Study of Some Hematological Parameters and Determination of Glysated Hemoglobin in β-Thalassemia

Zaineb Moneb Al-Rubae
Wasan A.K. M.
College of Education\Baghdad University
Zyad Hussein Jawad AL-Qaisi
College of Education\Al-Mustansiria University

Abstract:

The aim of the present study is to evaluate glysated hemoglobin (HbA1c) in major ,minor thalassemic patients and compare that with control,in order to investigate the possibility of using such parameters as marker in comparison with there levels in sera of normal control.

Some hematological parameters as diagnostic factors for thalessemia were estimated in sera of 50 control, 50 minor and 50 major thalassemic patients.The age for the all subjects range from (4-18) .A significant elevation in the HbF % was found in major thalessemia with value of (90.33±28.22) comparing to minor group (2.56 ± 1.12) and control group (0.44 ± 0.02).The HbA_1 % for control, minor and major thalassemic patients were (7.94 ± 0.57), (11.63 ± 1.38) and (14.83 ± 1.09) respectively.

The value for HbA_{1c} % increased also for all studied groups (5.95 ± 0.50), (9.04 ± 1.15) and (11.83 ± 0.86) for control, minor and major thalassemic patients respectively.

A conclusion could be drawn from the results of present study that the significant elevation in HbA1c in serum of major thalassemic patients compared to control was found that shown the relation between β-globin ,glucose and iron which led to oxidative strees and insulin resistance that cause endothelial damage ,therefore, the evaluation of HbA1c needs further studies.

Introduction:

Thalassemia is the name of a group of genetic blood disorders (inherited) 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 chain in the same patient, but never α and β chain together, unmatched globins precipitate and damage red blood cell membranes causing their destruction while still in the marrow[1,2].

Glycoconjucates are a class of complex carbohydrates equivalent terms used to denote molecules containing one or more carbohydrate chains covalently linked to protein (to form glycoproteins or proteoglycans) or lipid (to form glycolipids)[3,4].

Glysated hemoglobin (HbA1C) is the nonenzymatic glycated product of the hemoglobin beta- chain at the valine N-terminal residue. The number 1C following HbA represents the order in which this hemoglobin is detected on chromatography. Hence, other hemoglobin peaks are referred to as $HbA_{1a1,}$ HbA_{1a2}, HbA_{1b}, and so fourth[5].

The A1C constitutes about 60-80 % of total glycated hemoglobin, this type of hemoglobin serve as the certain function of hemoglobin but it contain one molecule of sugar in its structure, therefore, it can be separated chemically. It is normally present, albeit at low levels, in circulating red cells because of the glycosylation reaction between hemoglobin and circulating glucose[6].

In the presence of excess plasma glucose, the hemoglobin beta-chain become increasingly glycosylated , making the A1C a useful index of glycemic control and usefulness HbA_{1c} in discriminating between iron deficiency and thalassemia[7].

The major hemoglobin in the fetus (HbF) is represented by $\alpha_2\gamma_2$ [8]. The HbF is raised (by about 1% - 3%) in one third to half of the people with β-thalassemia trait. A raised HbF (5% -20%) in the presence of hypochromic, microcytic, red cell indices however, is the hallmark of the α, β- thalassemia trait and it should be measured, whenever HbF is detected on Hb electrophoresis on cellulose acetate membrane.The clinical significance of conditions with raised HbF related to their circulation in disorder such as cell disease and beta thalassemia in which raised levels of HbF can lead to considerable amelioration of disease severity. Mature erythrocytes or red blood cells (RBCs) lack nuclei, mitochondria and ribosome. They are incapable of biosynthesis. Mammalian RBCs regulate the oxygen (O_2) affinity of hemoglobin. Abnormalities of RBCs proteins result in several importante disease[9]

Materials and Methods:

Selection of subjects and blood sampling:

Six ml of venous blood sample were obtained from 50 patients with β - thalassemia major, 50 patients with β- thalassemia minor as pathological control

group and 50 healthy individuals as control group.The age of all studied groups range from years.

The blood samples which collected from all subjects were transferred into tube with ethelen diamine tetra acetic acid (EDTA) as anticoagulant which used for determination of HbF% by HPLC –Variant,Bio Rad ,Italy while HbA_1% and HbA_{1c} were determined by using a kit from a Stanibo-Glycohemoglobin – Texas.

The Bio-Rad Variant Hemoglobin Testing System:

This system utilized the cation-exchange high performance liquid chromatograp- by (HPLC) for automated analysis on normal and abnormal hemoglobin in adult and neonatal blood samples[10].

