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Serum ferritin ,Iron and haematological parameters in Beta thalassemia transfusion dependent

A research

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أهداء:

من قال أنا لها " نالها "
لم تكن الرحلة قصيرة ولاينبغي لها ان تكون
لم يكن الحلم قريباً ولا الطريق كان محفوفاً بالتسهيلات لكن فعلتها ونلتها.

الى من دعمتني بلا حدود واعطتني بلا مقابل (أمي) أهديك هذه الانجاز الذي لولا تضحيتك لما كان له وجود

الى ملاكي الطاهر وقوتي بعد الله داعمي الاول الى من مد يده دون كلل أو ملل (أبي)

الى من قال فيهم
{ سَنَشُدُّ عَضُدَكَ بِأَخِيكَ}
الى من أمن بقدراتي ولم يدخر جهداً لمساعدتي (أخوتي)

الى من قدم لي العون والتشجيع والمساعدة في اتمام هذه البحث
(الدكتورة شيماء عبيد)

بسم الله الرحمن الرحيم

شكر و تقدير

الى قسم علوم الحياة (رئاسة و اساتذة)

الى الدكتورة شيماء عبيد (مشرفة البحث)

الى كادر مستشفى مرجان في محافظة بابل ومدينة الطب في بغداد السلام(أطباء وممرضين)

الى كل من ساعدني ولو بكلمة

لكم مني كل الشكر والاحترام

الخلاصة

تناولت الدراسة الحالية احد اهم الامراض الوراثية وهي الثلاسيميا . ان استمرار العناية والتشخيص المبكر للثلاسيميا لها اهمية قصوى في عدم استمرارية مثل هذه الامراض في جميع سكان العالم.

تضمنت الدراسة اربعين شخصا مصاب بمرض بيتا ثلاسيميا الكبرى وكان مدى اعمارهم من 5–36 سنة , ممن يراجعون مركز الثلاسيميا في " مستشفى بابل للولادة والاطفال في محافظة بابل وثلاثون متطوع من الاصحاء كمجاميع سيطرة .أجريت الدراسة في تشرين الثاني 2023 الى شهر شباط 2024

شملت الدراسة الحالية دراسة المعايير الدموية مثل كريات الدم الحمراء , خضاب الدم , مكداس الدم , خلايا دم البيض, الصفائح الدموية , الهيموكلوبين ومستوى الفيرتين والحديد في الدم .

دلت نتائج الدراسة على وجود انخفاض معنوي ($P<0.05$) في معدل اعداد كريات الدم الحمر , مكداس الدم , خضاب الدم بينما أظهرت ارتفاع معنوي ($P<0.05$) في معدلات الصفائح الدموية , مستويات الحديد والفرتين في المصل .

Chapter One Introduction

Thalassemia

Thalassemia (from Greek, thalassa: sea, eima: blood; British spelling, "thalassemia").The thalassemia are hereditary anemia's produced by mutations that affect the synthesis of the globin, the protein component of the hemoglobin .thalassemia produce massive public health problems in many parts of the world (Vichinsky,2005).

Thalassemia Classification

There are two types of thalassemia ,α and β-thalassemia (classification accordance to which chain of the globin molecule is affected), both have different genetic defect, disease manifestation ,prenatal diagnosis and management, also different from each other epidemiologically.(Mok *et al*.,2011; Thein., 2013)

❖Alpha-thalassemia

The α-globin chains production is determined via two genetic locus of each chromosome 16, totally four alleles. Alpha- thalassemia caused from removal of the genes or mutation. The pattern of abnormalities is ethnically specific (Old .,2003).

α–thalassemia are categorized according to the number of α–globin genes that are removed (or mutated) (Forget & Bunn, 2013).

* Deletion of one gene (–α / αα) called α+– thalassemia.

* Deletion of two genes (– –/αα or –α/–α) called α0–thalassemia

* Deletion of three genes (– –/–α) called HbH disease

* Deletion of four genes(– –/– –) called Hydrops fetalis with Hb Bart’s

❖β-thalassemia classification

The most common β–thalassemia phenotypes are:

*** β–thalassemia minor or β–thalassemia trait** (heterozygots for a defected β–gene): They are characterized by minimal anemia ,microcytic and hypochromic ,also have

increased of HbA2 and fetal hemoglobin level that vary from normal to slightly raised. (Forget &Bunn, 2013).

