حالة الحديد كدليل لتأخر النمو و النضج الجنسي لدى الاكراد المصابين بفقر الدم البحري الكبرى

أطروحة مقدمة من قبل

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إلى مجلس كلية العلوم - جامعة بابل

وهي جزء من متطلبات نيل درجة دكتوراه فلسفه في

علوم الحياة (علم الحيوان)

بإشراف

د. إسماعيل كاظم عجام

ذو الحجة ١٤٢٧ كانون الثاني ٢٠٠٧

الخلاصة

تم الكشف على اربعة وسبعون مريضا (٤٠ ذكرا و ٣٤ من الاناث) مصابا بفقر الدم البحري المتجانس الزيجات نوع بيتا. استهدفت هذه الدراسة معرفة تاثير حالات الحديد المتراكم على العوامل المختلفة كالهورمونات وصفات الكيمياء السريرية ودراسة الصفات الدموية للمصابين بالثالاسيميا الكبرى.

يمكن استخلاص النتائج التالية.

١- ثبت ان هناك ادلة على وجود primary hypothyroidism في المرضى المصابين بفرط الحديد المتراكم نتيجة نقل الدم المتكرر مقارنة بمجاميع السيطرة ، عن طريق ارتفاع مستوى الهورمون محفز الدرقية TSH وانخفاض مستوى الهورمون الدرقي T_4.

٢- تشير النتائج الى ارتفاع معنوي في مستوى الهورمون محفز الجريب FSH و انخفاض معنوي في نسبة هورمون تيستوستيرون Testosterone في الذكور، بينما ظهر انخفاض معنوي في

مستوى الهورمون اللوتيني LH و استروجين Estrogen في الاناث Primary). amenorrhea)

٣- تاخر في النمو او قصر القامة نتيجة تراكم الحديد الذي يزداد مع انخفاض معنوي في مستوى هورمون النمو GH مقارنة مع مجاميع السيطرة.

٤- ظهرت زيادة معنوية في نسبة الهيموكلوبين HbF و HbA_2 في كلا الجنسين.

٥- انخفض عدد كريات الدم الحمر (RBCs)و حجم خلايا الدم المتراصة (PCV)و معدل حجم الكرية (MCV)و معدل تركيز الهيموكلوبين في الكرية الحمراء (MCHC) بشكل المعنوي و ارتفعت نسبة الخلايا الشبكية و (RDW-CV) مقارنة مع مجاميع السيطرة في دراسة الصفات الدموية لمرضى ثالاسيميا.

٦- في دراسة تاثير مرض الثالاسيميا الكبرى على الصفات الدموية للمصابين، ظهر ارتفاع معنوي في عدد خلايا الدم البيض (WBCs)و نسبة الخلايا اللمفاوية و النسبة المطلقة للخلايا اللمفاوية و نسبة الخلايا الحمضية والنسبة المطلقة للخلايا الحمضية و النسبة المطلقة للخلايا احادية النواة(في الاناث) بينما لوحظ انخفاض معنوى في نسبة الخلايا المتعادلة.

٧- تبين من فحوصات المسحة الدموية لهولاء المرضى, بان هناك زيادة معنوية في نسبة الخلايا الهدف و نسبة الخلايا صغيرة الحجم و الخلايا ذات الصبغة الباهتة و في كلاالجنسين. و لوحظت بعض خلايا كبيرة الحجم في مسحات الدم لعدد من المرضى.

٨- وجد ان استئصال الطحال يزيد من عدد الخلايا الدم البيض والاقراص الدموية و كما يزيد من عدد كريات الدم الحمر المشوهة و غير الناضجة.

٩- لوحظ ارتفاع تركيز كلوكوز الدم و قيمة (٢h GTT) و كذلك نشاط الكبد لدى المرضى المصابين بالثالاسيميا.

١٠- ارتفع تركيز فريتين المصل، و تركيز الحديد و مستوى فوسفات المصل و انخفض مستوى كالسيوم المصل و TIBC مقارنة مع مجاميع السيطرة في دراسة الصفات الكيمياوية للمرضى المصابين بالثالاسيميا الكبرى.

١١- تبين من هذه الدراسة ان كمية الحديد المتراكم له علاقة عكسية مع عددكريات الدم الحمر و نسبة الهيموكلوبين و نسبة حجم كريات الدم المتراصة و متوسط الهيموكلوبين في الكرية (MCH) و نسبة

الخلايا المتعادلة، بينما كانت العلاقة طردية مع نسبة الليمفوسايت و الخلايا الحمضية و النسبة المطلقة لليمفوسايت و نسبة الخلايا صغيرة الحجم و الخلايا ذات الصبغة الباهتة و عدد الخلايا الشبكية و قيمة (RDW-CV) في المرضى المصابين بالثالاسيميا.

١٢- ان كمية فريتين المصل المتراكم لها علاقة طردية مع كلوكوز الدم و وظيفة الكبد في الاناث و ٢h GTT في الذكور وكمية الحديد و كانت العلاقة عكسية مع TIBC و طول جذع المرضى المصابين بالثالاسيميا.

١٣- هناك علاقة طردية بين طول الجذع و عدد كريات الدم الحمر و تركيز الهيموكلوبين و حجم كريات الدم المتراصة و الوزن الكلي و الطول بين المصابين.

١٤- تبين ان لكمية الحديد المتراكم علاقة طردية مع عدد وحدات الدم المنقولة.

Iron Status as a Predictor of Impaired Growth and Puberty in Kurdish Thalassemia Major Patients

A THESIS

SUBMITTED by SARBAZ IBRAHIM MOHAMMED YAGAN

TO THE COUNCIL OF THE COLLLGE OF SCIENCE UNIVERSITY OF BABYLON IN PARTIAL

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FOR THE DEGREE OF DOCTOR

OF PHILOSOPHY IN

BIOLOGY (Zoology)

Supervised by

Dr. Ismael K. Ajam

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

الَّذِي خَلَقَنِي فَهُوَ يَهْدِين* وَالَّذِي هُوَ يُطْعِمُنِي وَيَسْقِينِ *وَإِذَا مَرِضْتُ فَهُوَ يَشْفِينِ*وَالَّذِي يُمِيتُنِي ثُمَّ يُحْيِينِ * وَالَّذِي أَطْمَعُ أَنْ يَغْفِرَ لِي خَطِيئَتِي يَوْمَ الدِّين*

صدق الله العظيم

سورة الشعراء ﴿ ٧٨-٨١﴾

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ABSTRACT

A total of seventy-four homozygous β-thalassemic patients (٤٠ males and ٣٤ females) at different stages of the disease were collected at random and twenty-seven healthy individuals as a control group during the study period, in order to study the effects of iron status on different variables such as hormones, biochemical and haematological characteristics of these patients. The following results were obtained.

١- There was evidence of primary hypothyroidism as defined by a high baseline TSH and a low or normal $T_٤$ in children with multi-transfused iron loaded thalassemia in comparison with those of the controls.

٢- Result indicate that FSH value increased significantly and testosterone level fall in male, but in females with primary amenorrhea, there were significant decrease in LH level and estrogen level.

