STUDY OF DIFFERENT CLINICAL AND DEMOGRAPHIC CHARACTERS OF PATIENTS WITH THALASSEMIA AND THEIR RELATION TO HEMOGLOBIN, SOME MINERALS AND TRACE ELEMENTS AND ALBUMIN LEVELS IN THEIR BLOOD

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Abstract

Background: Thalassaemia is considered the most common genetic disorder worldwide. β thalassaemia has emerged as a huge public health problem worldwide. The classic changes of untreated thalassaemia major are now regularly seen only in countries without resources to support long-term transfusion programs.

Objective: To study the different clinical features of patients with anemia attending the center for anemia of Mediterranean origin in Ibn-Albalady Hospital in Baghdad for blood transfusion. To correlate different clinical features with the different demographic characters among the sample patients and with the blood levels of hemoglobin, some trace elements, minerals and albumin.

Methods: Cross-sectional study was conducted 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 sample. Tests were done for different serum levels of trace elements, minerals, albumin and hemoglobin.

Results: showed that out of 157 patients studied, there were 112 (71.3%) from Baghdad and 107 (68.2%) from urban areas. The mean age on diagnosis was 1.6 years and that thalassemia major was found in 121 (77.1%), there were 108 (68.8%) who require blood transfusion between 2-4 weeks, and desferol treatment frequency was >4 times\ week in 99 (63.1%) and under nutrition was found in 76 (48.4%) patients.

Conclusion: More centers for thalassemia are to be established in different areas in our country, with increase efficiency as to include gene frequency. Programs based on carrier screening and counseling of couples at marriage, preconception or early pregnancy to be established. Prenatal diagnosis by mutation analysis on PCR amplified DNA from chorionic villi.

Key word: Mineral, Trace elements, Thalassemia

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Introduction

Thalassaemia is 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]. The estimated genetic frequencies range from 5% to 10% in some areas[2]. Thalassaemia syndrome is described as a series of genetic disorders of hemoglobin synthesis which have in common a reduce output of the globins chain production, it is inherited as an autonomic recessive basis, that to find heterozygous β thalassaemia in both parents of a child with β thalassaemia major[3]. William et al[4] describes the

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distribution of thalassaemia where the gene frequency for the different thalassaemia and structural hemoglobin variants are high. According to him as the social conditions improve in developing countries and childhood mortality due to infection and malnutrition declines, children with thalassaemia who would previously have died early in life are now surviving long enough to require treatment. The reason for high frequency of thalassaemia throughout the tropics, reflect the advantages that carriers are protected from the consequences of infection with Plasmodium falciparum malaria[5].

Thalassaemia is classified into α, β, δ β, δ, γ δ β subtypes[6]. The severe in effective erythropoiesis results in erythroid marrow expansion to as 30 times the normal level. Both an increase in plasma volume because of shunting through expanded marrow and progressive spleenomegaly exacerbate anemia[7]. Increased erythropoietin synthesis may stimulate the formation of extramedullary erythropoietic tissue, primarily in the thorax and Para spinal region. Marrow expansion also results in characteristic deformities of the skull and face, as well as osteopenia and focal defects in bone mineralization[8].

Marrow hyperplasia leads ultimately to increased iron absorption and progressive deposition of iron in tissues. The marked increase in survival, to the fifth decade of life, patient with well-managed β thalassemia in developed countries represent one of the most dramatic alteration in morbidity and mortality associated with a genetic disease in these countries. Still more than 75 years after the fascinating initial description of peculiar bone changes and other signs and symptoms of the disorder, the β thalassaemia has emerged as a huge public health problem worldwide[7]. The classic changes of untreated thalassaemia major are now regularly seen only in countries without resources to support long-term transfusion programs[9].

The intention of this study is first, to study the different clinical features of patients with anemia attending the center for anemia of Mediterranean origin in Ibn-Albalady hospital in Baghdad for blood transfusion. Second, to correlate different clinical features with the different demographic characters among the sample patients and thirdly, to correlate the different clinical features with the blood levels of hemoglobin, some of the trace elements and minerals, and albumin

Subjects & methods

A coss-sectional study was conducted for the period from 1/9 to 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. Well-studied questionnaire form were used to obtain information regarding different socio-demographic characters.

Blood samples for hemoglobin estimation and estimation of various levels of mineral, trace elements and albumin were taken from the entire studied sample. The blood was left at room temperature for 10 minutes for clotting, centrifuged at 3000 rpm for 10 minutes, and then serum was separated and stored at -20°C until used.