* **β–thalassemia intermedia (TI)**: is a less common clinical phenotype. its characterized by hemoglobin levels about (7–10 g/dl) . β–thalassemia intermedia patients display a moderate to severe, β–thalassemia intermedia patients usually do not require blood transfusions therapy to maintain acceptable hemoglobin levels in patients, while blood transfusion could be necessary if the anemia get worse due to associated complications (Forget & Bunn, 2013).

patients had a milder than TM disease because the mutation lead to a decrease in β globin chain production. Most patients with thalassemia intermedia carry two mutant β–globin genes, but, rare cases of thalassemia intermedia are resulting from heterozygosity for a single mutation β–globin gene association with the creation of a highly un stable β–globin subunit (dominant β–thalassemia allele). (Forget & Bunn, 2013; Musallam *et al*., 2013).

*** β–thalassemia major (TM)** or Cooley anemia (compound heterozygotes or homozygote) :Three alleles combination are responsible for phenotype of thalassemia "βº/βº, βº/β+, and β+/β+" , it is the greatest severity form of beta-thalassemia. At birth the β-TM patients has normal hematology parameters because of normal γ-globin synthesis,

Diagnosis of β-thalassemia

1- Clinical Diagnosis

Usually suspected in an infant under two years of age with severe microcytic anemia, hepatosplenomegaly and mild jaundice (Higgs *et al.*, 2012).

2- Hematology Diagnosis

Is characterized by low level of hemoglobin (<7 g/dl), mean corpuscular Hb (MCH) >12<20 pg , mean corpuscular volume (MCV) > 50 <70 fl. In peripheral

(hypochromia, microcytosis, ,poikilocytosis ,anisocytosis, speculated tear drop and elongated cell), and nucleated erythrocyte (i.e.,erythroblasts).The erythroblasts numbers is correlated to the degree of anemia and is noticeably increasing after splenectomy (Taher *et al*., 2012).

3- Hb- electrophoresis

4-Molecular Genetic Analysis

The prevalence of a limited number of mutations in each population has considerably facilitated molecular genetic testing. Generally mutations happen to the β-globin gene are detected by PCR- technique.

Management of Beta-thalassemia

- **Prevention Strategies**

The prevention of beta-thalassemia is based on community awareness of the disease, prenatal screening, detection of carriers and genetic counselling. (Peters *et al.*, 2012).

- **Blood Transfusion**

Patients with β-thalassemia major often need blood transfusions every 2-3 weeks to sustain a Hb level above 9.5 gm/dl and maintain normal growth. At six months of age ,they may require blood transfusions (Abdul-Fattah et al., 2008).

- **Chelating Therapy**

When serum ferritin level exceed 1000 mg/L, and this happen after 10 to 20 erythrocyte transfusions, the iron overload must be treated (Peters *et al*.,2012). Between five and eight years old, chelation therapy is usually started. defarasirox, deferoxamin and deferipron, alone or in combination are the three major iron chelators available. These are characterized by different absorption capacity through intestine (Taher *et al*., 2012).

Some studies indicated that deferoxamine is considered the only existing iron chelater, and deferiprone combined with silymarin are better iron chelator in iron loaded thalassemia patient than Deferiprone (Uygun & Kurtoglu, 2013 ;Hagag *et al*., 2014).

- **Bone marrow Transplantation**

The bone marrow transplant is only a therapeutic treatment in childhood of β -TM .Transplantation of hematopoietic stem cell will usually are excellent results in low risk person, clear as those with no portal fibrosis on liver biopsy, no hepatomegaly, and regulation chelation therapy, or at most, two of these deformities (Cao & Galanello, 2010).

- **Splenectomy**

Can be considered if hypersplenism causing a noticeable increased in blood transfusion requirements. In common, it must be delayed for as long as possible, in order to prevent life threatening infections, thromboembolic complications and pulmonary hypertension (Peters *et al*., 2012). At present-day, treatments under investigation are the induction of HbF, antioxidants and stem cell gene therapy (Arumugam & Malik, 2010).

❖Markers that measured in the study

❖Ferritin

What is the ferritin test؟

A ferritin test measures the amount of ferritin in the blood. Ferritin is a blood protein that contains iron. This test can be used to find out how much iron the body stores.