٣- Iron overload lead to growth retardation or short stature defined by significantly lower GH concentration than those for the controls.

٤- HbF % and HbA_2 level were increased and Hb decreased significantly in both sexes of patients.

٥- The red cell count, haematocrite value (or PCV), MCH and MCHC were significantly decreased and reticulocyte %, RDW-CV increased in both sexes.

٦- Disease significantly increased WBCs, lymphocyte %, lymphocyte absolute value, eosinophil %, eosinophil absolute and monocyte absolute in female and significantly reduced neutrophil % in both sex with the exception that the monocyte absolute is not significantly changed in males.

٧- Examination of blood smears of the thalassemic patient's shows a significant increase in target cell %, microcytes % and hypochromic % in both sexes. An appreciable number of patient's blood smears had shown macrocytosis.

٨- Splenectomy has increased the white cell and the platelet counts; it has also increased red cell distortion and normoblastaemia.

٩- The fasting serum glucose concentration, ٢hr oral glucose tolerance value and liver function test was higher in homozygous β-thalassemic patients.

١٠- Serum ferritin, serum iron, serum phosphorus level increased and TIBC, serum calcium reduced significantly.

١١- There was negative correlation between s. ferritin and RBCs counts, Hb, HCT value, MCH value, MCHC value, neutrophil % and positively with

lymphocyte %, eosinophil %, absolute lymphocyte, absolute eosinophil count, absolute monocytes, microcytes %, hypochromic %, reticulocyte count and RDW-CV in poly-transfused patients.

١٢- There was positive correlation between s. ferritin and s. fasting glucose, GTT (in female), liver function test (in male), s. iron and negatively with TIBC levels, spinal trunk height in our patients.

١٣- There was positive correlation between height spinal trunk and RBCs count, Hb concentration, HCT value, total weight, height in patients with beta-thalassemia major.

١٤- In multi-transfused beta-thalassemic patient's s. ferritin level & s. iron concentration (only in male) increased significantly by increasing unit of blood transfusion.

I certify that this thesis (**Iron Status as a Predictor of Impaired Growth and Puberty in Kurdish Thalassemia Major Patients**) was prepared under my supervision at the Dept. of Biology, College of Science, University of Babylon and do hereby recommend to be accepted in partial fulfillment of the requirement for the degree of Doctor of Philosophy Science in **Biology / (Zoology)**.

Signature:

Adviser: Professor Dr. Ismael K. Ajam

Date: / /

In view of the available recommendations, I forward this thesis for debate by the examining committee.

Signature:

Name: Professor Dr. Karim H. Rashid

Head of Biology Department

Date: / /

٢.٦.١.١	Serum Iron and Total Iron Binding Capacity (TIBC)	٢١	٢
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ABBREVIATIONS

*	P<٠.٠٥
**	P<٠.٠١
#	Absolute
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
$Ca^{+٢}$	Calcium Ion
$CD^{+٤}$	Receptors on the surface Helper T Cells
$CD^{+٨}$	Receptors on the surface Cytotoxic T Cells

DLC	Differential Leukocyte Count
DW	Distilled Water
ELISA	Enzyme-Linked Immunosorbent Assays
FSH	Follicle Stimulating Hormone
GH	Growth Hormone
GnRH	Gonadotropin-releasing hormone
GOT	Glutamate-Oxaloacetate Transaminase
GPT	Glutamate-Pyruvate Transaminase
GTT	Glucose Tolerance Test
HbF	Fetal Haemoglobin
Hbs	Hemoglobin's
hCG	Human chorionic gonadotropin
HCT	Haematcrite (PCV)
IGF-١	Insulin-like Growth Factor-type ١
IL-١	Interleukine-١
IL-٦	Interleukine-٦
IFN-γ	Interferon-gamma
LH	Luteinizing Hormone
LSD	Least Significant Difference Test
M ± SEM	Means ± Standard Error Means
MCH	Mean Corpuscular (Cell) Haemoglobin
MCHC	Mean Corpuscular (Cell) Haemoglobin Concentration
MCV	Mean Corpuscular (Cell) Volume
MPV	Mean Platelet Volume
N	Number of Sample
N.S	No Significant
OGTT	Oral Glucose Tolerance Test

PCV	Packed Cell Volume (Haematocrite)
PDW	Platelet Distribution Width
P-LCR	Platelet-Large Cell Ratio
PLT	Platelet
r	Correlation
rpm	Round per minute
RBC	Red Blood Corpascular
RDW-CV	Red Cell Distribution Width-Cell Volume
ROS	Reaction Oxygen Species
RT	Room Temperature
SDS	Standard Deviation Score
T_4	Thyroxin Hormone
TNF-α	Tumor Necrosis Factor-alpha
TIBC	Total Iron Binding Capacity
TSH	Thyroid Stimulating Hormone
U:L	Upper to Lower Segment ratio
UIBC	Unsaturated Iron Binding Capacity
WBC	White Blood Cell

INTRODUCTION

Thalassemia is the name of a group of genetic blood disorders characterized by anemia, due to enhanced red blood cell destruction. In ١٩٢٥, Thomas Cooley and Pearl Lee described a form of severe anemia, occurring in children of Italian origin and associated with splenomegaly and characteristic bone changes. The disease is common in Greece, throughout Africa,

Mediterranean countries, the Middle East and Southeast Asia, the disease was later termed thalassemia, from the Greek word for sea, thalassa. Adult haemoglobin consists of two type of polypeptide chains; alpha and beta, if the body doesn't produce enough of either of these two proteins, the red blood cells become defective and cannot carry sufficient oxygen. The human β-thalassemia syndromes are characterized by complete absence or decreased levels of β-globins chains and produce severe life-threatening anemia. The β-thalassemia in Iraqi Kurdish Jew results from single change in the T-A-T-A box from C-A-T-A-A-A-A to C-A-T-A-C-A-A. (Cooley & Lee ١٩٢٥; Ponez *et al.,* ١٩٨٢ and Haen, ١٩٩٥)

In Kurdistan region of Iraq, there are approximately ٢٠٠٠ thalassemic patients, and a bout ٤٥٠ of them were recorded and refered continuously to the Thalassemia-hemophilia Center in Erbil City for diagnosis and blood transfusion treatment. The hormonal and haematological analysis which was done for patients in the laboratory which belongs to this center uses very simple and limited methods and techniques. So this study was performed to introduce another new technique and more advanced tests on blood which were not previously used in this center and are very important and benefitial for patients who refered to this center. The aim of this study was to evaluate the prevalence of

١- Delayed puberty and growth failure in Iraqi Kurdish patients with beta-thalassemia major which has become an issue of interest in the last few years since life expectancy was increased by hyper blood transfusion and iron chelation therapy.
٢- Thyroid dysfunction in homozygous thalassemic patients.
٣- Hypocalcaemia and hypoparathyroidism.

٤- It is important to assess hypothalamic-pituitary-gonadal function in young women with β-thalassemia major, so that those with glandular dysfunction may be started on hormone replacement therapy.