Basal metabolic index (BMI) was classified according to the International accepted range of BMI[10] as follows: under weight <18.5, normal 18.5-24.9, over weight 25-29.9, obese 30.0-39.9 and extremely obese >40.

Chemicals and reagents

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

Measurement of trace elements

Serum trace elements were measured by flame atomic absorption spectrophotometry (Schimadzu AA 646). It is simpler and less tedious to perform than the flameless mode where the metal compound in the flame Hollow cathode lamp made of the same metal to be measured. The lamp was used to generate a wavelength of light specific for the same metal to be analyzed. This light is passing through the flame that contains the free atoms of the metal, which absorb a fraction of the light intensity. The light intensity reached to the recorder is directly proportional to the concentration of the free atoms in the flame, which in turn reflects the original concentration of the metal in the solution.

Dilution of the serum was made by deionized water according to the sensitivity of the atomic absorption spectrophotometer in order to avoid the viscosity and to decrease the interference of the protein in serum[11]. Single element hollow cathode lamps were used as line-radiation sources and were operated at currents or energies recommended by the manufacture. Optimum working conditions are given in Table 1.

Table 1: Optimum working conditions for Flame AAS of the elements (Zn, Cu, Fe, Mg, Ca)

Element	Wave length (nm)	Slit width (A˚)	Air Flow (L/min)	Acetylene Flow (L/min)	Lamp Current (mA)	Burner Height (mm)
Zn	213.9	3.8	10	2.4	6	4
Cu	324.7	3.8	10	2.3	7	4
Fe	248.3	1.9	10	2.7	5	6
Mg	285.2	3.8	10	2.4	5	5
Ca	422.7	3.8	10	2.6	8	10

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 were 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[12]. This effect is due to decrease viscosity and difference in droplet formation and this technique is widely used[13].

Determination of Iron

Serum iron measured using Olson and Hamlin method[14] as follow: Five hundred micoliters of 20% trichloroacetic acid were added to 500 micoliters of serum and heated at 90ºC for 15 minutes, cooled, centrifuged and the iron level in the supernatant determined by flame atomic absorption spectrophotometer at 248.3 nm.

Determination of total iron binding capacity

It is the approximate estimate of serum transferrin, 1 ml of serum was added to saturated iron solution mix them, let stand for 20 minutes. Then add 170 mg of magnesium hydroxycarbonate, wait 20 minutes, shaking intermittently, centrifuge for 10 minutes, and pipette 1 ml of supernatant and measure iron, treating it as serum[15].

Serum Selenium

Selenium in serum was measured by atomic absorption (flameless)[16].

Determination of Magnesium and Calcium

Samples used for determination of magnesium and calcium were diluted 1:50 with 1% lanthanum chloride solution to exclude the effect of serum

phosphate[15]. Standard solution of 1000 ppm was diluted with deionized water. Working standards of magnesium were (0,5,10,15,20,25, and 30 µ mol\L). Working standards of calcium were (0,20,40,60,80,100, and 120 µ mol\L)

Serum Albumin:

Albumin in serum was measured by Bromocresol Green (BCG) method[15] 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 bromocresol green (3,3',5,5',tetrabromo-m-cresolsulphonphthalin). The albumin-BCG-complex absorbs maximally at 578 nm.

Hemoglobin determination

The cyanomethaemoglobin using Drabkin test was applied. Five ml 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, 10 gm \ 100 ml, 7.5 gm\ 100 ml and 5 gm \ 100 ml) mix volume of standard solution of hemoglobin solution with two volume of Drab kin solution[15].

Statistical analysis:

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

Results

The present sample constitutes 157 patients studied randomly from the Thalassaemic Center (center for anemia of Mediterranean origin) in Ibn- Al-Balady hospital \Baghdad.

Table 2 shows that there were 99 (63.06%) males, and 58 (36.94%) females in the sample, there were 73 (46.5%) patients below the age of 10 years with the mean age 10.8 years, minimal age in years was 0.75 and maximal age 28.0, while there were 88 (56.1%) of patients below the age of one year on diagnosis, and 129 (82.2%) of patients below the age of 3 years on diagnosis, the mean age on diagnosis was 1.6 year mode 0.5, the minimal age in years was 0.08 and the maximal age was 17.

In the sample there were 112 (71.3%) patients from Baghdad, 21 (13.4%) from Diyala, 12 (7.6%) from Wasit, 4 from Karballa, 2 (1.3%) from Najaf and from Anbar, while there was only one patient (0.6%) from Karkuk, Babel and Theekaar governorate. The sample also shows that there were 107 (68.2%) patients from urban and 50 (31.8%) patients from rural areas.

