Study of some clinico-pathological parameters of Thalassemia patients in AL-Zahraa hospital

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Abstract:

Thalassemia: is a genetic disorder caused by a defect in production of one or more globin chains of hemoglobin (Hb). Thalassemia is classified into two major groups, namely, α-thalassemia and β-thalassemia according to the particular type of globin chain affected. Various complications caused by this disease including progressive liver failure and abnormal kidney function. One hundred samples of thalassemia were analyzed at AL-Zahraa hospital education in AL-Najaf province. The patients are aged (10-30) years. The results are compared with control group (50) persons. This study included some of the clinic-pathological and hematological parameters such as some liver enzymes, ferritin, complete blood count, blood film, also age and blood group. The results were (50%) male and (50%) female patients while the control group are (25%) male and (25%) female persons. Most Patients were (64%) in the age of (10 -15 years), followed by (26%) in the age group of (16 -20 years) and only (5%) in age (21-25 years), also (5%) for age (26 -30 years). Most frequent blood group in this study was O+ is (30%) followed by B+ group (27%) and the other blood groups A+, AB+, A-, O-, AB-, B- were (25%), (11%), (3%), (2%), (1%), (1%) respectively. Levels of Serum Glutamic Oxaloacetic Transaminase, Serum Glutamic Pyruvic Transaminase and ferritin, in addition to, platelets and White Blood Cells count are higher than normal while Hemoglobin, Hematocrit and Red Blood Cells are decreased. Peripheral blood film of thalassemia patients showing microcytic and hypochromic anemia, in addition to, nucleated Red Blood Cell and large number of target cells. This study demonstrates that thalassemia patients have difference in liver function, serum content ferritin and hematological characteristics in comparison with control group.

Keywords : Thalassemia, Peripheral blood film, Ferritin, clinico-pathological parameters.

Introduction

Thalassemia: is a genetic disorder caused by a defect in production of one or more globin chains of hemoglobin (Hb)¹. Thalassemia is classified into two major groups, namely, α-thalassemia and β-thalassemia according to the particular type of globin chain affected¹,².

α-Thalassemia is mainly caused by a deletion of the α-globin gene, and can be classified into four groups depending on one, two, three, or all four of the α-globin alleles, namely, α-
thalassemia 2 trait (-α/αα) or ‘silent carrier’, α-thalassemia 1 trait (−/αα), Hb H disease (−/−α), and Hb Bart’s hydrops fetalis syndrome (−/−−), respectively[1].

β-thalassemia is generally caused by point mutations of the β-globin gene leading to a reduction (β+) or absence (β0) of β-globin chain production. β-Thalassemia can be classified into three clinical conditions of increasing severity, β-thalassemia minor (β-thalassemia trait), β-thalassemia intermedia, and β-thalassemia major[2].

The clinical manifestations of thalassemia can vary from asymptomatic individuals to those with severe anemia, depending on the degree of gene defect[3]. Various complications caused by this disease including progressive liver failure[4] and abnormal kidney function. The treatment of thalassemia patients involves many aspects, among them blood transfusion, but have harmful effects including iron overload[4]. Trace metals, especially iron, are implicated as causative agents in excessive generation of free radicals which capable of causing oxidative damage to erythrocytes[5]. Iron metabolism in human is unidirectional because of being unable to be eliminated by the excretory route. Therefore, excess of iron is deposited in vital organs such as heart, liver and spleen as ferritin[6-8].

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are enzymes found mainly in the liver. AST and ALT formerly are called serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transaminase (GPT), respectively. The levels of (AST) and (ALT) provide important indicators of potential liver disease. In the serum of healthy adults, AST has a concentration of around 5 to 40 U/L, while ALT has a concentration ranging from 5 to 35 U/L. However, when the liver is diseased or damaged, the levels of AST or ALT rise. Therefore, the amount of AST and ALT in the blood is directly related to the extent of the tissue damage[9]. Relatively simpler way of knowing the liver damage is by estimation of liver enzymes such as (AST) and (ALT) which are raised due to oxidative injury and direct toxic effect of iron on liver cells[10].