Analytical methods for hematological parameters:

HPLC- (Bio-Rad Variant Hb testing system with β- thalassemia short program). For HbA_2 and HbF quantification, all operations of the system are controlled automatically by a built in microprocessor. The only manual step required is the preparation of sample hemolyzates, typically by adding 5µl of well mixed blood to 1 ml of hemolyzing solution. It uses the principle cation-exchange HPLC to separate HbA, HbA_2 (coelute E), HbF, HbS, HbD and C on a non porous cation-exchange resin.As the elute moves through the flow cell, a dual wavelength filter. Photometer detects the absorbance of Hb at the primary wavelength (415 nm) and correct for non specific absorbance at the secondary wavelength (690 nm). A presumptive identification of each components is determined automatically by comparing the retention (elutetion) time to the preprogrammed retention times for HbA, HbA_2, HbS, HbD and HbC. HbE can not be separated from HbA_2 or identified as HbE, but should be suspected if the peak identified as HbA_2 is highly increased, A built in integrator collects and reduces the data from each analysis and a printer produces a report showing the identification and area percent relative to the total area of the HbS separated during the 6.5 min program. The system is calibrated at beginning of each run with the Bio-Rad Variant hemoglobin A_2/F calibrator, a solution labeled by the manufacture with assigned values for the two hemoglobin components.

Determination of Glycohemoglobin (HbA_1&HbA_{1C}):

Glycohemoglobin(HbA_1&HbA_{1C})were determined in whole blood using colorometric method[11].

Hemolyzed whole blood is mixed with weakly binding cation exchange resin. The non glycosylated hemoglobin (HbAo) binds to the resin, leaving (HbA_1) free to be removed by means of a resin separator in the supernatant.The percent of HbA_1 is determined by measuring the absorbance values at 415 nm of the HbA_1 fraction and the total hemoglobin fraction, calculating the ratio of absorbances (R), and comparing this ratio to that of glycohemoglobin standard carried through the same procedure. Results are express as HbA_1 but can be converted or derived as HbA_{1c} by using a conversion factor .

Statistical Analysis:

Data presented were the means and standard deviations. Study of T-Test (p) was used to compare the significance of the difference in the mean values of any two 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 analysis (ANOVA)[12].

Results and Discussion:

Table (1) showed the percentage of HbA_1%, HbF% and HbA_{1c}% which expressed as mean± SD in 50 patients with major thalassemic, 50 minor thalassemic and 50 control group.

Table (1): Normal hemoglobin HbA_1%, HbF % and HbA1c% levels in sera of control, minor and major thalassemic patients.

Groups	HbA_1 %	HbF %	HbA_{1c} %	t-Test
Major-Thalassemic	14.83 ± 1.09	90.33 ± 28.22	11.83 ± 0.86	P≤ 0.05
Pathalogical control (minor)	11.63 ± 1.38	2.56 ± 1.12	9.04 ± 1.15	P≤ 0.05
Control	7.94 ± 0.57	0.44 ± 0.02	5.95 ± 0.50	P≤ 0.05
				**p ≤ 0.05

***p represented p value between minor and major thalassemia*

A significant elevation was found in HbA_1% in both patients groups compared to control group. Also a significant increase was found for HbA_1 in major thalassemic patients compared to minor thalassemic .

A Significant elevation in HbF% levels was noticed in thalassemic patients compare to control group which the values are (0.44 ± 0.02), (2.56 ± 1.12) and (90.33 ± 28.22) for control, minor thalassemic patients and major thalassemic patients respectively. Also significant elevation in HbF% for major thalassemic patients compared to pathological control (minor) was found.The synthesis of fetal hemoglobin (HbF) is normaly reduced to a very low levels (less than 0.6%) of the total hemoglobin in adults.The HbF is restricted to sub population has 0.3 % to 4.4 % F- cells.The result of the present study showed the high concentration of HbF due to cell- wide HbF distribution rather than the occasional occurrence seen with other disease or in specific fetal cells. Even with increased production of hemoglobins A_1, and F there are still excess alpha chains. Excess α- chains precipitate in the developing normoblast which cause membrance damage and inflexibility leading to premature RBC destruction and bone marrow production of abnormal cells (ineffective erythropoiesis that contributes to the anemia because the abnormal cells are destroyed in the marrow)[13].

The results demonstrated a significant elevation in HbA_{1c}% levels in sera of pathological controls (minor thalassemia) and patients with major β-thalassemia compare to control group. The results are in agreement with some studies[14,15].Because A1c is based on the total hemoglobin, both qualitative and variations in hemoglobin can affect the A1c value . There are many pathophysiological process that can affect the A1c value within structure of hemoglobin itself[16-18]. Some researches demonstrated the relationship between iron and glucose metabolism, because iron modulates insulin action in the human[19,20]. Another explanation could be due to genetic reasons that both β- globin and insulin genes are present on short arm of the chromosome 11 [21] . Insulin stimulates ferritin synthesis and facialitates iron uptake by the cell through the translocation of transferrin receptors from the intracellular compartment to the cell surface. Conversely, iron influences metabolism. Iron in a potent prooxidant that increases the cell oxidative stress, causing inhibition of insulin internalization and actions, results in hyperinsulinemia and insulin resistance. The increased oxidative stress and insulin resistance cause tissue damage and diabetes mellitus[22,23].