Table 2: Distribution of the sample (157 patients) according to different variables

Variables	Number	percent	Cumulative percent
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 10.8			
Mode 10.0			
STD 10.0			
Minimum 0.75			
Maximum 28.0			

Table 2: *Continued*

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			
STD 1.07			
Minimum 0.08			
Maximum 17.0			
Sex			
Male	99	63.1	63.1
Female	58	36.9	100.0
Address			
Baghdad	112	71.3	71.3
Wassait	12	7.6	79.0
Karkuk	1	0.6	79.6
Diala	21	13.4	93.0
Karballa	4	2.5	95.5
Najaf	1	0.6	96.2
Salah-Aldeen	2	1.3	97.5
Anbar	2	1.3	98.7
Babil	1	1.6	99.4
Thee-Kaar	1	0.6	100.0
Residency			
Urban	107	68.2	68.2
Rural	50	31.8	100.0

Table 3 shows that 121 (77.1%) of patients were suffering from thalassaemia major, 31 (19.7%) from intermedia, and one only from thalassaemia minor, there were also four with Alpha thalassaemia in the sample.

Table 3: The distribution of the sample according to the diagnosis

Variables	Number	percent	Cumulative percent
Thalassemia Major	121	77.1	77.1
Intermediate	31	19.7	96.8
Minor	1	o.6	97.5
Alpha thalassemia	4	2.5	100.0

Table 4 demonstrate that the mean hemoglobin level was 8.4 g\ 100 ml among the patients with the mode being 8.0, and the minimal hemoglobin was 5.0 g\ 100 ml and the maximal hemoglobin reading was 11.0 g\ 100ml.

Table 4: Distribution of the sample according to anemia and age groups

Age groups\years	Frequency	%	Mean Hb	St.d	Minimum	Maximum
< 5	26	16.6	9.1	0.84	7.5	10.5
5-9.99	47	29.9	8.3	1.0	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 5 showed that among the sample group the dominating blood group was group O (51 patients 32.5%), then group B (50 patients 31.8%), while blood group A was found in 42 patients (26.8)%), group AB was found in 14 patients (8.9%). Rh factor was mostly positive in 144 patients (91.7%), while it was negative in 13 (8.3%) patients.

Table 5: Distribution of the sample according to their blood group & Rh factors

Variables	Number	Percent	Cumulative percent
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 6 showed that there were 108 (68.8%) of patients who require blood transfusion between 2-4 weeks, 48 (30.6%) of patients require transfusion in > 4 weeks, while only one patient require transfusion in < 2 weeks time. Desferol treatment were taken > 4 times \week in 99 (63.1%) patients, between 3-4 times \week in 46 (29.3%) of patients, and < 3 times \week in 12 (7.6%) patients.

Table 6: Show type and frequency of treatment given to sample group

Type of treatment &frequency	Number	Percent	Cumulative percent
Blood transfusion			
<2 weeks	1	0.6	0.6
2-4 weeks	108	68.8	69.4
> 4 weeks	48	30.6	100.0
Total	157	100.0	
Desferol			
< 3 times\ week	12	7.6	7.6
3-4 times\ week	46	29.3	36.9
> 4 times\ week	99	63.1	100.0
Total	157	100.0	

Table 7 showed that 76 (48.4 %) of patients were having under nutrition according to the International accepted range of BMI, while 74 patients (47.1 %) were regarded as normal, and only 7 patients (4.5%) were regarded as over weight.

Table 7: Distribution of patients in the sample according to basal metabolic rate (BMI)

BMI	Frequency	Percent	Cumulative Percent
Under weight	76	48.4	48.4
Normal	74	47.1	96.1
Over weight	7	4.5	100
Total	157	100	---

Table 8 showed that there were 126(80.3%) of patients who were pale at time of examination, 93(59.2%) with jaundice, 95(60.5%) with frontal bossing, 76(48.4%) of patients with mongoloid features, 69(43.9%) with liver enlargement, 78(49.7%) with spleen enlargement, while there were only 5(3.2%) with heart failure and 2(1.3%) with diabetes mellitus.

