Biochemical Study on Splenectomy and Non Splenectomy Iraqi Major Thalassemic Patients

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Abstract

In this study the activities of alanine transaminase (ALT) and aspartate transaminase (AST) were evaluated, in addition to total protein and albumins in sera of sixty one subjects whose ages were ranged between(4-16) years. These subjects were, twenty eight major thalassemic patient (12 with splenectomy and 16 non splenectomy) and fifteen with minor thalassemia. eighteen healty subjecte as control.

The result revealed a significant elevation in the activities of both aminotransferases enzymes (AST and ALT) in the sera of all the alassmic patient groups compared with control. Also a significant increase in the activity of ALT in sera of non-splenectomy compared to splenectomy major thalassemic patient , which could be an indicative of the severity of liver dysfunction.

No significant differences in the albumine levels of all patient groups compared to control was noticed. A significant low level of total protein in non splenectomy major thalassemic compared to control was found , while no significant difference between total protein level in the sera of seplenectomy major Thalassemic compared with control was found. A conclusion could be obtained for the low levels of total protein and the normal level of albumin in sera of non splenectomy major thalassemia is the reduction in some protein fractions of the globulin part of the serum.

Introduction

The thalassemias are hereditary hemolytic diseases in which an inbalance occurs in the synthesis of globin chains. As a group they are the most common single gene disorders in humans. Normally , synthesis of the α and β globin chains are coordinated, so that each α – globin chain has a β- globin chain partner.This leads to the formation of α_2 β_2 (HbA). In the thalassemias , the synthesis of either the α- or the β- globin chain is defective. A thalassemia can be caused by a variety of mutations, including entire gene deletions, or substitutions or deletions of one to many nucleotides in the DNA [1]. In these disorders, synthesis of β- globin chains is decreased or absent, typically as a result of point mutations that affect production of functional mRNA, however, α – globin chain synthesis is normal. α- Globin chains cannot form stable tetramers and, therefore precipitate, causing the premature death of cells initially destined to become immature red blood cells [2] . This defect results in an excess of the other chain which precipitates within the red cell membrane causing hemolytic anemia bringing about cell death within the bone marrow and premature removal of circulating red cells by the spleen[3]. β- thalassemia is the most familiar type, in which the β- globin chain synthesis is impaired. A deficiency of β chains, α chain synthesis continuos producing an increased proportion of HbF and α chain production increases the amount of HbA_2[4]. The severity of the disease depends on the amount of HbA and HbF, which present [3].

Amino transferses catalyze the transfer of an α- amino group from an α – amino acid to an α- keto acid .These enzymes , also called transaminases, generally funnel α- amino groups from a variety of amino acids to α- ketoglutarate for conversion in to NH_4^+. Aspartate

amino transferase (AST), one of the most important of these enzymes, catalyzes the transfer of the amino group of aspartate to α-ketoglutarate .

Aspartate + α- ketoglutarate $\rightarrow$ oxaloacetate + glutamate

Alanine amino transferase (ALT) catalyzes the transfer of the amino group of alanine to α- ketoglutarate.

Alanine + α- ketoglutarate $\rightarrow$ pyruvate + glutamate

These transamination reactions are reversible and can thus be used to synthesize amino acids from α- ketoacids[5]. The proteins are substances made up of smaller building blocks called amino acid. And are important constituents of all cells and tissues. Human serum contains more than 125 identified proteins. So there are many different kinds of proteins in the body with many different functions[6]. The major site of synthesis of the plasma proteins is the liver. The second major site is the immune system[7]. The concentration of total protein in human plasma is approximately 7.0 – 7.5 g/dl and comprises the major part of the solids of the plasma. The proteins of the plasma are actually a complex mixture that includes not only simple proteins but also conjugated proteins such as glycoprotein and various types of lipoproteins[8] . Total protein level depends on the balance between their synthesis and their catabolism or loss from body . A total serum protein test measures total amount of protein in blood serum as well as the amounts of albumin and globulin which are the main two groups of protein[9] . Albumin has a single polypeptide chain of 580 amino acids. It is a very stable protein with a high net negative charge at physiologic pH with a M.Wt. of 66,000 D. Albumin forms the largest portion of the serum proteins it is produced and degraded by the liver. It carries many small molecules[10] . It is a collective term, used to refer to proteins other than albumin with the exception of the immunoglobulin and some complement proteins which are formed by immune system , most of the globulins are produced in the liver[11].