٥- Assess the pattern of iron status and elevated fetal haemoglobin level among homozygous beta-thalassemia and their correlations to different hormonal, biochemical, and haematological parameters.

٦- Incidence of Insulin Dependent Diabetes Mellitus (IDDM), Impaired Glucose Tolerance Test (IGTT) and associated factors in transfusion dependent -thalassemia major patients who had been observed in hospital.

It seemed mandatory that this attempt should be followed in the future, by gene mapping studies to uncover the genetic background and hence, for more precise identification of the disease pattern in Iraq. As it will be shown throughout the review of literature, β-thalassemia major had been studied extensively in different areas of the world.

REVIEW OF LITERATURES

٢-١- Thalassemia

The thalassemias are a heterogeneous group of haematological disorders resulting from mutations of the globin genes coding for the two pairs of polypeptide chains of haemoglobin. There is either no or reduced production of one or more of these globin polypeptides. This leads to less haemoglobin, an excess of the polypeptides unaffected by the mutation resulting of in inclusion body formation, erythroid cell dysfunction and (premature) destruction (Figure-١). Thalassemias are defined according to the particular polypeptide which is

absent or depleted; i.e. alpha-thalassemias from the reduced output of alpha polypeptide chains are encoded in duplicate on chromosome ١٦, and beta-thalassemias from reduced beta polypeptide chain is encoded in a cluster on chromosome ١١, production (Weatherall, ١٩٩٧). Haemoglobin disorders, both structural haemoglobinopathies and thalassemias, are the most common inherited diseases of man. The ß thalassemias result from over ١٥٠ different mutations of the ß globin genes that result either in the absence of the ß globin chains (ß° thalassemia) or a reduction in their output ($ß^+$ thalassemia). This results in imbalanced globin chain synthesis and production of an excess of α-chains, which precipitate in the red cell precursors, leading to their destruction in the bone marrow or peripheral blood. This process causes severe anemia, which in turn leads to increased erythropoietin production and expansion of the ineffective bone marrow, bone deformities, splenomegaly, and growth retardation. Treatment by regular blood transfusion reverses these pathological mechanisms so that growth and development is normal. But if excess iron derived from transfusion is not removed, patients die in the second or third decade from iron loading of the myocardium, liver, and endocrine organs (Brittenham *et al.*, ١٩٩٤ and Weatherall, ١٩٩٧).

Most of the patients with homozygous ß˙-thalassemia have typical manifestations of severe thalassemia syndrome (thalassemia major) because there is no Hb A as a consequence of the absence of ß-globin chain production. The onset of the disease usually begins in the first year of life. If blood hyper-transfusion and iron chelation are not administered, the patients usually have full blown manifestations and die before the age of ١٠ years due to heart failure and severe infections. Patients with detectable HbA and HbF may have inherited homozygous $ß^+$-thalassemia or compound heterozygosity for ß˙-thalassemia and $ß^+$-thalassemia. Most of these patients are presented as thalassemia intermedia. The clinical manifestations are milder, reflecting the modest reduction of beta-

globin chain synthesis. Massive erythropoiesis is observed in the bone marrow and extramedullary sites including liver, spleen, lymph node and others. Erythropoietic masses in the spinal canal can cause spinal cord compression or convulsions when they appear intracranially. Some patients who live into their third or fourth decades may develop a terminal wasting syndrome with increased skin hyper-pigmentation, poor appetite, weight loss, increasing anemia and eventually death. This syndrome is believed to be due to multiple organ failure caused by uncontrolled tissue oxidation from chronic severe iron overload (Greenberg *et al.*, ٢٠٠١). Therefore beta-thalassemia major patients require life-long transfusion therapy and bone marrow transplantation for successful control of the disease (Clarke and Higgins ٢٠٠٠).

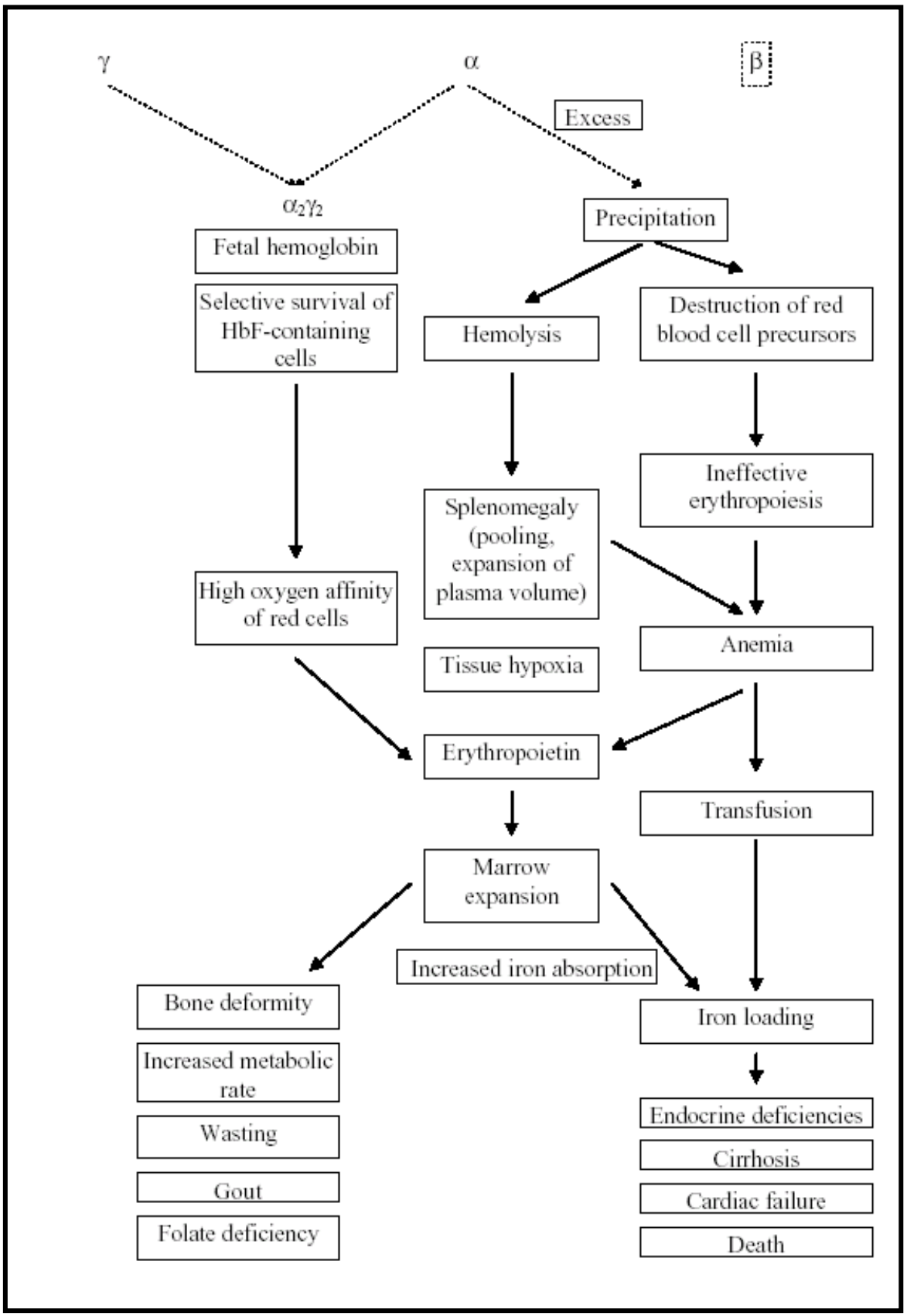
γ
α
β
Excess
$\alpha_2\gamma_2$
Precipitation
Fetal hemoglobin
Selective survival of HbF-containing cells
Hemolysis
Destruction of red blood cell precursors
Ineffective erythropoiesis
Splenomegaly (pooling, expansion of plasma volume)
High oxygen affinity of red cells
Anemia
Tissue hypoxia
Erythropoietin
Transfusion
Marrow expansion
Increased iron absorption
Bone deformity
Iron loading
Increased metabolic rate
Endocrine deficiencies
Wasting
Cirrhosis
Gout
Cardiac failure
Folate deficiency
Death