Table 8: Distribution of patients in the sample according to the presence or absence of some clinical manifestation

Variables		Number	Percent	Cumulative percent
Pale	Yes	126	80.3	80.3
	No	31	19.7	100.0
Jaundice	Yes	93	59.2	59.2
	No	64	40.8	100.0
Frontal bossing	Yes	95	60.5	60.5
	No	62	39.5	100.0
Bronze skin	Yes	84	53.5	53.5
	No	73	46.5	100.0
Mongoloid features	Yes	76	48.4	48.4
	No	81	51.6	100.0
Liver enlargement	Yes	69	43.9	43.9
	No	88	56.1	100.0
Spleen enlargement	Yes	8	49.7	49.7
	No	59	50.3	100.0
Heart failure	Yes	5	3.2	3.2
	No	152	96.8	100.0
Diabetes	Yes	2	1.3	1.3
	No	155	98.7	100.0

When the relationship between different clinical manifestation was correlated (Table 9a) it was found that, pale skin was correlated with bronze skin, mongoloid features, diabetes mellitus and heart failure. Jaundice was correlated with frontal bossing, bronze skin, mongoloid features and enlarged spleen. Frontal bossing was correlated with jaundice, bronze skin, mongoloid features; enlarge spleen & frequency of treatment with desferol. Bronze skin was correlated with pale skin, jaundice, frontal bossing, mongoloid features, enlarge spleen. Mongoloid features were correlated with pale skin, jaundice, frontal bossing, and bronze skin and enlarge spleen. Enlarge liver was correlated with enlarge spleen and heart failure. Enlarge spleen was correlated with jaundice frontal bossing mongoloid features bronze skin enlarge liver and heart failure.

Table 9a: The association between different manifestations of patients in the sample

Variables	Pale	Jaundice	F. Bossing	B. Skin	M. Feature	E. Liver	E. Spleen
Pale							
P. Correlation	----------	-.086		-.233**	-.251**	-.306**	-.507**
Significant	-	.285	-.009	.003	.002	.000	.000
Number		157	157	157	157	157	157
Jaundice							
P. Correlation	-.086	----------	.084	.239**	.081	.093	.074
Significant	.285	-	.298	.003	.312	.248	.355
Number	157		157	157	157	157	157

Table 9a: *Continued*

F. Bossing							
P. Correlation	-.073	.311	----------	.112	.045	.029	-.021
Significant	.361	.000		.161	.572	.718	.790
Number	157	157		157	157	157	157
Bronze skin							
P. Correlation	-.302**	.448**	.007	---------	.033	-.028	-.059
Significant	.000	.000	.929		.682	.725	.463
Number	157	157	157		157	157	157
M. Feature							
P. Correlation	-.224**	.414**	.117	-.049	----------	-.049	-.094
Significant	.005	.000	.145	.544		.546	.240
Number	157	157	157	157		157	157
Enlarge liver							
P. Correlation	-.044	.108	-.002	.115	.064	----------	-.087
Significant	.581	.179	.976	.156	.430	-	.282
Number	157	157	154	154	154		154
Enlarge spleen							
P. Correlation	-.083	.254**	.095	.139	.236**	.018	----------
Significant	.300	.001	.237	.082	.003	.824	
Number	157	157	157	157	157	157	
Diabetes							
P. Correlation	-.229**	-.021	.173*	.172*	.030	.217*	.155
Significant	.004	.791	.044	.046	.727	.011	.072
Number	157	157	136	136	136	136	136
Heart failure							
P. Correlation	-.183*	.150	.036	.137	.051	.170	.033
Significant	.021	.060	.662	.093	.539	.038	.687
Number	157	157	150	150	150	150	150

*= Significant at 0.05 level
** = Significant at 0.01 level

Table 9b showed that diabetes mellitus was found in patients with pale skin & heart failure. Heart failure was found in patients with pale skin; enlarge liver & spleen, diabetes mellitus. Treatment with blood transfusion was correlated with mongoloid features, while desferol treatment was correlated with frontal bossing; mongoloid features, and enlarges liver. Blood groups were correlated with enlarge liver; while Rh factors were correlated with enlarge liver & spleen. Basal metabolic rate (BMI) was correlated with jaundice, frontal bossing, mongoloid features, and enlarge spleen.