Thalassemia is characterized by iron overload condition and this iron overload is both due to increased absorption of iron from gut and from frequent blood transfusions. This state of iron overload affects almost all systems of body directly or indirectly. Liver is the earliest site of iron overload in regularly transfused patients and common cause of morbidity. Iron overload occurs both in hepatocytes and reticuloendothelial cells. The iron induced liver injury is characterized by development of fibrosis and eventually cirrhosis[10]. The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in Beta Thalassemia Major. The association between serum ferritin and levels of body iron are well established and the test is easy to perform compared with other tests for iron overload[11,12].

The complete blood count (CBC) is one of the most frequently ordered and most time-honored laboratory tests in the hematology laboratory. This evaluation consists of nine components and offers the clinician a variety of hematological data to interpret and review that directly relate to the health of the bone marrow, represented by the numbers and types of
cells in the peripheral circulation. Most physicians reported that the most preferred information was the Hgb, Hct, platelet count, RBC count and WBC count\cite{13}.

A microscopic examination of appropriately prepared and well-stained blood smear slides by a pathologist or a knowledgeable laboratory professional is useful for clinical diagnosis\cite{13}. Normal red cells are normocytic normochromic. This means that the average size of a red cell in an adult is the size of the nucleus of a mature lymphocyte. Variation in shape refers to poikilocytosis including target cells, elliptocytes, spherocytes, dacryocytes (teardrop red cells), bite cells and others. Each of these poikilocytes is associated with one or more underlying clinical conditions\cite{13}. In thalassaemia trait the peripheral blood film showing microcytes, hypochromasia, target cells and basophilic stippling or some of them\cite{14}.

The aim of this study is to evaluate some of clinico-pathological parameters and hematological features in thalassemia patients and compare them with the control group of AL-Najaf province’s individuals.

Material and Methods

Study design of this study is control case.

This study carried out on (150) samples in which:

- (50) Samples of non-thalasemia persons as control (25 male and 25 female), control group were aged (10-30) years. The information and blood samples of control group were collected from them in college of Health & Medical Techniques/Kufa, Al-sader medical city and Al-Hakeem hospital over a period of 3 months (from 12/11/2017 to 12/2/ 2018).
- (100) sample of thalasemia patients (50 male and 50 female). Thalassemia patients were aged (10-30) years. The information and blood samples of thalassemia patients were collected from them in the thalassemia center in AL-Zahraa education hospital over a period of 3 months (from 12/11/2017 to 12/2/ 2018). The included thalassemia patients had detailed clinical evaluation including a detailed history and physical examination. Some patients were scrutinized for skeletal face changes (thalassemic faces). The ages at diagnosis were recorded.

Blood collection

At every turn, under sterile conditions, about 5 mL of blood was collected through venipuncture (2.0 ml into EDTA tube for hematological purposes, 2 ml into serum tube for AST & ALT analysis and 1 ml into other serum tube for ferritin analysis). Both serum tubes after clotting occurs were then centrifuged for 15 min at 3,000 rpm.

procedure:
1. Hematological tests

A. Complete Blood Count (CBC):
1) After blood sample is collected about 2.0 ml in to EDTA tube through venipuncture, shake the tube slightly to mix the sample with anticoagulant.
2) Labeling the tube with the information including; number of patient, date and time of collection, sex and age.
3) Should be ensure that the sample has not clot before the sample deals with the instrument.
4) Analysis the sample with CBC instrument (Cel-dyn Rubby) (table-1).