Experimental Part

- Blood collection and subject samples : five mL of venous blood sample was obtained from twenty eight subjects of both sexes , whose β-thalassemia major (splenectomy and non splenectomy) condition was confirmed by evalution of HbF , HbA and HbA_2 using the Bio. Rad variant hemoglobin testing system from Bio. Rad company (Italy) , at Ibn-AL Balady hospital in addition to fifteen minor thalassemic subjects as pathological control were included in the present study. Also eighteen healthy subjects were enrolled as control group . All blood samples , which were collected from the above subjects were allowed to coagulate at room temperature within 30 minutes, centrifuged for 10 minutes at 2500g. The resulting serum was separated and divided to parts and frozen until time of analysis. Information of all subjects enrolled in the study are shown in table (1)
- Determination of amino transferases activities the dermination were performed using a ready Kit purctuesed from Randox laboratories , England.
- Determination of AST activity.
  Colorimetic method for determination of serum aspartate amino tranferase by monitoring the concentration of oxaloacetate hydrazine formed with 2,4 dinitrophenyl- hydrazine (DNPH)[12].
- Determination of (ALT) Activity.
  The Activity of ALT is measured by monitoring the concentration of pyruvate hydrozone formed with (DNPH)[12].
- Determination of total protein.

The concentration of total protein was determined according to the Randox Kit[13], in which cupric ions, in an alkaline medium, interact with protein peptide bonds resulting in the formation of a coloured complex .

- Determination of Albumin levels.
  Plasma albumin level was determined by the dye-binding technique using bromocresol green (BCG), provided by a ready kit from Randox laboratories, England.

In the assay medium albumin will bind BCG in phosphate buffer (pH 4.2), thus yielding a blue complex. The increase in the absorbance, at 630 nm , is proportional to the amount of albumin present in the sample. Human albumin is used as a standared[14].

Statistical analysis

The results were analyzed by student -t- test with p values ≤ 0.05 considered significant using Excel XP 2000 program.

Results and Discussion

Table(2): showes the activity of AST and ALT in sera of major thalassemic patients (splenectomy and non splenectomy) , minor thalassemia and healthy control.

A high significant increase in the activity of AST and ALT for both major thalasemic group compared to that for minor thalassemic and control were found. The result of the present study is in a good agreement with result reported earlier ,the reason for this elevation in the activities are due to symptoms of liver damages. As liver is the major site of iron storage , the liver is a conspicuous victim of excess iron deposition[15].

However the incidence and prevalence of iron- induced organ injury was not influenced by the ethnicity of the patients in multiple large thalassemia trials[16]. Plasma ALT is a marker of liver injure which increased in thalassemia and considered to be an indicative of liver dysfunction and leakage of liver metabolites into the plasma[17].

Results of serum total protein and albumin are shown in table (3).From table (3) the mean values of albumin in sera of patient with β-thalassemia are not siginfingthy different as compared to control group. These results agree with a studied parameter reported that patient with β- thalassemia major (splenectomy and non splenectomy) showed similar serum albumin level compatable to healthy individuals[18]. On the other hand reports showed lower level of albumin in the thalasseamic patient due to the presence of antibody in the sera of patient under blood transfusion[19].

The total protein concentration in sera of all pataient groups except non splenectomy showed non significant differences with that for healthy control while a significant reduction was found in the non splenectomy compared to control the possible cause reported to be secondarily decreased synthesis of protein by the liver and which imposed to damages by transfusion therapy leading to iron over load[20].

The reduction in total protein and the near normal value of albumin could be due to the decrease in the globulin synthesis because the latter consist of different types (i,e α_1 , α_2 , β and δ) globuline so the reduction in any one of these globulin could result in a decrease of total level.