Table 9b: The association between different manifestations of patients in the sample

Variables	DM	Heart failure	Freq. Blood transfusion	Freq. Desferol	Blood group	Rh. Factor	Hb	BMI
Pale								
P. Correlation	-.229**	-.183**	-.111	-.091	-.055	-.073	-.090	.007
Significant	.004	.021	.165	.292	.504	.375	.265	.930
Number	157	157	157	136	150	150	156	151
Jaundice								
P. Correlation	-.021	.150	.078	.111	.060	.088	.070	-.384**
Significant	.791	.060	.331	.198	.464	.286	.385	.000
Number	157	157	157	136	150	150	156	151

Table 9b: *Continued*

F. Bossing								
P. Correlation	-.027	.072	.95	.173*	.036	.039	.113	-.176*
Significant	.761	.368	.237	.044	.662	.634	.160	.031
Number	157	157	157	136	150	150	156	151
Bronze skin								
P. Correlation	.106	.096	.139	.172*	.137	.155	.159*	-.116
Significant	.187	.230	.082	.046	.093	.059	.048	.157
Number	157	157	157	136	150	150	156	151
M. Feature								
P. Correlation	.117	.115	.236**	.030	.051	.066	.122	-.202*
Significant	.144	.153	.003	.727	.539	.426	.129	.013
Number	157	157	157	136	150	150	156	151
Enlarge liver								
P. Correlation	-.014	.205*	.018	.217*	.170*	.194*	.105	-.141
Significant	.863	.010	.824	.011	.038	.017	.193	.085
Number	157	157	157	136	150	150	156	151
Enlarge spleen								
P. Correlation	.001	.183*	.063	.155	.033	.230**	.121	-.223*
Significant	.993	.022	.430	.072	.687	.005	.133	.006
Number	157	157	157	136	150	150	156	151
Diabetes								
P. Correlation	----------	.303**	.072	-.074	.095	.033	.011	.009
Significant	--	.000	.370	.393	.249	.691	.888	.917
Number		157	157	136	150	150	156	151
Heart failure								
P. Correlation	.303**	----------	.115	.042	.135	.047	-.022	-.145
Significant	.000		.115	.623	.100	.572	.788	.076
Number	157		157	136	150	150	156	151

*= Significant at 0.05 level
** = Significant at 0.01 level

Table 9c showed the correlation between different clinical features and blood level of trace elements, minerals and albumin and it showed that serum copper was correlated with enlarge liver. Serum Selenium level in blood was correlated with frontal bossing, bronze skin, mongoloid features. Hemoglobin concentration in blood was correlated with bronze skin. Serum iron & total iron binding capacity (TIBC) were correlated with pale skin only.

Table 9c: The association between different manifestations of patients in the sample

Variables	Cu	Zn	Selenium	Ca	Mg	Albumin	S. Iron	TIBC
Pale								
P. Correlation	.077	-.046	.093	-027	.073	.053	-.205*	.250**
Significant	.336	.570	.244	.740	.367	.506	.013	.002
Number	157	157	157	157	157	157	146	146
Jaundice								
P. Correlation	-.031	-.077	-.120	.094	-.062	.001	-.125	-.042
Significant	.701	.336	.135	.242	.443	.513	.133	.612
Number	157	157	157	157	157	157	146	146
F. Bossing								
P. Correlation	.039	.080	.197*	.042	-.090	.053	-.091	.011
Significant	.627	.321	.014	.600	.264	.513	.274	.899
Number	157	157	157	157	157	157	146	146

Table 9c: *Continued*

Bronze skin								
P. Correlation	.027	.078	-.168*	.057	.001	.023	-.101	-.067
Significant	.740	.330	.036	.475	.993	.779	.224	.420
Number	157	157	157	157	157	157	146	146
M. Feature								
P. Correlation	-.002	.083	-.169*	.089	.022	.107	-.129	-.026
Significant	.978	.303	.034	.270	.784	.182	.121	.753
Number	157	157	157	157	157	157	146	146
Enlarge liver								
P. Correlation	-.164*	.078	.019	-.135	.021	.028	.108	.104
Significant	.040	.331	.814	.091	.799	.729	.193	.212
Number	157	157	157	157	157	157	146	146
Enlarge spleen								
P. Correlation	-.086	.073	.002	-.085	-.107	-.004	.000	.029
Significant	.284	.360	.980	.290	.183	.960	.999	.724
Number	157	157	157	157	157	157	146	146
Diabetes								
P. Correlation	.005	.006	.020	.086	.086	-.079	.082	.122
Significant	.947	.937	.806	.284	.284	.323	.323	.142
Number	157	157	157	157	157	157	146	146
Heart failure								
P. Correlation	.011	.008	.084	.096	-.012	-.037	.143	.146
Significant	.894	.917	.294	.232	.877	.650	.085	.078
Number	157	157	157	157	157	157	146	146

*= Significant at 0.05 level
** = Significant at 0.01 level

Tables 10a and b showed that the age of the patients was correlated with pale skin, jaundice, frontal bossing, bronze skin, mongoloid features, liver and spleen enlargement, frequency of blood transfusion, diabetes mellitus, heart failure, BMI, and hemoglobin level ion blood. Sex of the patients was correlated with bronze skin, hemoglobin level and blood groups. Address, residency, and diagnosis of the disease were not correlated with any of the studying variables. Age on diagnosis was correlated with BMI only.

