A Comparative Biochemical Study of Proteins Profile in Iraqi Children and Adolescent with β–Thalassemia

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Abstract

The aim of the present research is to study different protein fractions in sera of children and adolescent with β –thalassemia major and minor and to compare the results with that of healthy control.One hundred fifty children and adolescents were enrolled in this study,including 50 patients with β- thalassemia major , 50 patients with β- thalassemia minor as pathological control group and another apparently 50 healthy individuals as a control group. The age of all studied groups ranged from (4-18)years.Total protein, albumin and immunoglobulins were estimated in sera of all subjects. A Significant decrease was found in the total protein and albumin levels in sera of both major and minor thalassemic patients compared to normal groups. A Significant increase in immunoglobulin levels (IgG, IgM and IgA) was found in the sera of major and minor β-thalassemia patients compared to control.Different protein parts in sera of all subjects were detected using cellulose acetate electrophoresis.The results revealed significant reduction in β- globulin fractions in β- thalassemia major patients compared to the normal and pathological control groups. Significant elevations in γ-globulins fractions were observed in both major β- thalassemia and minor β- thalassemia as compared to normal control group. As a Conclusion the alteration in some protein parts occurred which is more obvious in major thalassemia patients compared to the normal and pathological control groups.

Key words: Protein Parts , Elecrtrophoresis , β-Thalassemia.

الخلاصة

جمعت نمادج الدم من ١٥٠ شخصا"من الصغار في العراق ، خمسون منهم مصابا بمرض فقر دم البحر الابيض المتوسط الشديد، وخمسون منهم مصابا بمرض فقر دم البحر الابيض المتوسط الخفيف و الذين اعتبروا كمجموعة سيطرة مرضية ، و٥٠ شخصا من الاصحاء كمجموعة سيطرة طبيعية.تتراوح اعمار المجاميع المدروسة بين (٤-١٨)سنة. الهدف من البحث دراسة مختلف اجزاء البروتين في مرضى فقر دم البحر الابيض المتوسط نوع بيتا من الاشخاص الصغار في العراق ومقارنتها مع المجموعة الضابطة. تم قياس كل من البروتين الكلي والالبومين والامينوكلوبيولينات في مصل كل من المجاميع المرضية والضابطة. اشارت النتائج الى وجود نقصان معنوي في مستوى البروتين الكلي والالبومين مع زيادة واضحة في مستوى كل من الامينوكلوبيولينات (IgM وIgG وIgA)في كل من مجاميع المرضى المدروسة مقارنة مع المجموعة الضابطة الطبيعية. تم دراسة اجزاء البروتين في مصول مرضى فقر الدم البحر الابيض المتوسط و المجموعة الضابطة الطبيعية بتقنية الترحيل الكهربائي و اشارت النتائج الى عدم وجود نقص في كلوبيولين –الفا بينما هناك نقص واضح في كلوبيولين- بيتا مع زيادة واضحة في كلوبيولين- كاما بين مجاميع المرضى الشديدة والخفيفة. تم الاستنتاج ان التغير في اجزاء البروتين معنوية وخاصة في مرضى فقر دم البحرالابيض المتوسط الشديدة مقارنة مع مرضى فقر دم البحرالابيض المتوسط الخفيف والمجموعة الضابطة الطبيعية .

Introduction

Thalassemia is the name of a group of geneticlly (inherited), blood disorders , all of which involve under production of haemoglobin , and partial or complete failure of synthesis a specific type of globin chain.The defect may affect the α, γ and δ chain or may affect some combination of the β, γ and δ chains in the same patient, but never α and β chain together, unmatched globins could precipitate and damage RBC membranes causing their destruction while still in the marrow[1,2].Beta (β)- thalassemia manifest clinically has three major groups: 1-β-thalassemia major, 2- β- thalassemia intermedia and 3-β- thalassemia minor (trait)[2].β -thalassemia major occurs at a high gene frequency throughout the Mediterranean populations, the Middle East, India and Southern China through Thailand populations[3].The prevalence of β–thalassemia in Iraq have not taken much intention in previous studies in spite of the large population affected by this haematological disease.Proteins are substances that made up of smaller building blocks called amino acids[3], which are an important constituents of all cells and tissues. Human serum contains more than 125 well identified

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proteins. So there are many different kind of proteins in the body with many different functions,for the example:- enzymes, some hormones, hemoglobin, immunoglobulin (antibodies)[4]. The major sites of synthesis of plasma proteins are the liver and the immune system[5]. Total protein level depend on the balance between their synthesis and their catabolism or loss from body . A test for total serum protein measures total amount of protein in blood serum as the amounts of albumin and globulins[6]. Albumin has a single polypeptide chain of (580) amino acids. It is a very stable protein with a high net negative charge at the physiological pH. It has a molecular weight of (66)KDa . Albumin molecule could serve as hormones and various metabolites as well as drugs and antibiotics carrier. Albumin also functions in the maintenance of proper osmotic pressure[7].The immunoglobulins which are antibodies, are a heterogeneous group of plasma proteins produced by B-lymphocytes.These proteins are important in preventing and fighting infections. Elevation in the serum levels of immunoglobulin are seen in infectious diseases and thalassemia[8].Many studies have been carried out to evaluate changes of the immune system in thalassemia patients, considering the humoral and cellular immune system; but no consistent defect in white cells or immune functions had been documented[8].The aim of the present research is to study different protein fractions in sera of children and adolescent with β -thalassemia major and minor and to compare the results with that of healthy control.