If a ferritin test shows that the blood ferritin level is low, it means the body's iron stores are low. This is a condition called iron deficiency. Iron deficiency can cause anemia.If a ferritin test shows high ferritin levels, it most often means swelling in the body, called inflammation. Conditions that can cause inflammation include liver

disease, rheumatoid arthritis and other inflammatory conditions, and overactive thyroid, called hyperthyroidism. Rarely, a high ferritin level could be from a condition that causes the body to store too much iron. Some types of cancer also can cause the blood ferritin level to be high (Abdul-Fattah et al., 2008).

Why it's done?

A ferritin test can diagnose or suggest:

- Iron deficiency anemia.
- A condition that causes the body to absorb too much iron from food, called hemochromatosis.
- Liver disease.
- A rare type of inflammatory arthritis called adult Still disease.

A health care professional might also suggest a ferritin test for people who have a condition that results in too much iron in the body, such as hemochromatosis. Ferritin tests can help watch the condition and guide treatment.

How you prepare?

If your blood sample is being tested only for ferritin, you can eat and drink as usual before the test. If your blood sample will be used for other tests, you might need to fast for a time before the test. A member of your health care team will tell you what to do.

What you can expect?

During the ferritin test, a member of your health care team puts a needle into a vein in your arm and takes a sample of blood. The blood sample is sent to a lab for study. Most people can go back to your usual activities right away.

Results ferritin test

The typical range for blood ferritin is:

For men, 24 to 336 micrograms per liter. **For women**, 11 to 307 micrograms per liter.

Low results

Results that are lower than the typical range show iron deficiency. These results also can mean anemia. If your ferritin level is low, your health care professional will work to find the cause.

Higher than expected results

There can be several causes for a high ferritin level. More testing might be needed to pin down the cause. Causes include:

1. Hemochromatosis.
2. A group of conditions caused by not having enough of an enzyme that affects the nervous system and skin, called porphyria.
3. Rheumatoid arthritis or another ongoing condition that causes swelling, called inflammatory.
4. Liver disease.
5. Hyperthyroidism.
6. Leukemia.
7. Hodgkin's lymphoma.
8. Repeated blood transfusions.
9. Alcohol misuse.
10. Taking too many iron supplements.

❖ Iron test

What are iron tests?

Iron tests measure different substances in the blood to check iron levels in your body. Iron is a mineral that's essential for making red blood cells. Red blood cells carry oxygen from your lungs to the rest of your body. Iron is also important for healthy muscles, bone marrow, and organ function. Iron levels that are too low or too high can cause serious health problems.

Different types of iron tests include:

1- Serum iron test, which measures the amount of iron in the blood
2- Transferrin test, which measures transferrin, a protein that moves iron throughout the body
3- Total iron-binding capacity (TIBC), which measures how well iron attaches to transferrin and other proteins in the blood
4- Ferritin blood test, which measures how much iron is stored in the body

Some or all of these tests are often ordered at the same time. Other names: Fe tests, iron indices

What are Iron test used?

Iron tests are most often used to:

1- Check if your iron levels are too low, a sign of anemia
2- Diagnose different types of anemia
3- Check if your iron levels are too high, which could be a sign of hemochromatosis. This is a rare genetic disorder that causes too much iron to build up in the body.
4- See if treatments for iron deficiency (low iron levels) or excess iron (high iron levels) are working.

Why do I need an iron test?

You may need testing if you have symptoms of iron levels that are too low or too high.

Symptoms of iron levels that are too low include:

Pale skin , Fatigue , Weakness , Dizziness , Shortness of breath , Rapid heartbeat

Symptoms of iron levels that are too high include:

Joint pain , Abdominal pain , Lack of energy , Weight loss.

Will I need to do anything to prepare for the test?

Your health care provider may ask you to fast (not eat or drink) for 12 hours before your test. The test is usually done in the morning. If you have any questions about how to prepare for your test, talk to your health care provider.

Are there any risks to iron tests?

There is very little risk to having a blood test. You may have slight pain or bruising at the spot where the needle was put in, but most symptoms go away quickly.

What do the results of Iron test mean?

If one or more iron test results show your iron levels are too low, it may mean you have:

-Iron deficiency anemia, a common type of anemia. Anemia is a disorder in which your body doesn't make enough red blood cells.

• Complete Blood Counts (CBC)

What is the CBC test?

A complete blood count (CBC) is a blood test. It's used to look at overall health and find a wide range of conditions, including anemia, infection and leukemia.