Table 10a: The occurrence of different clinical manifestation in the sample of patients in association with different variables

Variables	Pale	Jaundice	F. Bossing	B. Skin	M. Feature	E. Liver	E. Spleen
Age							
P. Correlation	.160*	-.234**	-.208**	-.233**	-.251**	-.306**	-.507**
Significant	.045	.003	-.009	.003	.002	.000	.000
Number	157	157	157	157	157	157	157
Sex							
P. Correlation	-.081	.090	.084	.239**	.081	.093	.074
Significant	.331	.262	.298	.003	.312	.248	.355
Number	157	157	157	157	157	157	157
Address							
P. Correlation	.011	-.085	.010	.112	.045	.029	-.021
Significant	.895	.291	.903	.161	.572	.718	.790
Number	157	157	157	157	157	157	157

Table 10a: *Continued*

Residency							
P. Correlation	-.133	-.036	.007	.048	.033	-.028	-.059
Significant	.097	.633	.929	.550	.682	.725	.463
Number	157	157	157	157	157	157	157
Diagnosis							
P. Correlation	-.005	-.112	.117	-.049	.049	-.049	-.094
Significant	.949	.162	.145	.544	.546	.546	.240
Number	157	157	157	157	157	157	157
Age on diagnosis							
P. Correlation	-.042	-.094	-.002	.115	.064	-.018	-.087
Significant	.603	.244	.976	.156	.430	.820	.282
Number	154	154	154	154	154	154	154

*= Significant at 0.05 level
** = Significant at 0.01 level

Table 10b: The occurrence of different clinical manifestation in the sample of patients in association with different variables

Variables	Freq. Blood	Freq. desferol	DM	Heart failure	BMI	Hb	Blood group	Rh factor
Age								
P. Correlation	-.256*	.024	-.191*	-.203**	.346**	-.216**	-.021	-.119
Significant	.001	.784	-.017	.011	.000	.007	.798	.146
Number	157	136	157	157	151`	156	150	150
Sex								
P. Correlation	-.066	.147	.087	.064	.121	.230**	.197*	.055
Significant	.411	.087	.279	.428	.139	.004	.016	.501
Number	157	136	157	157	151	156	150	150
Address								
P. Correlation	.014	-.064	.058	.031	-.010	.034	.094	.091
Significant	.864	.459	.469	.701	.903	.671	.251	.269
Number	157	136	157	157	151	156	150	150
Residency								
P. Correlation	.030	-.041	-.044	.032	-.002	-.058	-.073	-.021
Significant	.710	.636	.582	.693	.984	.469	.375	.801
Number	157	136	157	157	151	156	150	150
Diagnosis								
P. Correlation	.104	-.020	.045	-.072	-.015	-.029	-.049	-.053
Significant	.193	.815	.574	.369	.850	.720	.550	.523
Number	157	136	157	157	151	156	150	150
Age on diagnosis								
P. Correlation	-.003	-.038	-.100	-.048	.163**	-.067	.146	-,089
Significant	.967	.666	.217	.553	.047	.413	.078	.286
Number	154	133	154	157	148	153	147	147

*= Significant at 0.05 level
** = Significant at 0.01 level

Table 10c showed that only age on diagnosis and frequency of desferol treatment were correlated with serum level of magnesium, while there were no significant correlation between other variables studied with serum level of mineral, trace element and albumin level in blood.

Table 10c: The blood levels of different trace element in patients in association with different variables

Variables	Cu	Zn	Selenium	Ca	Mg	Albumin	S. Iron	TIBC
Age								
P. Correlation	.017	-.076	.052	-.084	.090	.034	.126	.073
Significant	.834	.342	.521	.297	.263	.677	.130	.379
Number	157	157	157	157	157	157	146	146
Sex								
P. Correlation	-.001	.097	-.030	-.156	.020	-.063	-.120	-.126
Significant	.994	.226	.708	.052	.808	.432	.150	.129
Number	157	157	157	157	157	157	146	146
Address								
P. Correlation	-.043	-.043	.007	-.001	-.016	-.117	-.039	-.112
Significant	.592	.592	.926	.989	.840	.146	.643	.180
Number	157	157	157	157	157	157	146	146
Residency								
P. Correlation	.072	-.063	-.037	-.099	-.135	-.140	.078	.060
Significant	.370	.436	.642	.215	.092	.081	.349	.473
Number	157	157	157	157	157	157	146	146
Diagnosis								
P. Correlation	-.037	.065	.063	.030	-.038	-.061	.051	.053
Significant	.645	.416	.435	.711	.634	.444	.541	.522
Number	157	157	157	157	157	157	146	146
Age on diagnosis								
P. Correlation	-.004	-.062	-.095	-.055	.161*	-.072	.076	.072
Significant	.957	.448	.242	.501	.046	.375	.364	.393
Number	154	154	154	154	154	154	144	144