Materials and Methods

Selection of subjects and blood sampling

Six ml of venous blood sample was obtained from 150 children and adolescent attending Ibn Al-Baladi Hospital . 50 patients were with β -thalassemia major, 50 patients were with β- thalassemia minor (as pathological control group) and 50 apparently healthy individuals as control group.The age of all studied groups were ranging from (4-18) years.The blood samples were transferred into plain tubes, allowed to stand for 15 minutes at room temperature then centrifuged at 3500 rpm for (10) minutes. The resulting serum was separated and frozen at (-20 ^{0}C) till used for the estimation of total ptotein (TP), albumin, IgM, IgA, IgG and performing electrophoresis for sera.

Determination of Total Protein(TP)

The concentration of total proteins was determined according to the colorimetric method described by Gornall A.[9-10] The peptide bonds of proteins react with Cu^{2+} in alkaline solution to form a colored complex in which the absorbance at 550 nm was proportional to the concentration of total protein in the specimen. The biuret reagent contains sodium potassium tartrate to complex cupric ions and maintains their solubility in alkaline solution .

Determination of Albumin

Albumin concentration in serum was measured using manual procedure, TECO diagnostics kit.Serum albumin binds selectively to the dye bromcresol green at the pH 4.2. The absorbances of the resulting albumin-dye complex, was read at 630 nm, was proportional to the albumin concentration[9].

Determination of IgM, IgA and IgG

Immunoglobulins (IgM, IgA and IgG) were determined by using immunoturbidimetric method[11].Immunoglobulins (IgM, IgA and IgG) form a complex with antibodies in solution. The absorbance of the complex could be measured spectrophotometrically at 340 nm. Immunoglobulin concentrations for each sample was estimated by the equation obtained from a comparable standard curve for each type.

Serum Protein Electrophoresis

Electrophoresis is referring to the transport of electrically charged particles in an electric field,that can be utilized for the characterization of their components after a comparison to references.To perform an electrophoretic separation requires the support material (cellulose acetate) which was made with buffer previously placed in the electrode chamber then sample (serum) was applied to the support, and electrophoresis was performed by conducting to electric power for 40 min, using a constant voltage (150 v). The support was then removed from the electrophoresis unit stained with 1% penceau S. After washing out excess dye, the support media was dried then the electrograms were scanned[9].

Statistical Analysis

Data presented as means and standard deviation. Study of T-Test (p) was used to compare the significance of the difference in the mean values of any groups($p \leq 0.05$) were considered statistically significant. The overall predictive values for the results in all studied groups were performed according to biostatistics by Daniel in 1987[12] .

Results and Discussion

Table (1) summarizes the results of estimated levels of serum total protein (TP) ,albumin ,globulin and (A/G) ratio expressed as (mean ± SD) in sera of normal control,thalassemia minor and β- thalassemia major .

Table 1 : Total protein (TP), albumin,globulin and (A/G) ratio levels in sera of β- thalassemia major, minor thalassemia patients and normal control .

Groups	TP gm/dl (No.=50)	Albumin gm/dl (No.=50)	Globulin gm/dl (No.=50)	(A/G)ratio	t-Test
thalassemia major	5.46± 0.54*	3.83± 0.54*	1.626± 0.011*	2.35 *	P≤ 0.05
(thalassemia minor)	6.88 ± 0.66*	4.55± 0.49*	2.335± 0.150*	1.94 *	P≤ 0.05
Control	7.99 ± 0.59	5.21 ± 0.48	2.775 ± 0.402	1.88	P≤ 0.05

P significant difference from control values.
*: significant difference between minor and major thalassemia patients.

The (Mean ± SD) level of TP in sera of control group, and β- thalassemia patients (minor &major) in gm /dl were (7.99 ±0.59), (6.88 ± 0.66) and (5.46 ± 0.54), respectively. The results showed a significant decrease of TP in β- thalassemia major and minor comparing with control. Also a significant decrease in TP of β- thalassemia major compared to minor was found . These results are in agreement with one study who claimed that the decrease in serum total protein is due to secondarily decreased synthesis of protein by the liver[13].The results of serum albumin measurements revealed that the mean values of albumin in the three studied groups in gm/dl were (5.21 ± 0.48) , (4.55 ± 0.49) and (3.83 ± 0.54) , respectively.The low levels of albumin may roughly be balanced by arise in immunoglobulin levels . This is quite common combination;where most of individual proteins , other than albumin , make relatively small contribution to total protein because of quite large percentage change in the concentration of one of them may not be detected as change in total protein so only low albumin levels are of clinical importance[14].The (A/G)ratio of β- thalassemia patients (major &minor) were 2.35 and 1.94 respectively decreased significantly to 1.88 in the control group. A ratio much less than one can give clues about problems in the body[15,16].The serum levels of IgG, IgM and IgA in sera of control,minor and β- thalassemia major patients are shown in table (2). Furthermore,the results showed high levels of IgG, IgM and IgA in sera of thalassemia patients compared to control. Also a significant higher increase in immunoglobulins in sera of major thalassemic patients was noticed compared to thalassemia minor patients.