Figure (١): Schematic Representation Summarizing the Pathophysiology of β-thalassemia (adapted from Weatherall, ١٩٩٧)

٢-٢- Geographical Distribution

There is a characteristic geographic distribution of the thalassemias and haemoglobinopathies (Figure ٢).The thalassaemias are distributed across the Mediterranean region, the Middle East, the Indian subcontinent, and throughout Southeast Asia in a line stretching from southern China down the Malaysian peninsula to the Indonesian islands. These are inherited in a simple autosomal recessive Mendelian fashion. In many of these countries gene frequencies for the different thalassaemias and structural haemoglobin variants are high. As social conditions improve in developing countries and childhood mortality due to infection and malnutrition declines, children with thalassemia who would previously have died early in life are now surviving long enough to require treatment. The reason for the very high frequency of thalassemia is that carriers are protected from the consequences of infection with *Plasmodium falciparum* malaria (Weatherall, ١٩٩٧ and Pallister, ١٩٩٩).

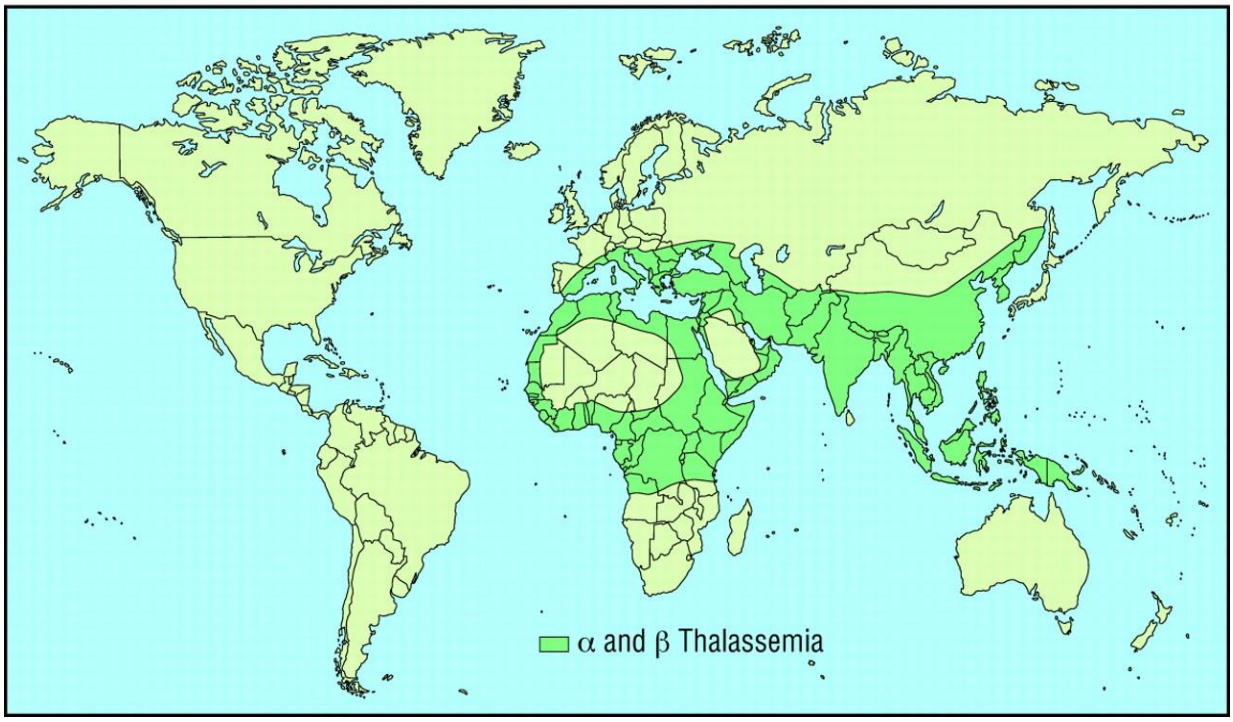

Figure (٢): Geographical Distribution of the Thalassemia (Weatherall, ١٩٩٧).

The geographical distribution of the patients indicated that incidence of the disease percentage in Erbil Center (٢٨ %), in surrounding region of Erbil (٤٦.٦ %), while in immigrants from Sulaymania and Kurkuk was (٢٥.٤ %), (Rashid, ١٩٩٧). The estimated prevalence rates of β- and α-thalassemia traits in Baghdad would be ٤.٤٪ & ١٪ respectively. Baghdad, virtually at the heart of Iraq, has a population of about ٤ million people. The bulk of these people have become permanently integrated over the decades, many through intermarriage, with the population of the city. Thus, unlike most other cities in Iraq, Baghdad's population is rather heterogeneous with most ethnic groups being represented to one extent or another (Yahya *et al.,* ١٩٩٦).

On the other hand, malaria was endemic throughout Iraq until the late ١٩٥٠s, in view of the theory of malaria selection (Weatherall and Clegg, ١٩٨١)

which has been offered as an explanation of the high prevalence rates observed in many parts of the world. The prevalence rate of β-thalassemia trait in Baghdad is intermediate between those reported from Saudi Arabia and that from Turkey (Yahya *et al.,* ١٩٩٦).

Iran has a large number of major thalassemia patients; among some ethnic groups the gene frequency has remained high. Iranian Jews represent an ancient community with a high degree of inbreeding. Although the community has remained relatively isolated, it has had strong ties with Babylonian Jewry in Iraq. As a consequence, β-thalassemia is prevalent among both the Iranian and Iraqi Jewish communities (Zlotogora, ١٩٩٥). Interestingly, the highest occurrence (٧.١ %) of β-thalassemia in Southern Anatolia was mostly observed in the Kurdish people living in that region (Kocak *et al.,* ١٩٩٥).

٢-٣- Diagnoses of Thalassemia;

Prior to the consideration of blood transfusion therapy, it is critical to confirm that the patient has thalassemia major and to eliminate other concomitant causes of anemia. Once transfusion has been started, it is more difficult to establish the correct diagnosis and almost impossible to diagnose thalassemia-intermedia (Cape Town, ٢٠٠١).

