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 their levels in sera of normal control.

Some hematological parameters as diagnostic factors for thalassemia were estimated in sera of 50 control, 50 minor and 50 major thalassemic patients. The age for all subjects range from (4-18). A significant elevation in the HbF % was found in major thalassemia with value of (90.33±28.22) comparing to minor group (2.56 ± 1.12) and control group (0.44 ± 0.02). The HbA1c % 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 HbA1c % 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 stress and insulin resistance that cause endothelial damage, therefore, the evaluation of HbA1c needs further studies.
Study of Some Hematological Parameters and Determination of Glysated Hemoglobin in β-Thalassemia

Zaineb Moneb Al-Rubae, Wasan A.K. M., Zyad Hussein Jawad AL-Qaisi

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].

Glycoconjugates 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].

Glycated 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 α$_2$γ$_2$[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 important 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
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The blood samples which collected from all subjects were transferred into tube with ethylened 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 \(\beta\)-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 5\(\mu\)l 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.
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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≤ 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 HbA₁%, HbF% and HbA₁c% which expressed as mean± SD in 50 patients with major thalassemic, 50 minor thalassemic and 50 control group.

Table (1): Normal hemoglobin HbA₁%, HbF % and HbA1c% levels in sera of control, minor and major thalassemic patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>HbA₁ %</th>
<th>HbF %</th>
<th>HbA₁c %</th>
<th>t-Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major-Thalasemic</td>
<td>14.83 ± 1.09</td>
<td>90.33 ± 28.22</td>
<td>11.83 ± 0.86</td>
<td>P≤ 0.05</td>
</tr>
<tr>
<td>Pathalogical control (minor)</td>
<td>11.63 ± 1.38</td>
<td>2.56 ± 1.12</td>
<td>9.04 ± 1.15</td>
<td>P≤ 0.05</td>
</tr>
<tr>
<td>Control</td>
<td>7.94 ± 0.57</td>
<td>0.44 ± 0.02</td>
<td>5.95 ± 0.50</td>
<td>P≤ 0.05</td>
</tr>
</tbody>
</table>

**p represented p value between minor and major thalassemia

A significant elevation was found in HbA₁% in both patients groups compared to control group. Also a significant increase was found for HbA₁ 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 normally 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 A₁, and F there are still excess alpha chains. Excess α- chains precipitate in the developing normoblast which cause membrane 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\textsubscript{1c} % levels in sera of pathological controls (minor thalassemia) and patients with major \( \beta \)-thalassemia compared 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 processes that can affect the A1c value within the 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 \( \beta \)-globin and insulin genes are present on short arm of the chromosome 11\[21\]. Insulin stimulates ferritin synthesis and facilitates 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\[22,23\].

References:
6- American Diabetes Association, (2005), 2, 568.
11- Bishop L., Fody P. and Lary Schoeff, Clinical Biochemistry: Principles Procedures, Correlation, 5\textsuperscript{th} ed., Lippincott Williams & Wilkins (2005), 77.
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دراسة بعض الدوال الهيماتولوجية وتقدير الهيموكلوبين المسكر في مرضى فقر الدم البحر الأبيض المتوسط من نوع بيتا

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الخلاصة:

الهدف من هذه الدراسة تقييم الهموغلوبين المسكر في مرضى فقر دم البحر الأبيض المتوسط الكبيرة والصغرى مقاورة مع مجموعة السيطرة للبحث من امكانيات استخدامها كدليل مقارنة مع مستويات مجموعة السيطرة.

يتضمن هذا البحث دراسة بعض الدوال الهيماتولوجية في الدم الكلي لمرضى فقر دم البحر الأبيض المتوسط الكبيرة والصغرى. أجريت هذه الدراسة على 150 شخصًا، 50 منهم مصابين بمرض فقر دم البحر الأبيض المتوسط الكبيرة، و 50 مصابا بمرض فقر دم البحر الأبيض المتوسط الصغرى، الذين اعتبروا كمجموعة سيطرة مرضية، و 50 شخصًا من الأصحاء كمجموعة سيطرة طبيعية. تتراوح اعمار المجموعات المدروسة بين (4-60) سنة.

تم تقييم نسبة الهموغلوبين المسكر HbA1c والهموغلوبين في دم HbF والهموغلوبين في مجموعة HbA1c من مجموعات المرضى ومجموعة السيطرة حيث وجدت زيادة معنوية في نسبة كل من HbA1c في مجموعات المرضى مقارنة مع الأصحاء.

تم الاستنتاج من نتائج البحث وجود زيادة معنوية في نسبة الهموغلوبين المسكر في مرضى فقر دم البحر الأبيض المتوسط الكبيرة مقارنة مع مجموعة السيطرة، بينما وجود علاقة بين بينو -كلوبين والسكرواحديد والذي يؤدي إلى الشد التاكسدي ومقاومة الانسولين وبالتالي تحطم الأنسجة.