Table 2 : Serum immunoglobulin levels (IgA, IgM and IgG) of control, pathological control and β- thalassemia major patients.

Groups	IgG mg/dl (No.=50)	IgM mg/dl (No.=50)	IgA mg/dl (No.=50)	t-Test
thalassemia major patients	1519 ± 37.60*	220 ± 6.55*	241.6 ± 7.48*	P≤ 0.05
thalassemia minor Patients	1182 ± 18.41*	148.68 ± 4.13*	202 ± 11.12*	P≤ 0.05
Control	1004 ± 19.00	120.6 ± 3.04	168.6 ± 5.05	P≤ 0.05

P significant difference from control values.
* significant difference between minor and major thalassemia patients

Such observations can be attributed to many factors. For instance repeated blood transfusion in β- thalassemia patients would result in a continuous exposure to various antigens and might lead to increased levels of serum immunoglobulins.Repeated infections also stimulate the immune system and may result in increased immunoglobulin levels [17,18]. Iron overload was suggested by some investigators as an important contributing factor in altering the immune parameters in thalassemia patients that results in increased

*migration of T helper cell*s to the gut and lymph nodes and this causes an increase in serum immunoglobulin levels in thalassemia patients[19,20].Figure (1) showed sera electrophoresis pattern of thalassemia major ,minor and control groups. Abnormal pattern of serum protein in sera of patients groups is obvious by the decrease shown in the albumin band and change in the pattern of β and γ globulins which include main proteins specially those of immunoglobulins.The decrease in albumin levels of patient groups could be due to decrease rate of synthesis ,or to an increase in catabolism rates which occurred in many diseases[14].Meanwhile results revealed a mild reduction in β globulin that may be due to the presence of transferrin (the iron bound protein)in this band,which is considered as to be the major component of the β-globulin fraction and appears as a distinct band on high resolution serum protein electrophoresis[22]. Also figure (1A) of electrophoresis showed a highly significant increase in γ- globulins in major β- thalassemia major patients compared to normal control, while figure (1B) showed a mild increase in γ-globulins in pathological control compared to normal control.The increase in γ-globulins related to hemoglobinopathy diseases[9].

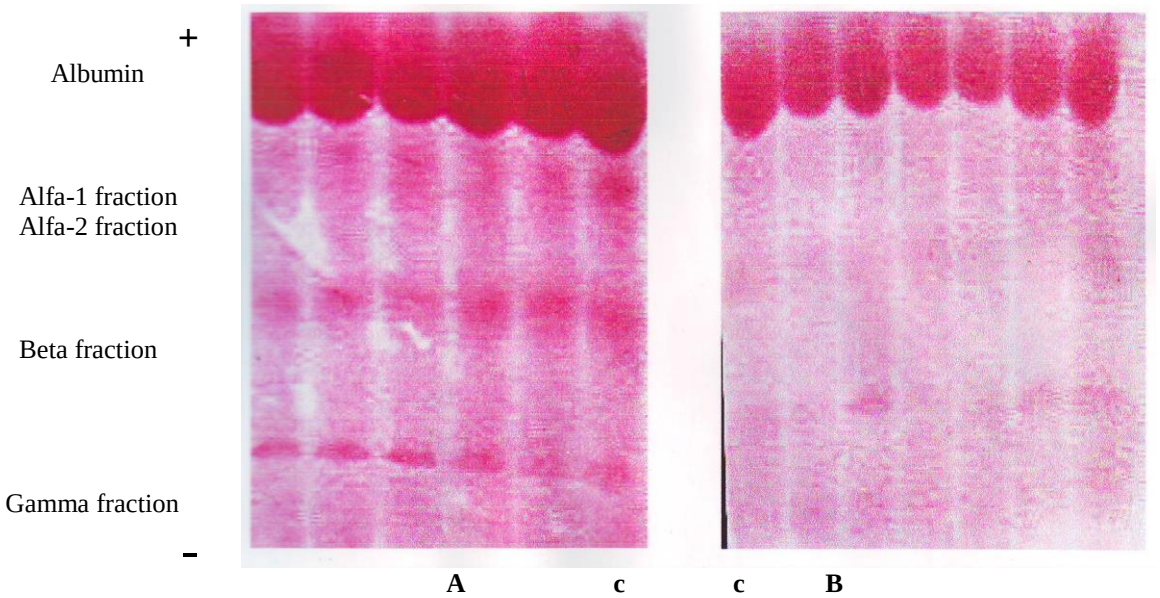

Figure 1 : Serum protein Electrophoresis Pattern (c control)
A- (left):Normal control and major β-thalassemia patients
B-(right):Normal control and pathologicalcontrol (minor)

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