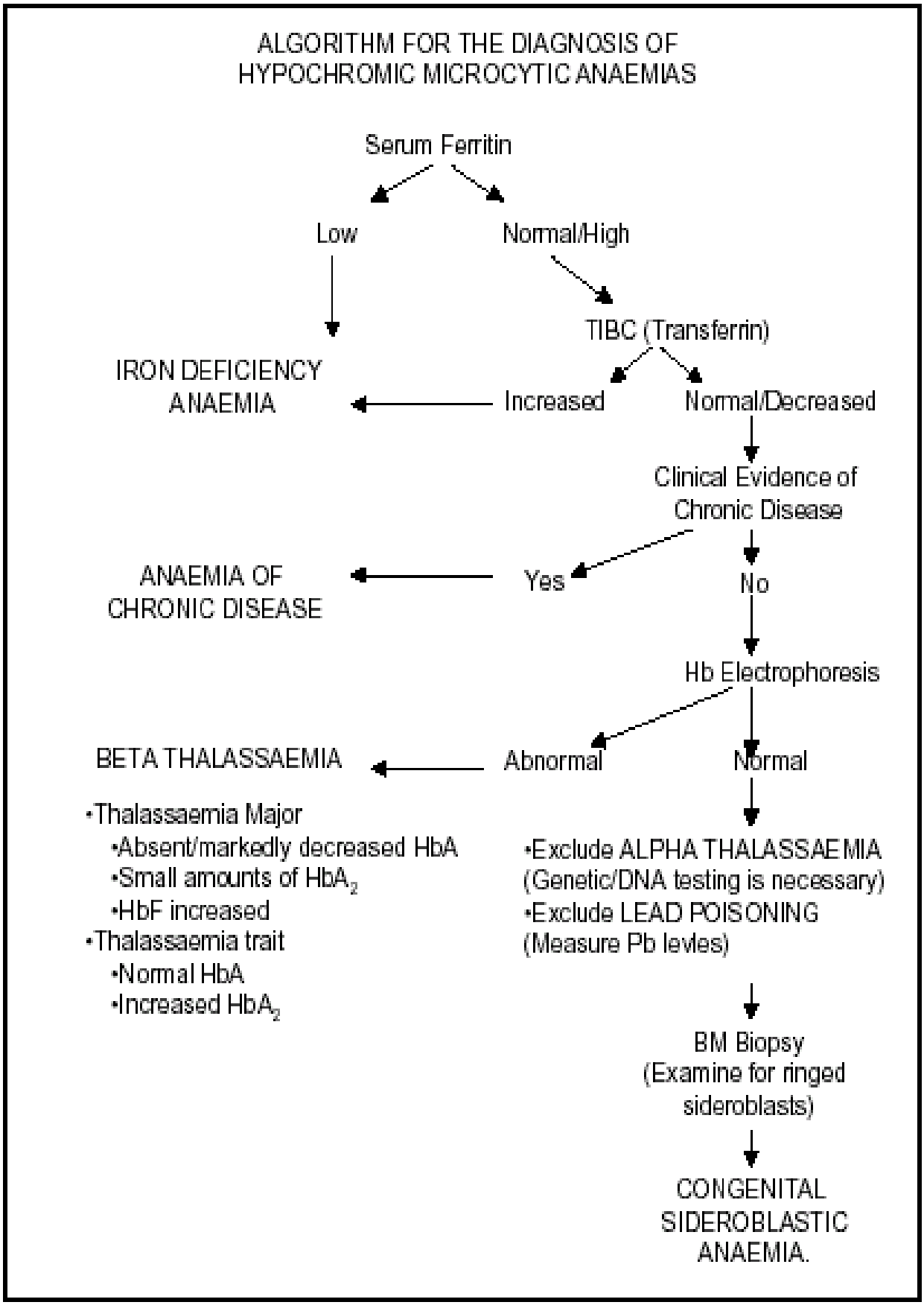
ALGORITHM FOR THE DIAGNOSIS OF
HYPOCHROMIC MICROCYTIC ANAEMIAS
Serum Ferritin
Low
Normal/High
IRON DEFICIENCY ANAEMIA
TIBC (Transferrin)
Increased
Normal/Decreased
Clinical Evidence of Chronic Disease
ANAEMIA OF CHRONIC DISEASE
Yes
No
Hb Electrophoresis
BETA THALASSAEMIA
Abnormal
Normal
•Thalassaemia Major
•Absent/markedly decreased HbA
•Small amounts of HbA2
•HbF increased
•Thalassaemia trait
•Normal HbA
•Increased HbA2
•Exclude ALPHA THALASSAEMIA (Genetic/DNA testing is necessary)
•Exclude LEAD POISONING (Measure Pb levles)
BM Biopsy (Examine for ringed sideroblasts)
CONGENITAL SIDEROBLASTIC ANAEMIA.

Figure (٣): An Approach to the Diagnoses of β-thalassemic Anemia (Cape Town, ٢٠٠١)

An approach to the diagnoses of β-thalassemic anemia involved:

- In addition to complete blood count, hemoglobin electrophoresis is the first diagnostic test. Fractions of hemoglobin A, A_2, F, and other variants are measured (Figure ٣).
- Measure serum ferritin, total iron binding capacity, and serum iron,
- Both β and α globin gene mapping should be determined on all patients to confirm the diagnosis and determine the presence of co-inherited α-globin gene defects that may affect prognosis and management (Cape Town, ٢٠٠١).

٢-٤- Blood Content:

٢-٤-١- Endocrine Complication:

Endocrine dysfunction hypothyroidism, hypogonadism, diabetes mellitus, low bone mass and hypoparathyroidism are well recognized in patients with transfused-dependent thalassemia and are thought to reflect the sequence of iron overload (Cunningham *et al*., ٢٠٠٤ and Shalitin *et al*., ٢٠٠٥).

٢-٤-١-١- Growth Complication:

Growth hormone (hGH, somatotropin), secreted from the anterior pituitary, is a polypeptide with two intra-chain disulfide bridges, which circulates free or bound to a number of different GH-binding proteins. GH exerts its effect directly on target organs such as bones and muscles and indirectly through the release of somatomedins, a family of insulin-like growth

factor (IGF) hormone, produced in the liver. In particular, somatotropin C (IGF-١) is essential for bone growth during childhood (Ganong, ٢٠٠٥; Guyton and Hall, ٢٠٠٦)

Homozygous thalassemias frequently suffer from growth retardation, short statures and delayed pubertal development. This is believed to be due to a direct effect of iron overload on the endocrine mechanisms of puberty (El-Hazmi *et al*., ١٩٩٤), or chronic cellular hypoxia due to severe anemia has been considered as a possible cause, and the effects of deferoxamine (Fuchs *et al*., ١٩٩٧). Reduced GH secretion and low IGF-١ in thalassemia patients are related to a neurosecretory dysfunction due to iron overload rather than to liver damage (Roth *et al*., ١٩٩٧). Short body building children with thalassemia had significantly decreased GH and insulin-like growth factor١ (IGF-١) (Soliman *et al*., ١٩٩٩).