*= Significant at 0.05 level
** = Significant at 0.01 level

Discussion

The present sample was 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, for this reasons we can see that patients included in this sample were from ten governorate, but mostly from Baghdad 121(77.1%), and then from Diyala 21(13.4%), Wassiet12 (7.6%).

This could be explained by the fact that patients living in Baghdad and Deilla can more easily come to the center for blood transfusion and treatment and that doctors in these areas are more aware about this condition, so the diagnosis and treatment are more available to patients. On the other hand, patients living in far areas probably will not have the chance for early diagnosis and treatment and die from the disease in early age, or it is very difficult for their families to come to the center in proper time for blood transfusion and therapy. In addition, the prevalence of the disease might be higher in these areas than in other areas in our country, the same observation could be applied to the distribution of the sample according to the residency of patients as the number of patient were 107 (68.2%) from urban areas while they were 50 (31.8%) patients from rural areas.

The finding that more than half of the patients 88 (56.1%) were diagnosed before the age of one year and 129 (82.2%) were diagnosed before the age of three years, this agree with the fact that 77.1% of patients in the sample were suffering from thalassaemia major, most of those patients usually developed severe anemia early in their life[17].

The patients age were mostly <10.0 years (73 patients 46.5%), 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 thalassaemia major with shorter life period, in a study done by Zurlo etal[18], 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[19] showed that patients who adhere fully to treatment usually complete their education, work, and find a partner, and are expected to live at least until their mid- forties.

In the past decade, treatment of patients with beta-thalassemia has changed considerably, with advances in red cell transfusion and the introduction of iron chelation therapy. This progress has greatly increased the probability for a thalassemic child to reach adult age with a good quality of life[20]. Currently all newborns in the United States are screened for hemoglobinopathies, if a newborn screen returns with large amounts of fetal hemoglobin, alpha hemoglobin, or hemoglobin E, further investigation for thalassemia takes place[21].

In the present sample male patients 99 (63.1%) constituted higher number than females 58 (36.9%), this could be explained by the fact that people especially in developing countries are more concern about their male children than female children.

Treatment of patients with thalassemia major has improved dramatically during the past 40 years; however, the status of these patients remains poorly characterized[22].

The mean Hb. level in the present sample was 8.4 g\dl with the minimal reading of 5.0 g\dl and maximum of 11.0 g\dl, this result is expected since those blood samples were taken from patients who were coming for blood transfusion and accordingly their Hb. level were expected to be low, when the Hb. level was correlated with the clinical manifestation, it was found that it correlated significantly with bronze skin only, while pale looking was correlated with bronze skin, mongoloid feature, diabetes mellitus, heart failure which signified severe complication of thalassemia, and again the patients examined were in the majority suffering from thalassemia major.

Blood group O was the dominating blood group among the sample of patients then comes blood group B, A, AB. The National blood 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), the 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 form, since we could not find a reference which touch this particular point (blood group difference in thalassemic patient and general population).

As for treatment with blood transfusion, it was found that most of the patients (108 68.8%) where having blood transfusion between 2-4 weeks and desferol treatment > 4 times \ week (99 patients 63.1%), which indicate reasonable treatment program, this does not coincide with the severity of clinical manifestation found among the patients, which could be explained by the fact that those patients probably need more aggressive treatment than the one planned for them, on the other hand regular blood transfusion will inevitably lead to multi-organ

hemosiderosis and are attended by risks of blood-born infections[23].

In the present sample the BMI showed that around half of the patients were underweight and only 7 patient were overweight, which means that a good number of patient were suffering from growth retardation, this finding coincide with the finding that large proportion of patients were suffering from thalassemia major with symptomatic manifestation of the disease, and probably those patients are in need of more aggressive treatment than the present one.