A complete blood count test measures the following:

-Red blood cells, which carry oxygen

-White blood cells, which fight infection

_Hemoglobin, the oxygen-carrying protein in red blood cells

-Hematocrit, the amount of red blood cells in the blood

-Platelets, which help blood to clot.

Why it's done?

A complete blood count is a common blood test done for many reasons:

-To look at overall health. A complete blood count can be part of a medical exam to check general health and to look for conditions, such as anemia or leukemia.

-To diagnose a medical condition. A complete blood count can help find the cause of symptoms such as weakness, fatigue and fever. It also can help find the cause of swelling and pain, bruising, or bleeding.

_To check on a medical condition. A complete blood count can help keep an eye on conditions that affect blood cell counts.

_To check on medical treatment. A complete blood count may be used to keep an eye on treatment with medicines that affect blood cell counts and radiation.

How you prepare?

If your blood sample is being tested only for a complete blood count, you can eat and drink as usual before the test. If your blood sample also will be used for other tests, you might need to fast for a certain amount of time before the test. Ask your health care provider what you need to do (Abdul-Fattah et al., 2008) .

Aim of study

The aim of current study was to illustrate certain biological aspects of Iraqi Beta thalassemia patients that dependent on transfusion , and this achieved by using following objectives :

1- Study hematological parameters which included :
   PCV , Hb level and CBC .
2- Study Ferritin and Iron test

Chapter Tow

Materials and Methods

Materials and Methods :

Experimental Design :as in figure (1)