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Table (1): information of thalassemic patients and healthy control.

Groups	No.	Mean age (year)	range of age (year)
Major thalassemic (splenectomy)	12	9 ± 2.2	6-11
Majar thalassimic (non–splenectomy)	16	8 ± 1.8	4-12
Minor thalassemia	15	14 ± 3.7	9-16
Healthy control	18	12 ± 2.3	7-16

Table(2) : AST and ALT actives in sera of all groups .

Groups	No.	AST mean ±SD(U/L)	P	ALT mean ±SD(U/L)	P
Major thalassemic (splenectomy)	12	46.8 ± 19.1	< 0.05 S	51.8±13.1	< 0.05 S
Major thalassemic (non splenectomy)	16	48.7 ± 11.7	< 0.05 S	58.7 ± 21.3	< 0.05 S
Minor thalassemic	15	10.3 ± 2.3	S < 0.05	8.4 ± 3.1	< 0.05 S
Health control	18	7.9 ± 1.8	< 0.05 S	5.6 ± 1.9	
			p^* >0.05 NS		p^* < 0.05S

* represent p value between both major thalassemic patients (splenectomy and non splenectomy).

Table (3): serum total protein and albumin in sera of all studied groups.

Groups	Total protein (gm/dL)± SD	p	Albumin (gm/dL)± SD	p
Major thalassemic (splenectomy)	7.12 ± 0.39	> 0.05 NS	4.08 ± 0.41	> 0.05 NS
Major thalassemic (non splenectomy)	6.21 ± 0.81	< 0.05 S	4.03 ± 0.46	> 0.05 NS
Minor thalassemic	7.09 ± 0.37	> 0.05 NS	4.12 ± 0.51	> 0.05 NS
Health control	7.18 ± 0.28	> 0.05 NS	4.01 ± 0.31	> 0.05 NS
		p^* <0.05 S		p^* < 0.05 NS

دراسة كيموحيوية لمرضى فقر دم البحر الابيض المتوسط لكل من الخاضعين وغير الخاضعين لرفع الطحال في العراق

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الخلاصة

تمت في هذه الدراسة قياس فعالية انزيمي الانين ترانزمينز (ALT) ،والاسبارتيت ترانزامنير (AST) فضلا عن تقدير مستوى البروتين والالبومين في مصل الدم واحد وستون أنموذجا تتراوح اعمارهم من (4–16) سنة في هذه الشريحة ثمانية وعشرون يعانون من مرض فقر دم البحر الابيض المتوسط منهم اثنا عشر خضعوا لعملية رفع الطحال ،وستة عشر لم يخضعوا لها . وهناك خمسة عشر مريضاً يعاني من فقر دم البحر الابيض المتوسط الخفيف كما شملت الدراسة ثمانية عشر أنموذجا لاصحاء كمجموعة سيطرة .

إظهرت النتائج زيادة معنوية في فعالية انزيمي AST وALT في مصل دم كل من مجاميع المرضى مقارنة مع مجموعة السيطرة كذلك وجدت زيادة معنوية في فعالية ALT في مصل دم المجموعة التي لم تخضع لرفع الطحال نسبة الى المجموعة الخاضعة للرفع والتي يمكن ان تكون دلالة على شدة التلف الحاصل في الكبد .

لم تظهر اختلافات معنوية في مستوى الالبومين بين مجاميع المرضى مقارنة بمجموعة السيطرة ظهر انخفاض معنوي في البروتين الكلي في المرضى غير خاضعين لعملية رفع الطحال مقارنة مع الاصحاء، بينما لم تظهر فروقات معنوية في المرضى الخاضعين لعملية رفع الطحال مقارنة مع الاصحاء .

يمكن ان نستنتج من انخفاض البروتين الكلي وبقاء مستويات الالبومين في مصل دم المرضى غير الخاضعين لرفع الطحال بأن هناك انخفاضا في بعض اجزاء من الكلوبيولينات في مصل الدم .