Banani *et al*., (٢٠٠٠), found that a significant increase in GH may be explained on the basis of the triggering effect of splenectomy upon an already hyperplastic pituitary gland. They have claimed that there is a correlation between the serum ferritin levels, the age of the patient and the possibility of endocrine deficiencies. Shalitin *et al*., (٢٠٠٥) reported that endocrine factors contributing to stunted growth in thalassemia include impairment of the GH-IGF-١ axis secondary to hemosiderosis of the pituitary gland and liver. Cavallo *et al.,* (١٩٩٧) suggested that GH impairment at different levels (hypothalamic or pituitary) and a reduced IGF-١ synthesis were the main causes of stunted growth in polytransfused β-thalassemia patients. Although normal rates of prepubertal linear growth may be observed in patients maintained on regular transfusion programs and poor pubertal growth have been observed in well-transfused patients (Olivieri and Brittenham, ١٩٩٧**).** Growth retardation in

iron-overloaded patients is the result of growth hormone deficiency in up to ٣٠٪ of patients. Height gain can be successfully achieved in these patients with growth hormone treatment (Wonke *et al.,* ١٩٩٨).

In patients with beta-thalassemia major had GH deficiency, lower insulin like growth factor-١ ($p<٠.٠٠١$) and insulin like growth factor binding protein-٣ ($p<٠.٠٠٣$) levels than those without growth retardation (Wu *et al.,* ٢٠٠٣).

٢-٤-١-٢- Gonadal Complication:

FSH and LH, secreted by the gonadotropin cells of anterior pituitary gland, glycoprotein hormones each with a molecular weight of ٣٠ k Dalton, are required in homeostasis of fertility regulation via the hypothalamic-pituitary-gonadal axis which has been well established in both women and men. FSH contains two different subunits (α & β) linked by no covalent bounds. The α-subunit shares structural homology with LH, while the β subunit is unique. In women, FSH exerts its effect directly on ovarian granulosa cells, essential for growth and maturation of ovarian follicles while LH is required for rupturing of Graafian follicle and ovulation (Griffin and Ojeda ٢٠٠٠ & Ganong, ٢٠٠٥)

١٧beta-Estradiol is the most potent natural estrogen produced by the Graafian follicle of the ovary and the placenta and in smaller amounts by the adrenals, and the male testes. Target organs for ١٧beta-Estradiol include the follicles, uterus, breast, vagina, urethra, hypothalamus, pituitary and skin. Testosterone secreted by the interstitial cells of Leydig in the testes, exerts its effect on the development of adult primary and secondary sexual characteristics (Ganong, ٢٠٠٥)

Delayed puberty or primary amenorrhea defined as no evidence of pubertal development by the age of ١٣ year in girls or by the age of ١٤ years in

boys is extremely common in patients with transfusion dependent thalassemia major; children with thalassemia at puberty LH pulsatility index is below the mean for pubertal stage compared to normal children and FSH level were normal for pubertal stage in ٧٦٪ patients and only ١٢٪ of the patients had FSH level significantly lower than expected for their stage of puberty and ١٢٪ had high FSH levels which suggest early gonadal failure (Oerter *et al.,* ١٩٩٣). Low serum FSH, LH, and testosterone values indicated hypogonadotrophic hypogonadism (Trainer and Besser, ١٩٩٥) while a high FSH and LH and low testosterone indicated primary hypogonadism (Islam and Trainer ١٩٩٨).

Basal serum LH and FSH were significantly lower compared with those values in normal pubertal controls and did not differ significantly from those found in prepubertal controls; on the other hand basal serum estradiol concentrations of all thalassemia patients with primary amenorrhea were below the normal values (De Sanctis *et al.,* ١٩٨٨), and also LH, FSH, testosterone, and estradiol were perfectly correlated with each other (Yazigi *et al.,* ٢٠٠٢) and Papadimas *et al.,* (٢٠٠٢) have reported a higher incidence of hypogonadism in Greek subjects, manifested as menstrual irregularities and hypoestrogenemia was demonstrated in ٩٥ % of cases. However, only ٧٤ % of patients developed primary or secondary amenorrhea.

El-Hazmi *et al.,* (١٩٩٤) showed that the levels of LH, FSH and testosterone were lower in β-thalassemia major patients with a negative correlation with plasma ferritin level. Shalitin *et al.,* (٢٠٠٥) concluded that the mean serum ferritin level was significantly higher in the patients with hypogonadism than in those with normal puberty. Pignatti *et al.,* (٢٠٠٥) had reported hypogonadism in ٥٥٪ Italian patient with thalassemia major. Argyropoulou *et al.,* (٢٠٠٠) and Wang *et al.,* (١٩٨٩) reported that patients with β- thalassemia had subnormal

serum LH and FSH responses to gonadotropin hypogonadism, also Chatterjee *et al.,* (١٩٩٨) showed that patients had very low FSH and LH levels and failed to respond to GnRH challenge test indicating severe gonadotropin insufficiency. On the other hand patients had a very low testosterone level and also failed to respond to hCG test indicating primary testicular failure. Serum ferritin in patients with hypogonadism was significantly higher than patients without hypogonadism (Shamshiraz *et al.,* ٢٠٠٣).

٢-٤-١-٣- Thyroid Complication:

Thyroid hormones are secreted by follicular cells of thyroid gland**.** They have many major effects on the body; such as increases the overall metabolic rate in the body, sexual function in adult and growth stimulation in children. TSH, also known as thyrotropin, is an anterior pituitary hormone, a glycoprotein with Mw of about ٢٨.٠٠٠ Dalton. This hormone increases the secretion of $T^{٤}$ and $T^{٣}$ by thyroid glandular cells (Sembulingman and Sembuligman ٢٠٠٣ and Guyton and Hall, ٢٠٠٦)

Hypothyroidism resulting from hemosiderosis has been implicated; the objective is to estimate that the thyroid function of patients must correlate height with age, iron status and thyroid hormone level (Cunningham *et al.,* ٢٠٠٤). A remarkable change in TSH was observed in thalassemia patient after splenectomy (Banani *et al.,* ٢٠٠٠). There was evidence of primary hypothyroidism as defined by a high baseline TSH and a low $T_{٤}$ and $T_{٣}$ in children with thalassemia due to iron overload (Oerter *et al.,* ١٩٩٣ and Shalitin *et al,* ٢٠٠٥). There was no significant difference in mean serum ferritin among hypothyroid patients (Shamshiraz *et al,* ٢٠٠٣), and also in Lebanese β-thalassemia children had rare evidence of primary hypothyroidism compared to matched control (Yazigi *et al,* ٢٠٠٢).

The mean concentrations of serum T٤, T٣ and TSH levels were not significantly different from those of the controls in mild iron overload transfusion-dependent *ß*-thalassemic patients, but in severe iron overload the mean concentrations of T٤ and T٣ decreased significantly (*P*<٠.٠٥), and the mean concentration of TSH showed a ٢.٦-fold increase (*P*<٠.٠١) in comparison with those of the controls (Al-Hader *et al.,* ١٩٩٣).