Refrences :

1-Cappolini N.,Cohen A.,and Proter J.:Thalassemia International Federat, (2000),1(29),79-92.
2- Noguchi C., Butterwoth J.,Karawajew L.,Kupperrs R.,and Jacobsohn D.:Haematologica ,(2004),89,1281-1283.
3-Murray R., Granner D.,Mayes P.,and Rowel V.:" Harpers Illustrated Biochemistry",26th ed.,Mc Graw–Hill Companies ,(2003),111,494.
4- Campbell K. and Shown O.:"Biochemistry " 5th ed. ,Thomson Learning Academic Learning ,(2006), 442,458-459.
5- Huy A.,Tran D.,Silva M., and Nikolai P. :J. Clin Chem. ,(2004),140 –143.
6- American Diabetes Association ,(2005), 2, 568.
7- Aslan D.,and Gursel T.:Pediatr Hematol Oncol,(2006),23(4),307-315.
8-Stryer,L.:"Biochemistry",4thed.NewYork,W.Freeman Company,(2000);153-156.
9-Adamson J.: Am. J. Med, (1996);101:45-65.
10-Riou J., Gadart C., and Hurtrel D.:J. Clin. Chem.; (1997);43:34-39.
11- Bishop L., Fody P. and Lary Schoeff ,Clinical Biochemistry: Principles Procedures,Correlation",5th ed ,Lippincott Williams & Wilkins (2005),77.
12- Dainel W. : "Biostatestics: A Foundation for analysis in the Health science", 4th ed,(1987),127-139.
13- Christopher P., Randie R., Curt L., Thomas G., and William L. : J. Bio. Chem , (2004),350,123-128.
14- Castelli R.,Tempesta A.,Bianchi A., Ivaldi G., and Cappellinis U.: Diabetic Medicine, (2004), 21(4),377-379.
15-Roseman S.:J.Bio.chem,(2001),276,41527.

16- Karger R., Schmidt J., Weber C., Frietsh T., and Kretschmer V.: Transfution, (2006),46,811-817.
17- Symeonidis A., Kourakils A., Psiroyiannis A., Vagenakis A., and Zoumbos N.: Hematology,(2006),85(2),79.
18- Fleming R., Sly W. : Annu Rev Physiol,(2002), 64,663-680.
19-Goldstein D., Lorenz R., Malone J., Mathan D., Peterson C. : Diabetes, (2000), 23,805 – 825.
20- Barbieri M., Ragno E., Benvenutti E., Zito G., Corsi A., Ferrucci L: Diabetoliogia, (2001),44,1232-1237.
21- Kar B.: J. Asso phys. India, (2002),50,1368-71.
22-Mohapatra M.: Asso Phys. India,(2005),895-896.
23-Fernandez J., Bermejo A. and Ricart W.:Diabetes ,(2002),51,2348.

دراسة بعض الدوال الهيماتولوجية وتقدير الهيموكلوبين المسكر في مرضى فقر دم البحر الابيض المتوسط من نوع بيتا

زينب منيب الربيعي

وسن عبد الكريم محمد

قسم الكيمياء /كلية التربية ابن الهيثم/جامعة بغداد

زياد حسين جواد القيسي

قسم الكيمياء / كلية العلوم /الجامعة المستنصرية

الخلاصة:

الهدف من هده الدراسة تقدير الهيموكلوبين المسكر في مرضى فقر دم البحر الابيض المتوسط الكبرى والصغرى مقارنة مع مجموعة السيطرة للبحث من امكانية استخدامها كدليل مقارنة مع مستويات مجموعة السيطرة.

يتضمن هذا االبحث دراسة بعض الدوال الهيماتولوجية في الدم الكلي لمرضى فقر دم البحر الابيض المتوسط الكبرى و الصغرى . اجريت هذه الدراسة على ١٥٠ شخصا، ٥٠ منهم مصابا بمرض فقر دم البحر الابيض المتوسط الكبرى، و ٥٠ مصابا بمرض فقر دم البحر الابيض المتوسط الصغرى و الذين اعتبروا كمجموعة سيطرة مرضية ، و٥٠ شخصا من الاصحاء كمجموعة سيطرة طبيعية. تتراوح اعماركل المجاميع المدروسة بين(٤–١٨) سنة.

تم تقدير نسبة الهيموكلوبين المسكر HbA_{1c} والهيموكلوبين HbF والهيموكلوبينHbA_1 في دم مجموعة المرضى ومجموعة السيطرة حيث وجدت زيادة معنوية في نسبة كل من HbA_{1c} وHbF و HbA_1في مجاميع المرضى مقارنة مع الاصحاء.

تم الاستنتاج من نتائج البحث وجود زيادة معنوية في نسبة الهيموكلوبين المسكر في مرضى فقر دم البحر الابيض المتوسط الكبرى مقارنة مع مجموعة السيطرةوالتي بينت وجود علاقة بين بيتا –كلوبين والسكروالحديد والدي يؤدي الى الشد التاكسدي ومقاومة الانسولين وبالتالي تحطم الانسجة.