B. Blood film
1) Place a 1 × 3 cm glass microscope slide with a frosted end on a flat surface (usually the counter top of a laboratory bench).
2) Attach a label on the slide or write the specimen identification number, on the frosted surface.
3) Place a 50µ drop of blood approximately ¼” from the frosted slide, using a Pipette (P-100).
4) Hold the slide by the narrow side between the thumb and forefinger of one hand at the end farthest from the frosted end.
5) Grasp a Cover slips between the thumb and forefinger of the other hand at the frosted end.
6) Place the edge of the Cover slips on the lower slide in front of the drop of blood (side farthest from the frosted end).
7) Pull the Cover slips toward the frosted end until it touches the drop of blood. Permit the blood to spread by capillary motion until it almost reaches the edges of the Cover slips.
8) Push Cover slips forward at a 45° angle with a rapid, even motion. A well-made peripheral smear is thick at the frosted end and becomes progressively thinner toward the opposite end. The smear should occupy the central area of the slide and be margin-free at the edges. The ideal shape of smear is “tongue shape”.
9) After the smear is dried, place several drops of leishman stain (table-2-) until the slide is filled.
10) Wait for (10-12)min.
11) Place distal water same quantity of leishman stain (until slid is filled) and wait for (3-5)min.
12) Wash the slide with distal water or tap water and wait until the slide is dried.
13) Place oil immersion on the slide and then examine under microscope, 100X objective lens of the microscope is selected[15].
14) Picturing by (mobile J7) (table-1-) and recording the results.

C. ABO Group and Rh: according to manual kit (SPINREACT):
1) Place on labelled glass slide one drop of anti-A reagent, anti-B reagent and anti-D reagent (table-2-) in three positions on clean and dry slide.

2) Place one drop of blood on each reagent’s drop.

3) Using a clean applicator stick, mix reagent and cells.

4) Slowly tilt the slide back and forth for 30 seconds, with occasional further mixing during the 2 minute period, maintaining slide at room temperature.

5) Read macroscopically after 2 minutes over a diffuse light and do not mistake fibrin strands as agglutination.

6) Read and recording the results for agglutination macroscopically[16-23].

2. Biochemical tests:

A. AST(SGOT) and ALT(SGPT) tests:
1) After blood sample is collected about 2.0 ml in to serum tube through venipuncture, label the sample with the information including; number of patient, sex, date and time of collection and age.

2) Wait for clotting occurs.

3) The sample is centrifuged for 15 min at 3,000 rpm by Centrifuge (PLC_03) (table-1-)

4) Isolate the serum by Pipette (P-1000) in to the tube of instrument (special tube).

5) Analysis the serum sample with instrument (ARCHITECT c4000) (table-1-).

6) Read and recording the results.

B. Ferritin tests:

VIDAS Ferritin was an automated quantitative test for use on the VIDAS family instruments (table-1-) for the determination of human ferritin in human serum using the ELFA technique (Enzyme Linked Fluorescent Assay). Serum was used to determine Ferritin by VIDAS ferritin kit (BIOMERIUX -69280) (table-2-).

Principle

The assay principle combines a one-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA). The Solid Phase Receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre dispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument.

procedure

1- After blood sample is collected about 1.0 ml in to serum tube through venipuncture, label the sample with the information including; number of patient, sex, date and time of collection and age.

2- Wait for clotting occurs.

3- The sample is centrifuged for 15 min at 3,000 rpm.
4- Used one FER strip and one’ FER’ SPR for each sample, control or calibrator to be tested.

5- The test was identified by the FER code on the instrument. The calibrator must be identified by "S1". If the control needs to be tested, it should be identified by "C1".

6- For this test, the calibrator, control, and sample test portion was 100µl.

7- Insert the "FER" SPRs and FER" strips into the instrument. Check to make sure the color labels with the assay code on the SPRs and the Reagent Strips match.

8- Initiated the assay as directed in the User's Manual. The instrument performs all the assay steps automatically.

9- The assay will be completed within approximately 30 minutes. After the assay was completed, remove the SPRs and strips from the instrument.

10- Dispose of used SPRs and reagent strips in an appropriate method [24,25].

Statistics: All the results were analyzed statistically by SPSS version 20 program (2001).