The clinical picture of inadequately treated β thalassaemia is characterized by anemia, spleenomegaly, bone changes, and being prone to infection[24], the severity of the disease is extremely variable, and those so called major forms of the illness reflect the severe end of a spectrum that stretches from less severe anemias, which do not require transfusion, through intermediate forms of β thalassaemia to the completely symptom less conditions that are identified only by chance[25].

Clinically patient in the present sample showed high proportion of clinical manifestation of severe thalassaemia, for example, there were 126 (80.3%) of patients who were pale (the mean hemoglobin level was 8.4 g\100ml), 93 (59.2%) of them were with jaundice, 84 (53.5%) with bronze skin.

One of an important issue to be considered during follow up of patient with thalassaemia is bone abnormalities characterized by bone marrow expansion of the medullary cavity, and osteopenia with cortical thicking and trabecular coursing[26], in the present sample, it was found that 95 (60.5%) of patients had frontal bossing, and 76 (48.4%) had mongoloid features (complication of bone marrow expansion).

Hepatospleenomegaly had been mention in many articles[19,24,27-29] especially in those patients suffering from severe form of thalassaemia due to hypertrophy of ineffective bone marrow, in the present sample we find that 69 (43.9%) of patients suffers from liver enlargement, and 78 (49.7%) suffer from enlarge spleen.

There were 5 (3.2%) patients with heart failure in the sample group, it is well Known fact that if excess iron derived from transfusion is not removed patients die in the second or third decade from iron loading of the myocardium[30,31]. In a study done by Ferrara et al[32] 2004, they found that patients with thalassemia major in the study sample showed marked reduction in contractile state and milder LV than in thalassemia intermedia. It is important to notice that classic changes of untreated thalassaemia major are now regularly seen only in countries without resources to support long-term transfusion programs[9].

The striking increases in survival in patients with β thalassemia over the last decade have focused attention on abnormal endocrine function, for example diabetes mellitus was observed in 5% of adults which is due to exhaustion of beta cell and reduce circulating insulin concentration[33,34], in the present sample only 2 (1.3%) of patients were having diabetes mellitus, this could explain by the fact that most of the patients in this sample are of younger age with severe clinical symptom of thalassemia major, which makes the possibility of detecting diabetes mellitus among them less likely than those who are with better treatment programs and older in age.

Growth retardation in early childhood is a consequence of severe anemia. It can be prevented (although not corrected) by an aggressive blood transfusion program[35]. Even in children optimally transfused, however, the preadolescent

growth spurt is delayed and curtailed, so that full potential stature is rarely realized[36]. Growth failure occurs as a result of low somatomedin activity, because the liver synthesizes somatomedin, hemosiderosis has been held responsible for preadolescent growth failure[37].

Filosa[38] concluded that puberty positively influences the bone mineral density only at the start of puberty, while subsequently, the degree of osteoporosis is the expression of widespread and chronic systemic damage due to the hematological phenotype. In the present sample, it was found that 48.4% of patients suffer from under weight, which means that about half of the patients their BMI level were below the normal, this result is expected since 77.1% of patients suffer from thalassemia major and a good percentage of them show different clinical manifestation of thalassemia, also age on diagnosis of the patient was found to be correlated with BMI, which is an expected finding.

Again, the finding of significant correlation between age of patient with most of the clinical feature of thalassemia, type of treatment applied and diabetis mellitus was expected one, while sex was correlated with bronze skin, Hb level, and blood groups only probably because 63.1% of patients were males, and parents of thalassemic children do care about their male children more than the female ones, so different clinical features were correlated less frequently with the sex of the patients.

The finding that the address, residency and diagnosis of the disease were not correlated with any of the studied variables could be due to the fact that the sample size is not big enough to show differences and most of the patients were from urban areas, the same things could be said for the finding that age on diagnosis and treatment with desferol were correlated with magnesium level in the blood, while other variables were not correlated with any of the trace elements, minerals, or albumin level in the patient blood

Recommendation

1. It is very important that more centers for thalassemia are to be established in different areas in our country, with increase efficiency as to include gene frequency
2. To established programs based on carrier screening and counseling of couples at marriage, preconception or early pregnancy, which can be done by simple hematological analysis. These programs are operating in several Mediterranean at risk population, and are very effective, as indicated by increasing knowledge on thalassemia and its prevention by the target population and by the marked decline of the incidence of thalassemia major.
3. Prenatal diagnosis by mutation analysis on PCR amplified DNA from chorionic villi Molecular diagnosis of homozygotes and identification of carrier of beta thalassemia may lead to improved clinical management of patients with the disorder and prevention of the birth of affected homozygotes.

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