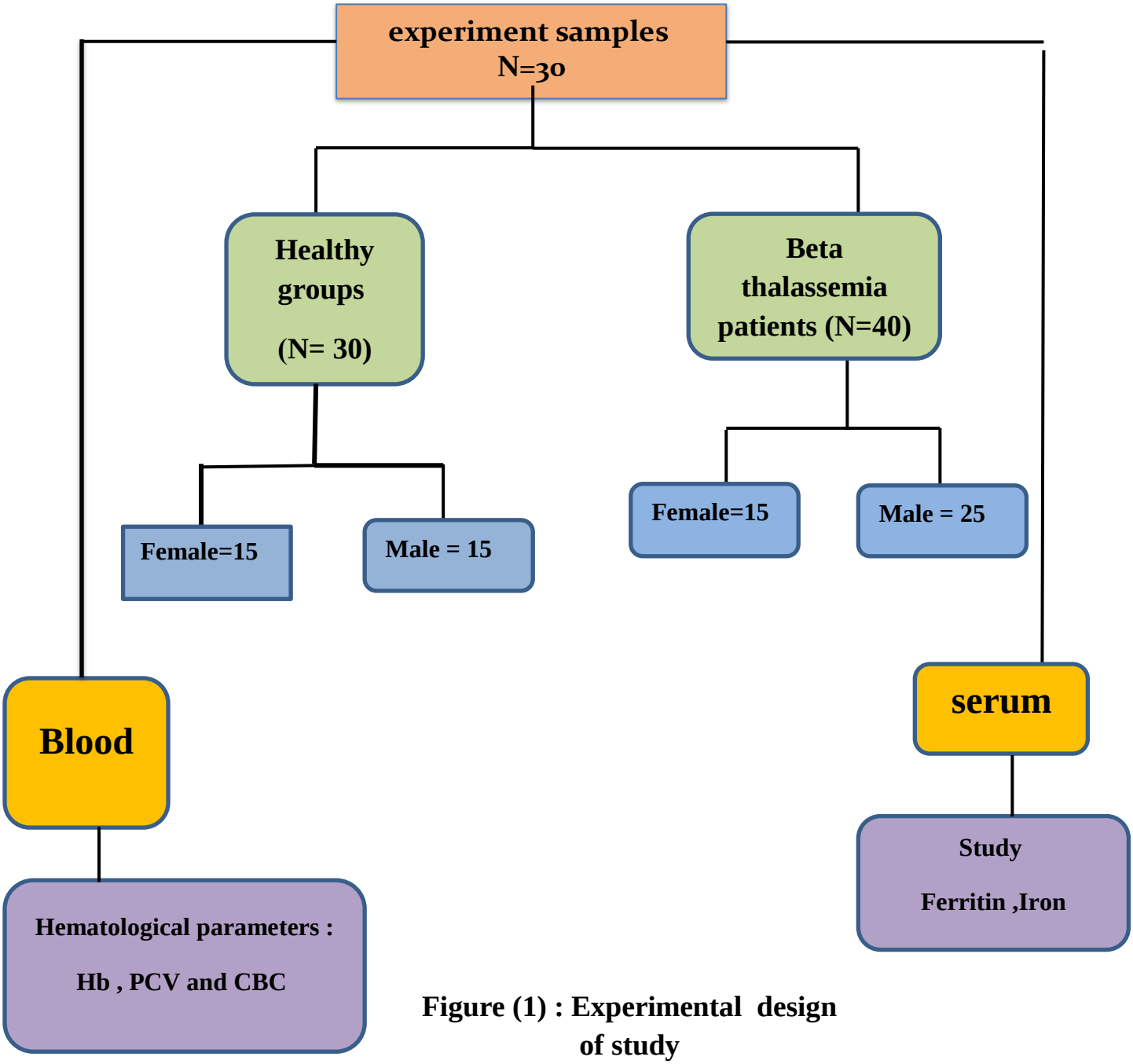


Figure (1) : Experimental design of study

❖Study subjects (Patients and Healthy) :

The study included 40 patients with β-thalassemia major with age 5-36 years old were obtained from the Thalassemia Center in Maternity and Children's Hospital in Al–

Hilla region , Iraq . They were Those attending for blood transfusion and iron-chelating therapy, in addition to (30) healthy subjects not suffer from any disease, served as a control group. This study was carried out during the period from September 2023 to February 2024.

❖Collection of blood samples :

Venous blood samples were drawn from patients and control subjects by using disposable syringes . Five ml of blood was obtained from each subject , 3 ml was placed into EDTA tubes and the remaining (2 ml) pushed slowly into disposable gel containing tubes. Blood in the EDTA tubes was used to determine CBC and the ESR while blood in the gel containing tubes was allowed to clot at room temperature for 15 minutes and then centrifuged at 3000 rpm for approximately 10-15. minutes , after that sera was obtained and stored at -20˚C until used.

❖Hematological Assessments :

The counting of the cellular blood components, the Analyzer Ruby, uses the impedance technique only. A cyanide free spectrophotometry method was used to measure hemoglobin by formation of oxyhemoglobin at 555 nm. Packed cell volume (PCV) was measured by volume integration. The sample volume was 10 µl. The instrument can determine the parameters in the research mode: white blood cells (WBC), the number of red blood cells (RBC), hemoglobin concentration (HGB), hematocrit value (HCT), platelet count (PLT). For platelet counting a floating threshold was used, whereas for RBC and WBC counts the thresholds are predefined.

Results :are provided within 1 minute on the display, printed out on the printer and stored in the resident memory or on a USB key. Results were presented with flags;

optionally reference ranges can be reported. the instrument uses three reagents: a diluent, a lysis reagent and a cleaning solution.

Procedure :

1. 10 µl of the EDTA blood sample was placed in the aspirator on the instrument.
2. The start key on the instrument was pressed and the blood sample was aspirated.
3. Results were provided within 1 minute on the LCD display, printed out on the printer and stored in the resident memory.

❖**Measurements of Serum Iron concentration**

Principle: Transferrin - bound iron is released at acid pH and reduced from ferric Fe3 to ferrous ions Fe2+ iron. These ions react with ferrrozine to form a violet colored complex which is measured spectrophotometrically at 560 nm. This absorbance is proportional to serum iron concentration in sample.

❖**Reagents composition**

1- Iron Buffer Reagent : Hydroxylamine hydrochloride 220 mM in acetate buffer, pH 4.5 with surfactant

2- Iron Color Reagent (Chromagen): Ferrozine 16.7 mM in Hydroxylamine hydrochloride.

3- Iron Standard: (500 µg/dl) ferrous chloride in Hydroxylamine hydrochloride.

❖**Procedure (Manual):**

Wavelength : 560 nm , Working temperature : 37^{o} C , Optical path : 1 cm , Assay type : endpoint , Direction : increasing

Prepare 3 sets of tubes according to following boards:

Composition	Blank	Standard	Sample
Iron Buffer Reagent	2500 µL	2500 µL	2500 µL
Distilled water	500 µL	-----	-----

Iron Standard	-----	500 µL	-----
Sample	-----	-----	500 µL

1- Zero spectrophotometer at 560 nm with the blank.
2- Read and record absorbance of all tubes.