This figure shows the number of patient groups according to gender where the males (50%) and the females were (50%).

Figure (1): The number of thalassemia patients according to gender.
This figure show the age categories of patients (10-15 years) were (64%) followed by (16-20 years) groups were (26%) and the (21-25 years), (26-30 years) were (5%) to both groups.

![Figure (2): Age categories of thalassemia patients.](image)

**Results**

This figure show the most frequency blood group was O+ = (30) followed by B+ = (27), A+ = (25), AB+ = (11), and A-,O-,AB-,B-,were (3),(2),(1),(1) respectively of thalassemia patients.

![Figure (3): The blood group Frequent of thalassemia patients.](image)
This figure show the levels of serum AST, ALT and ferritin, in addition to, platelets and WBCs count of thalassemia patients were higher than control group while HB, HCT and RBCs were decreased.

![Figure 4](image)

**Figure (4):** The levels of some clinico-pathological parameters of thalassemia patients in comparison with control group.

In this study the peripheral blood film of thalassemia patients show wide variations in the size (anisocytosis) and shape (poikilocytosis) of RBCs including microcytes, hypochromasia, target cells and nucleated RBCs, as in pictures (1,2,3and4) in comparison with control group where normocytic normochromic, cells as in pictures (5,6,7and8).

![Picture 1](image)

**Picture(1):** Peripheral blood film showing microcytes(M) and large number of target(T) cells in thalassemia patient (x 1000)

![Picture 2](image)

**Picture (2):** Peripheral blood film showing microcytes(M), hypochromasia(H), nucleated RBCs(NR) and large number of target cells(T) in thalassemia patient (x 1000)
Discussion

Thalassemia are inherited disorders characterized by abnormal production of hemoglobin, associated with low hemoglobin production and excessive destruction of red blood cell.

Present study was conducted to observe clinico-pathological parameters in 100 thalassemia patients attending thalassemia center in Al_Zahraa education hospital.

The present study comprised of (50%) male and (50%) female patients. The control group are (25%) male and (25%) female.

In this study the majority of the patients (64%) were in the age of (10 -15 years),Followed by (26%) in the age group of (16 -20 years),and only (5%)in age (21-25 years),also (5%) for age (26 -30 years) (figure 2).

Blood group in the present study found the most frequent comprising the blood group O+ is (30%) followed by B+ group (27%) and A+ group (25%) and AB+ group is (11%),and the blood group A-, O-, AB-,B- is 3%,2%,1%,1% respectively (figure 3).

This study shows (30 patients) of 100 possess Blood group O+, (27 patients) of 100 possess B+, and (25 patients ) were A+, and only (11 patients) have AB Blood group (figure 3) This results similar with studies of Gira p.Mankad et.al[26]
Thalassemia are group of hereditary and sever disorder resulting from the hemozygous state of one of thalassemia or hemoglobin lepore genes in infancy or childhood\cite{27,28} It is accompanied with metabolic irregular ions , iron overload ,chronic hypoxia and cell damage\cite{28},most patients are dependent on transfusion for their survival and bone marrow transplantation\cite{29,30}.

The present study found the higher level of serum AST and ALT in thalassemia patients (figure 4) indicate an abnormal muscle and liver function ,these finding are in agreement with the finding of Maher Y.Abdalla et.al\cite{31}

There is appositive correlation between serum ALT and AST concentration and serum ferritin levels in thalassemia patients compared to controls group(figure 4) these similar to the finding of Md.Fazlul karim et.al\cite{32}.

All hematological parameters including HB, HCT and RBCs, were found to be significantly.

Lower than the controls group, which are in accordance with the outcomes of the study conducted by filiz simsek et.al\cite{33}.

An increase in serum ferritin level in thalassemia patients have been observed in this study (figure 4), which is contingent with several other studies\cite{34,35}.