Hypothyroidism was documented by Agarwal *et al,* (١٩٩٢) in multi-transfused iron loaded thalassemia patients; ٨٠.٦٪ had normal $T_{٤}$, $T_{٣}$, TSH, compensated hypothyroidism; ١٢.٥٪ had normal $T_{٤}$, $T_{٣}$ and raised TSH; and ٦.٩٪ decompensated hypothyroidism had decreased $T_{٤}$ and increased TSH, and also Zervas *et al.,* (٢٠٠٢) indicated no correlation between serum ferritin levels and thyroid function status. There is no correlation between thyroid dysfunction and serum ferritin; considerable iron overload was present despite chelation therapy with desferioxamine, and this may have led to thyroidal tissue damage (Alexandrides *et al.,* ٢٠٠٠).

٢-٥- **Haematological Parameters**:

Beta-thalassemia comprises a group of different clinical and hematological pictures. Haemoglobinopathies that lead to decreased production of globin chains (α or β) produce a clinical syndrome characterized by anemia of variable severity (Clarke and Higgins, ٢٠٠٠). The effect of β-thalassemia on haematological parameters is related to the exact phenotype, which depends on the number of genes affected (Quadri *et al*., ٢٠٠٠). Thalassemia major is the most severe form of this syndrome and requires frequent blood transfusions (Hoffbrand *et al*., ٢٠٠٣).

٢-٥-١- Red Blood Corpuscular (RBC) Parameters:

٢-٥-١-١- Haemoglobin (Hb) Level:

Normal adult haemoglobin contains two α and two β-globin chains which are produced in equal amounts. The α-globin chain is encoded in duplicate on chromosome ١٦, and the non α-chains (β, δ, and γ) are encoded in cluster or chromosome ١١. A diploid cell has four α-globin genes and two β-like genes. The α and β chains consist of ١٤١ and ١٤٦ amino acid residues, respectively and the β chain differs from the δ and γ chains by ٣٩ and ١٠ residues, respectively (Clarke and Higgins, ٢٠٠٠).

In the thalassemia, production of one of these globin chains is deficient; consequently less than the normal amount of adult haemoglobin is produced. Thalassemia may be divided into two major categories, according to the globin chain that is deficient into α-thalassemia and β-thalassemia (Haen ١٩٩٥).

Galanello *et al*., (١٩٨٠) reported that Hb levels are reduced in β˚-thalassemia, and in non-splenectomized β-thalassemia major (Rashid ١٩٩٨), and they may also be associated with moderate to severe anemia (Clarke and Higgins, ٢٠٠٠).

On the other hand, Jaafer (١٩٨٩) who showed a significant difference in the Hb level between the control group and transfused patients, whereas, Rees *et al.,* (١٩٩٩) showed the mean Hb in the un- transfused group was significantly lower than that in the transfused group. Khider (١٩٨٦) mentioned that no significant difference in the mean Hb concentration was found between male and female patients and no significant correlation had been reported between serum ferritin levels and the pre-transfusional Hb.

٢-٥-١-٢- $HbA_٢$:

The minor haemoglobin component is haemoglobin A_2 (HbA_2) which has the peptide chain structure $\alpha_2\delta_2$. Only small amounts of δ chain, and consequently HbA_2, are synthesized at the time of birth, but levels reach adult values in early childhood (Haen, ١٩٩٥). HbA_2 levels are characteristically elevated in adults with homozygous β-thalassemia and no significant difference was found between both sexes before pubertal age (Rashid, ١٩٩٨). On the other hand, (Jaafer, ١٩٨٩) reported that the HbA_2 level was within the normal limits in patients with thalassemia.

٢-٥-١-٣- Fetal Haemoglobin (HbF):

Fetal haemoglobin (HbF, $\alpha_2\gamma_2$) accounts for up to ٩٠٪ of the circulating haemoglobin at birth, its synthesis starts to decline during the third trimester and over the first year of life it is gradually replaced by adult haemoglobin (HbA). Normal adults have less than ١٪ of HbF, apparently confined to a subset at red blood cells called F cells (Hoffbrand *et al.*, ٢٠٠٣). Gamma chain production compensates for beta chain loss, resulting in increased HbF (٢٠ % to ٩٠ %), (Powers, ١٩٨٩).

Rees *et al.,* (١٩٩٩) found that the HbF is significantly lower in the regularly transfused patients, making it possible to monitor the HbF level in both the transfused and non transfused patients. Jaafer, (١٩٨٩) who reported no correlation was found between HbF% and Hb level, age, sex, serum ferritin, serum iron, and TIBC, whereas, Khider, (١٩٨٦) showed significant correlation with the transfusional requirements as measured by the total number of blood transfusions received.

٢-٥-٢- Red Blood Corpuscular Indices and Morphology:

Red blood cells indices are important tests in the investigation of thalassemia. Blood pictures shows marked microcytosis with hypochromia, moderate degree of anisopoikilocytosis, tear drop cells, target cells are prominent, and the mean of corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are diminished (Sood, ١٩٨٥).

Jaafer, (١٩٨٩) noticed no significant differences in the mean values of MCV, MCH, and MCHC between the control group and transfused thalassemic patients, nor between the splenectomized and non splenectomized patients, but there was a significant decrease in RBC count and packed cell volume (PCV) in different ages of patients with beta-thalassemia (Rashid, ١٩٩٨). Patients with hereditary spherocytosis (HS)/β-thalassemia have a significantly lower MCV, MCH, MCHC, PCV, and high RDW (Akar and Gokge, ٢٠٠٢). In the *β* -thalassemia children MCV and MCHC were significantly lower than those in healthy controls (Meral *et al.*, ٢٠٠٠).

Thalassemic individuals have reduced RBC indices and blood pictures characterized by produce a uniform microcytic red cell population without a concomitant increase in red cell distribution width (RDW) and peripheral blood film shows frequent target cells (Clarke and Higgins, ٢٠٠٠). On the other hand, Quadri *et al.*,(٢٠٠٠) investigated α-thalassemia gene significantly reduces MCH, MCV, RDW, PCV and increases RBC count in both normal and sickle cell trait neonates. In Sardinia adult β-thalassemia heterozygote are known to have increased mean red cell count (RBCs), reduced MCV, MCH, MCHC and PCV (Galanello *et al.*, ١٩٨٠). There was no significant difference between splenectomized and non splenectomized patients in PCV and MCHC; Khider, (١٩٨٦) also showed the anemia associated with thalassemia was

characteristically hypochromic, microcytic with marked anisopoikilocytosis and an appreciable number of patients had macrocytosis in addition which possibly signifies inadequate folate and vit.B_{12} supply. The stained peripheral blood smears of splenectomized β-thalassemia shows hypochromic, microcytosis, anisopoikilocytosis, variable number of target cells, fragment cells and normoblasts (Weatherall and Clegg, ١٩٧٢). Other reports, Sears *et al*., (٢٠٠٣) showed that blood smears in patients with homozygous haemoglobin C-β thalassemia have an interesting admixture of both microspherocytes and target cells. Tillmann and Schroter, (١٩٧٩) showed in all splenectomized patients marked normoblasts and many distorted and bizarre-shaped cells were found in peripheral blood smear. In patients with thalassemia in whom yearly transfusion requirements exceed ٢٠٠ mL packed cells per kilogram body weight, splenectomy significantly diminishes RBC requirements and iron accumulation (Cohen *et al*, ١٩٨٩).