Composition	**Blank**	**Standard**	**Sample**
Iron Colour Reagent	**50 µL**	**50 µL**	**50 µL**
Chromagen			

3- Mix and place all tubes in heating path at 37° C for 10 minutes.
4- Zero instrument at 560 with reagent blank.
5-Read and record absorbance of all tubes.

Calculation

Abs. = Absorbance Std. = Standard

$$\text{Total Iron (mg\dl)} = \frac{\text{Abs 2 sample} - \text{Abs 1 sample}}{\text{Std2 sample} - \text{std 1 sample}} \times \text{Con. Std}$$

❖ **Measurements of Serum Ferritin**

VIDAS Ferritin was an automated quantitative test for use on the VIDAS family instruments for the determination of human ferritin in human serum or plasma using the ELFA technique (Enzyme Linked Fluorescent Assay). Serum was used to determine Ferritin by VIDAS ferritin kit (Biomerieux)

❖**Procedure**:

The assay principle comb munoassay 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 predispensed in the sealed reagent strips. All of the assay steps are performed automatically by the instrument. The reaction medium was cycled in and out of the SPR several times. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) was cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4- methyl–umbelliferone) the fluorescence of which was measured at 450 nm. The intensity of the fluorescence was proportional to the concentration of antigens present in the samples. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory and then printed out.

Statistical Analysis

The Statistical analyzes by SPSS 25 Version was used. The data were analyzed by using descriptive analysis to show the mean and standard deviation of variables. The significance of difference between mean values was estimated by two tailed student T-Test and ANOVA test , Significance was assumed for P values ≤ 0.05, (Al Rawi,2000).

Chapter Three

Results

RESULTS

1 . General characteristic of the study group

The genaral characteristic of the study group are revealed in Table (1)

Table 1: General characteristics of the patients and healthy groups.

Characteristics	Patients No. (%)	Control No. (%)
Gender		
Male	**25(62.5%)**	**15(50%)**
Female	**15(37.5%)**	**15 (50%)**
Age (years)		
5-10	**7 (20%)**	**2 (6.6%)**
11-19	**26 (65%)**	**10 (33.3%)**
≥ 20	**7 (15%)**	**18(60%)**

2 . General and some clinical characteristic of the patients

The general and clinical characteristic of the patients are summarized in Table (2)

Table 2 : General and some clinical features of the patients

Parents Consanguinity	
Relatives	**30 (75%)**
Non relatives	**10 (25%)**
Iron chelation	
Exjade (deferasirox) DFX	20 (50%)
deferoxamine (DFO)	15 (37.5%)
Mix	5(12.5%)
Spleen status	
splenectomy	25(62.5%)
un splenectomy	15 (37.5%)

The serum ferritin and Iron of the Beta Thalassemia patients as compared with the control are shown in Table (3) The hematological parameters of the Beta Thalassemia patients as compared with the control are shown in Table (4)

The most hematological parameters in our study revealed difference ($p < 0.05$) between β-thalassemia major patients and control group . Patients with β-thalassemia major had a significant decrease ($p<0.05$) in Hb , P.C.V % , RBCs compared with control group, while PLTs increased significantly ($p < 0.05$) as shown in Table(4)., S.iron , Ferritin increased significantly ($p < 0.05$) in patients group in comparison with control group table (3) ,

Table 3 : Serum ferritin and Iron values in patients as compared with healthy control

Parameters	**control (N=30)** **Mean ± S.D**	**patients (N=40)** **Mean ± S.D**
S. iron (µl/ml)	12.62 ± 7.63	18.6 ± 167.79
Ferritin (ng/ ml)	49.29 ± 5.57	3407.5 ± 265.02

Table 4 : Hematological values in Beta Thalassemia patients as compared with healthy control

Parameters	**Control (N=30)**	**Patients (N=40)**
	Mean ± S.D	**Mean ± S.D**
Haemoglobin (g\dl)	12.69 ± .44	7.29 ± .11
PCV%	40.70 ± .96	22.84 ± .37
WBC x 10^9\ L	8.23 ± .46	10.38 ± .63
RBCs (10_6/mm_3)	4.93± 1.89	3.58 ±1.6
Platelet x 10^9/L	216.70 ± 13.63	281.76 ± 17.00

The statistical analysis of Hb ,PCV ,RBCs , S.iron displayed non-significant difference among different age groups ,while WBCs levels showed significant ($p < 0.05$) increase in third age group (> 20 year) when compared with first age group (< 10 year) ,and second age group (11-19 year) ,while the results of PLTs showed significant increase ($p < 0.05$) in third age group (>20 year) when compared with first age group (<10 year) ,and second age group (11-19 year) but there were non-significant difference ($p > 0.05$) between first and second age, while, Ferritin level showed significant increase ($p< 0.05$) in third age group (>20 year)when compared with first and second age groups as show in Table (5,6).