Thalassemia is a world wide disorder and β-thalassemia are the most common microcytic hypochromic anemia (pictures 1,2,3 and 4 ) is hematological abnormality in clinical practice and usually is caused by thalassemia trait.

This study finding the total count of leucocytes is always increased (WBCs) in thalassemia patients, in (figure 4) shows significantly increase of WBCs level (4.5_11×10 3 cell/cmm).

The number of WBCs may appear raised due to the presence of large number of immature (nucleated RBC) \cite{26}.This study agreement with study of Gira p.Mankad et.al\cite{26}.

The total count of platelets was increased in thalassemia patients as in (figure 4) shows significantly high level of platelets.

**Conclusion**

This study show the majority and severity of disease occurs in the age (10_15 years) that show high percentage about 64%.

The blood group that most effected by this disease O+ about 30%,while the very rare blood group effected are B-,O- and this study show have 1% to this blood group.

The study also show higher level of serum ALT,AST and ferritin in the thalassemia patients in comparison with control group.
Hematological parameters of patients including [HB, HCT, RBCs] in this study show decrease in the level compared with control group.

The count of platelets and total count of leucocyte of thalassemia patients is also increased in the number in comparison with control group.

**Recommendations**

- Since thalassemia is a genetic disorder, there’s no way to prevent it. However, there are ways you can manage the disease to help prevent complications. In addition to hepatitis vaccines and ongoing medical care, diet and exercise may also be helpful.
- A low-fat, plant-based diet is the best choice for most people, including those with thalassemia.
- Should be avoid rich-iron food such as : fish and meet. also should be avoid fortified cereals, breads, and juices. They contain high iron levels.
- Should be ask the doctor for appropriate exercise.
- Should be perform hematological and biochemical tests monthly.
- Should be avoid the marriage between those families who have this disease to reduce the risk for the offspring.
- There are many drugs for reduce the iron in blood such as (Ferriprone oral, Exjade oral and desferal injection).

**References**

[1] Leung WC, Leung KY, Lau ET, Tang MH, Chan V. Alphathalassaemia. Semin Fetal Neonatal Med 2008;13:215–22.

[2] Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med 2005;353:1135–46.

[3] Winichagoon P, Fucharoen S, Weatherall D, Wasi P. Concomitant inheritance of alpha-thalassemia in beta 0- thalassemia/ Hb E disease. Am J Hematol 1985;20:217–22.

[4] Ambu R, Crisponi G, Sciot R, et al. Uneven hepatic iron and phosphorous distribution in beta-thalassemia. J Hepatol. 1995; 23:544–9.

[5] Widad NM, Al-Naama L, Meaad KH. Trace element in patients with beta thalassemia major. Haem. 2003; 6:376-83.

[6] Taher A, Isma’el H, Cappellini MD. Thalassemia intermedia: revisited. Blood Cells Mol Dis. 2006; 37: 12- 20.
[7]. Rund D, Rachmilewitz E. Beta-thalassemia. Engl J Med. 2005; 353: 1135-1146.
[8]. Origa R, Bina P, Agus A, et al. Combined therapy with deferiprone and desferrioxamine in thalassemia major. Haematologica. 2005; 90(10): 1309-14.

[9]. Huang, X.J.; Choi, Y.K.; Im, H.S.; Yarimaga, O.; Yoon, E.; Kim, H.S. Aspartate aminotransferase (AST/GOT) and Alanine aminotransferase (ALT/GPT) detection techniques. Sensors 2006, 6, 756–782

[10]. Richa J, Sachdeva A. Iron overload and its manifestations. In: sachdeva A, Jain R, Aggarwal RK, Yadav SP, Broker A, eds. Manual of Thalassemia IAP. Pediatric hemato oncology chapter of IAP. 2009:76-115.

[11]. Porter JB. Practical management of iron overload. British Journal of haematology, 2001; 115: 239 – 253.

[12]. Hoffbrand AV and Moss PA . Hoffbrand’s Essential Haematology . John Wiley & Sons Ltd 2016:7:44-43.

