.1	0.84	7.5	10.5
5-9.99	47	29.9	8.3	1.05	6.0	10.5
10-19.99	67	42.7	8.2	1.12	5.0	11.5
20-29.99	17	10.8	8.2	0.91	*7.0*	10.0
Total	157	100.0	8.4	1.07	5.0	11.0

Table (4): Shows serum levels of trace elements Minerals and albumin in the sample of patients

Variables	Mean±SD	Range	Normal value	P value
Copper umol No=157 ***dil factor=0	20.32±6.052	8.63-39.8	18	<0.001
Zinc umol\l No=157 Dil factor=10	0.994±2.221	0.27-28.47	15	<0.001
Selinum umol\l No=157 Dil factor=0	1.723±0.943	0.1-3.74	0.995	<0.001
Calicium Umol\l No=157 Dil factor=0.7	0.323±0.292	0.01-1.94	2.6	<0.001
Magnesium Umol\l No=157 Dil factor	0.507±0.196	0.20-2.54	0.9	<0.001
Iron umol\l No=146 Dil factor=5	13.088±5.212	6.21-30.26	21.8	<0.001
TIBC** Umol\l No=146 Dil factor=2**	43.164±11.095	13.23-77.3	61.0	<0.001
Albumin Umol\l No=146 Dil factor=0	4.166±0.766	0.38-6.10	4.5	<0.001

***=standard error**
****=standard deviation**
*****=dilution factor**
******=total iron binding capacty**

Table (5) Show the association between different Blood levels of element in the sample patients

variables		zinc	selenium	calcium	Magnesium	Albumin	.Iron	TIBC
Copper	**r**	-0.392**	0.0100	-0.090	0.150	-0.021	-0.093	-0.100
	p	0.0001	0.273	0.328	0.101	0.821	0.331	0.394
Zinc	**r**		0.009	0.027	-0.031	0.061	0.119	0.147
	p		0.926	0.769	0.735	0.506	0.212	0123
Selenium	**r**			-0.289**	-0.070	-0.020	0.128	0.098
	p			0.001	0.444	0.831	0.178	0.306
Calcium	**r**				0.108	0.109	-0.082	-0.044
	p				0.240	0.232	0.387	0.644
Magnesium	**r**					-0.001	-0.014	0.060
	p					0.987	0.880	0.527
Albumin	**r**						0.114	0.138
	p						0.231	0.146
Iron	**r**							0.832**
	p							0.0001

****=Significant at 0.001**

Table (6): Zinc and Copper status among patients according to their sex

Variables	Mean±SD	Range	Normal value	P value
Zn/Cu Male	17.86±28.68	0.83-126.0	1.00	> 0.05
Zn/Cu Female	23.75±29.05	0.89-147.96	1.00	

Discussion

The present sample taken from the Thalassaemic Centre in Ibn-Al-balady hospital in Baghdad; this center is the only center for thalassaemia in Baghdad .The name of the center was changed recently to Center of Anemia of Mediterranean Region,

In the present sample male constituted higher number then females, this could be explained; by the fact that people especially in developing countries are more concern about their male children than female children. The mean Hb level in the present sample was 8.4g\dl this result expected since those blood sampled taken from patients who were coming for blood transfusion and accordingly their Hb level is low. The low Hb level found in this study is in agreement with the results of other studies[3, 12, 13, and 14]. Blood group O was the dominating blood group among patients then comes blood group B, A, AB. Transfusion centre in Baghdad (1988-1993) recorded that blood group O shows the highest percentage (31%) among people attending the blood bank for giving blood then comes group A,B,AB (personal. contact), higher prevalence of blood group B in the thalassemic patients than group A could be due to chance only , or possibly that people with blood group B are more prone to develop thalassemia , a suggestion which need to be studied in a wider and more generalized from, since we could not find a reference which touch this particular point

The finding that more than half of the patients were diagnosed before the age of one year and 82.2% were diagnosed before the age of three years agreed with the fact that 77.1% of patients in the sample were suffering from thalassemia major, most of those patients usually developed severe anemia early in their life[15]. The patients ages were mostly <10.0 years, and there was no patient above the age of 30 years, these finding can be explained by the fact that, most of the patients included in the sample were those suffering from thalassemia major with shorter life period, in a study done by Zurlo etal[16],they found that the over all survival from birth for patients born in 1970-74 was 97.4% at 10 years and 94.4% at 15 years, the most common cause of death was heart disease, followed by infection, liver disease and malignancy. Modell [17] showed that patients, who adhere fully to treatment usually complete their education, work, find a partner, and expected to live at least until their mid-forties.

Zinc deficiency considered as one of the main factors contributing to growth and puberty disorders in thalassemic patients[18]. Our finding shows zinc deficiency, which agreed with the result obtained by Arcasoy et al they showed that there, was marked zinc deficiency in the presence of hyperzincuria[19], Kwan and colleagues[20] reported

that only 3 of their 68 thalassemic patients had zinc deficiency in their study. Deficiencies of zinc and copper in patients with thalassemia major have been under debate, in the present study we find increase level of serum copper, this result disagreed with the study done by Shamshirsaz[21]. Calcium deficiency also reported by other studies[13, 15]. Hypocalcaemia is a well-known complication of iron overload[22], iron overload occurs either from the transfusion of red blood cells or because there is increased absorption of iron from the digestive tract. Both of these occur in thalassemia. Hypocalcaemia could be due to hypoparathyroidism, which occur as a late complication of thalassemia, in our study this explanation is unlikely since most of our patients were young, and hypocalcaemia probably are caused by the fact that patients might be taking blood without taking iron chelating agents either because of ignorance or it is not always available. Magnesium deficiency was reported in other studies[23, 24] selenium showed higher significant level in the present study which disagreed with other studies[25, 26]

It is recommended to do wider studies, which include in them measurements of further biochemical parameters in patients with different types of thalassemia.

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