Table 5 : Serum ferritin and Irion values in Beta Thalassemia patients according to age

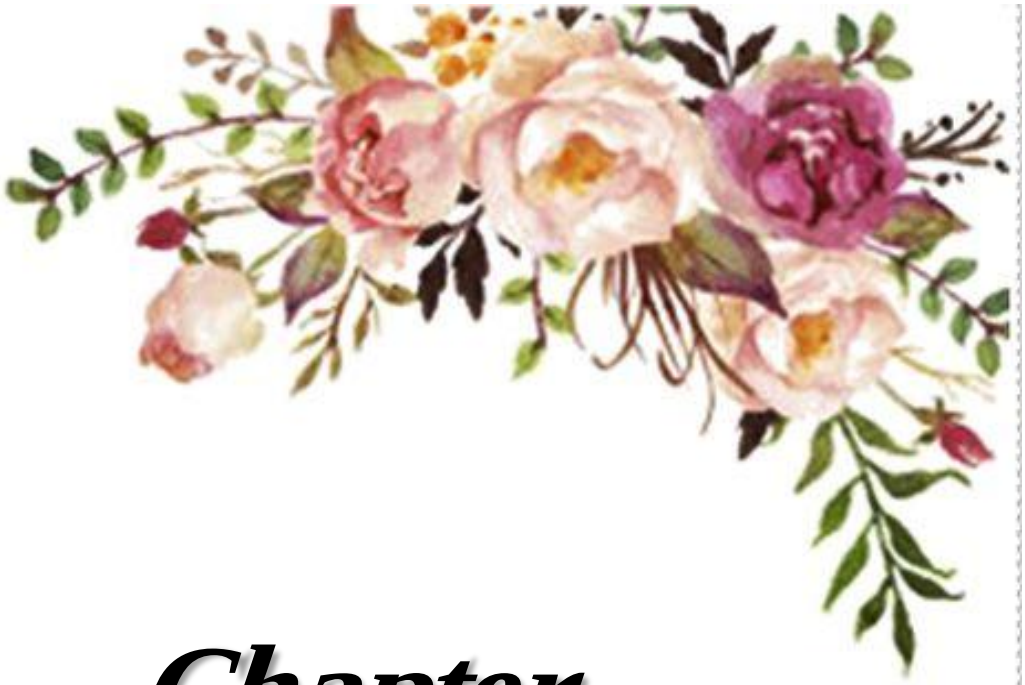
Parameters	**≤ 10 years (first group) n=8**	**11 -19 (second group) n = 26**	**≥20 years (third group) n=6**
S. iron (µl/ml)	17.9 ± 296.42 a	19.9± 240.0 a	18.5 ± 384.73a
Ferritin (ng/ ml)	2409.8 ± 304.94 a	3594.8 ±382.47 b	4256.4 ± 668.18 b

Different symbols mean significant differences at (p<0.05)

Table 6 : Hematological parameters in Beta Thalassemia patients according to age

Parameters	**≤ 10 years (first group) n=8**	**11 -19 (second group) n = 26**	**≥20 years (third group) n=6**
Haemoglobin (g\dl)	7.15± .24a	7.32 ± .16 a	7.38 ± .209 a
PCV%	22.60 ± .74 a	23.08 ± .50 a	22.62 ± .81a
WBC x 10^9\ L	8.01 ± .48 a	8.82 ± .85 a	16.09 ± 1.16 b
RBCs (10_6/mm_3)	3.44 ± .12 a	3.61 ± .09 a	3.68 ± .12 a
Platelet x 10^9/L	206.80 ± 19.90 a	277.41 ± 19.97 a	384.69 ± 46.35 b

Different symbols mean significant differences at (p<0.05)



Chapter Four

Discussion

Dissection

Hematological Parameters of the Study Groups

In the current study, the parameters of hematology showed significant decrease in Pcv , Hb levels and RBCs count, in.β-thalassemia major patients. These results agree with Arshad *et al*.,(2014), who reported that thalassemia patients might have abnormalities associated with lower Hb level, due to decreasing erythrocyte numbers and decreasing values of RBC indices (MCV, MCH, MCHC, HCT).Thus, these patients suffer from anemia that leads to less oxygen content in blood.