[13]. Fratantoro C, Land G W and etal. Hematology in practice. F. A. Davis 2012 :2:22-23.

[14]. Rozenberg G. Microscopic Haematology . Elsevier Australia 2011:3:27-29.

[15]. Lokwani DP. The ABC of CBC. J.P. Medical Ltd 2013:1:23.

[16]. Kholer G, Milstein C. Continuous culture of fused cells secreting antibody of predefined specificity. Nature 1975; 256, 495-497.

[17]. Messeter L et al. Mouse monoclonal antibodies with Anti-A, Anti-B and Anti-A,B specificities, some superior to human polyclonal ABO reagents. Vox Sang 1984; 46, 185-194.

[18]. Race RR, Sanger R. Blood Groups in Man, 6th Edition. Blackwell Scientific, Oxford 1975; Chapter 2.

[19]. Mollison PL. Blood Transfusion in Clinical Medicine, 8th Edition, Blackwell Scientific, Oxford 1987; Chapter 3.

[20]. Issitt PD. Applied Blood Group Serology, 3rd Edition. Montgomery Scientific, Miami 1985; Chapter 6.
[21]. BSCH Blood Transfusion Task Force. Guidelines for microplate techniques in liquid-phase blood grouping and antibody screening. Clinical Laboratory Haematology 1990; 12, 437-460.

[22]. Guidelines for the Blood Transfusion Service in the United Kingdom. H.M.S.O. Current Edition.

[23]. British Committee for Standards in Haematology. Blood Transfusion Task Force. Recommendations for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and cross matching. Transfusion Medicine, 1995, 5, 145-150.

[24]. Mary Ann Knovich, Jonathan A. Storey, Lan G. Coffman, and Suzy V. Torti, Frank M. Torti. Ferritin for the clinician. Blood Rev. 2009 May ; 23(3): 95–104.

[25]. Yutaka Kohgo, Katsuya Ikuta, Takaaki Ohtake, Yoshihiro Torimoto, Junji Kato. Body iron metabolism and pathophysiology of iron overload. Int J Hematol (2008) 88:7–15

[26]. Gira p. Mankad , Bhavik manked. and S.P. siugh July 2013.

[27]. Phumala N, Porasuphatana S, Unchern S. Hemin: a possible cause of oxidative stress in blood circulation of beta-thalassemia/hemoglobin E disease. Free Radic Res. 2003; 37: 129–35.

[28]. Weatherall DJ, Clegg JB. Thalassemia revised. Cell. 1982; 29: 7–9.

[29]. Ghavamzadeh A, Jahani M, Baybordi E. Bone marrow transplantation in Iran. Bone marrow transplantation. 1994; 13: 743-744.

[30]. Zakerinia M, Khojasteh HN, Ramzi M, et al. Bone marrow transplantation in thalassemia major patients using “short” anti-thymocyte globulin therapy in Shiraz, Southern Iran. Transplant Proc. 2005; 37:4477-81.

[31]. Abdalla M, Fawzi M, Salem R, et al. Increased oxidative stress and iron overload in jordanian β-thalassemic children. Hemoglobin. 2011; 35:67–79

[32]. Md.fazul karim,Md. Ismail,AKM Mahbub Hasan, Hossain Uddin shekhar.Apr.2015.

[33]. Simsek F, Ozturk G, Kemahl S, et al. Oxidant and antioxidant status in beta thalassemia major patients. Ankara Universitesi Tip Fakultesi Mecmuas. 2005; 58:34-38.

[34]. Rahul AG, Kumbar KM, Suryakar AN, et al. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. Indian J Clin Biochem. 2008; 23: 337-340.
[35]. Attia MMA, Sayed AM, Ibrahim FA, et al. Effects of antioxidant vitamins on the oxidant/antioxidant status and liver function in homozygous beta-thalassemia. ROMANIAN J. BIOPHYS. 2011; 21: 93-106.