Al-Hakeim *et al*.,(2017) also found a significant decrease in hemoglobin as compared with healthy control group. This result is expected mainly due to the hemolysis of RBC & iron released from the degraded abnormal hemoglobin molecules chain. Anemia can occur in severe iron overload .

Shanthi and his colleagues (2013) pointed out that decreased levels of PCV, RBCs and Hb , found in patients with β-thalassemia, may be caused by early breakdown and continuous degradation of erythrocytes due to the abnormal globin molecule which lead to erythrocyte break before maturation .

β-thalassemia major is characterized by failure of the hemoglobin synthesis leading to excess beta-globin chains, hemolysis and impaired erythropoiesis (Olivieri .,1999).

The studies of Yassin *et al*., 2013 ; Arshad *et al*., 2014;Sherief *et al*.,2017 found significant increase of WBCs & PLTs count in β-thalassemia major patients which agree with our study results. These are attributed maybe to ongoing severe anemia accompany by hyper-cellular (leukocytosis & thrombocytosis) resulting from stimulation of erythropoietin hormone which acts on bone marrow to increase proliferation of blood cells, or may result from the immune system activation by getting blood from varied donors.

Serum Ferritin and Iron

The highly significant increase of serum iron and ferritin in patients with β-thalassemia major compared with control group , indicated an existing iron overload which agree with (Al-Hakeim.,2017) in Iraq-Najaf..

The studies of Mourad *et al*.,(2003), Sherief *et al*.,(2017), showed a higher level of ferritin in thalassemic patients children than healthy children.

In one study carried out in one of Iraq neighboring country , Iran, the serum ferritin showed a higher concentration (3503 ± 201 ng/ml) in thalassemia major in many thalassemic patients (Ali *et al*.,2008).

The higher level of Serum ferritin level may be result from the leakage of tissue ferritin, as well as tissue. ferritin plays a role in intracellular iron management, furthermore the ferritin level in plasma represents, an equilibrium between its synthesis, which is directly related to intracellular iron concentration, and its clearance, mostly in liver & other organs (Kattamis *et al*.,2001).

El-Kinawy & Andrawes (2012) found, serum ferritin was significantly higher in the patient groups, especially β-thalassemia major and β-thalassemia intermedia. In addition some studies found that the serum ferritin levels were higher than our study (Cunningham *et al.,* 2004 ; Choudhry *et al.,* 2004). The deference in ferritin level in current study may be due to the differene in population ; number of blood transfusion ; age and duration of disease.

This iron overload may be in response to ineffective erythropoiesis, repeated transfusions and repeated infections. (Succar *et al*., 2011).

The iron intestinal hyperabsorption & abnormal molecular iron form, nontransferrin bound (NTBI) accumulation lead to form iron overload found in β-thalassemia patients , moreover nontransferrin –bound (NTBI) leads to the creation of free radicals and increase hemolysis process (Kosman, 2010).

The free iron has an important role in the cells membrane oxidation , one of the main path for removal of erythrocyte. This elevation of ferritin was the a most important risk factor for myocardial infarction (Piga *et al*., 2009).

Eissa and El-Gamal (2014) results agree with our results and elucidated that transcend iron overload leads to higher serum ferritin, Transferrin Iron Saturation Percentage (TISP) ,causing increased GDFf15 which indicates the development of retarded growth in these patients.

Haj Khelil *et al*. (2001) showed increased iron indices in patients with β-thalassemia may be cause to chronic blood transfusion or/ and erythrocyte hyper hemolysis .

All patients who had severe anemia due to ineffective erythropoiesis which was primary reason for iron overload and blood transfusion was secondary to it (Widad *et al*., 2003). Thus, increased iron might be increase the potential of oxidative injury to erythrocytes and cell organelles (Chakraborty *et al*., 2010).

Conclusions

1- Beta thalassemia is the most common type of thalassemia among patients.
2- Men are more susceptible to the disease than women.
3- Most cases of infection with the disease are from consanguineous marriages and failure to conduct pre-marital examinations.

Recommendations

1- Do not neglect conducting pre-marriage tests to limit the spread of genetic diseases

2- Giving hereditary diseases more medical care, with the necessity of providing special centers for them.

3- Conduct studies showing the effect of excess iron deposition on various organs of the body

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