٢-٥-٣- Reticulocyte Percentage (%):

The reticulocyte is immature erythrocyte which contains remnants of RNA strands. It is slightly larger and less regular in shape than the erythrocyte. The reticulocyte count is another important test in the investigation of thalassemia; if the reticulocyte count is raised, this suggests bone marrow activity, but in contrast suggests impaired bone marrow function (Haen, ١٩٩٥). Blood transfusion significantly reduces reticulocytes and erythropoietin (Rees *et al*., ١٩٩٩). In splenectomized patients marked reticulocytosis were observed in peripheral blood smears, and also similar results obtained by Jaafer (١٩٨٩), and (١٠٪) reticulocytosis (Sood, ١٩٨٥). While, characteristically the severely affected C-β thalassemia patients have moderate (٢-٧٪) reticulocytosis (Dacie,

١٩٨٨). However, there is no significant correlation found between serum ferritin levels and reticulocyte counts (Khider, ١٩٨٦).

Reticulocyte counts were not significantly different from control in thalassemic patients that indicate erythropoiesis was more completely suppressed, as reflected both by normal reticulocyte counts and near-normal transferring receptor levels (Tancabelic *et al.,* ١٩٩٩**)**.

٢-٥-٤- White Blood Cell Count (WBC):

The blood contains many types of leukocytes (or WBCs). They are true cells with a nucleus including; Neutrophils, cells with a lobulated nucleus and a cytoplasm filled with (azurophil) granules. Eosinophils, are characterized by the presence of yellowish, refractile granules and by bilobed nucleus. Basophils, which also contain coarse granules and kidney shape nucleus. Lymphocytes, are spherical cells, they contains large nucleus surrounded by narrow rim of cytoplasm. Monocytes are large cells and the nucleus is central and is ovoid or kidney-shaped (Lesson *et al.,* ١٩٨٥). The number of leukocytes changes under several conditions such as, age, environment, hormones, drugs and diet (Haen ١٩٩٥ and Hoffbrand *et al*., ٢٠٠٣).

Neutrophils, the body's primary defense against bacterial invasion, engulf and destroy bacterial and foreign particles by a process known as phagocytosis (Lee *et al.,* ١٩٩٣ and Rodak ١٩٩٥). Chemotaxis in patients with thalassemia major was found to be defective when compared with healthy controls, the total neutrophil count for patients and controls decreased but not significantly ($p < ٠.٠٥$). On the other hand there was a significant difference in neutrophilic chemotaxis between splenectomized and non-splenectomized patients (Shaiegan *et al.,* ٢٠٠٢). However, the number of

circulating T-lymphocytes, helper T-cells and B-lymphocytes was increased in some patients. This phenomenon probably reflects an unspecific stimulation of the antibody-producing cells by repeated blood transfusions (Speer *et al.,* ١٩٩٠).

Immune function studies on recipients of blood transfusion have revealed changes in several immune parameters including decreased helper, suppressor T-cell ratios in patients with thalassemia major (Grady *et al*., ١٩٨٥). Patients with thalassemia showed significantly increased absolute lymphocyte counts compared with reference group. All splenectomized patients with thalassemia showed significantly an absolute lymphocytosis, However ٣٨٪ non splenectomized thalassemia patients also had elevated absolute lymphocyte counts involving both B and T-cell subsets, but a greater increase occurred in the number of B-cells with ongoing B-cell stimulation associated with chronic exposure to red cell antigens (Hodge *et al*., ١٩٩٩).A moderate leukocytosis were observed in splenectomized patients (Jaafer, ١٩٨٩). Also no significantly increase was observed in WBC count in children with β-thalassemia compared with control (Rashid, ١٩٩٨). Neutropenia may result from hypersplenism (Bouroncle and Doon, ١٩٦٤) and leucocytosis (٥x١٠^{٣}- ٤٠x١٠^{٣} µl), with immature forms (Sood, ١٩٨٥).

Agranulocytosis has been considered the most serious side effect of deferiprone; the rates of agranulocytosis (absolute neutrophil count [ANC] <٥٠٠ x١٠^{٩}/L) and milder forms of neutropenia (ANC, ٥٠٠ to ١٥٠٠ x١٠^{٩}/L) were ٠.٢ and ٢.٨ per ١٠٠ patient-years, respectively. In ٣٤ years old patient with β-thalassemia major on regular transfusion, splenectomized, eleven months after the start of deferiprone therapy, who developed neutropenia (Cohen *et al.,* ٢٠٠٣ and Piga *et al.,* ٢٠٠٥).

٢-٥-٥- Platelet Count:

The Platelets are small, granulated bodies ٢-٤ µm in diameter; they have a half-life of about ٤ days, their function related to hemostasis. The megakaryocytes form platelets by pinching off bits of cytoplasm and ٧٥٪ excluding them into the circulation, but the remainder are mostly in the spleen, therefore splenectomy causes an increase in the platelet count (thrombocytosis) (Hoffbrand *et al*., ٢٠٠٣).

Abnormal platelet aggregation was found in patients with β-thalassemia major and transfusional iron overload. The platelet aggregation defects was not correlated with units of blood transfused, serum ferritin, splenectomy and liver function test, however platelet count was elevated in ٥٩ % patients (Husain *et al*., ١٩٧٩). The platelet counts and mean platelet volume (MPV) in the whole blood of both splenectomized and non-splenectomized patients were higher than those of healthy subjects (Unchern *et al.,* ٢٠٠٣). In addition to their increased number of platelets in splenectomized patients, this may explain the weak response of thalassemic platelets to aggregation; in ٢٠٠٢, Eldor and Rachmilewitz found the mean platelet life span in patients who underwent splenectomy was (١٠٧ ± ٣٦) hours compared to (٢٤٨ ± ٥١) hours in healthy individuals who underwent splenectomy because of trauma (*P*< ٠.٠٠١). Analysis of the data suggested that the shortened platelet life span was caused by enhanced platelet consumption.

In patients with beta thalassemia major and minor had defects of platelet function, the extent of which was directly related to the severity of haemorrhagic symptoms (Eldor, ١٩٧٨). In splenectomized patient there was moderate to high thrombocytosis, but normal platelet count was observed in non-splenectomized patients (Jaafer, ١٩٨٩) and also similar results were

obtained by Caocci *et al*., (١٩٧٨); Khider, (١٩٨٦) Canatan *et al*., (٢٠٠١) they showed high platelet counts in splenectomized patients and slightly reduced in non-splenectomized patients. Platelet count was normal, but decreases with splenomegaly (Sood, ١٩٨٥)

٢-٦- Biochemical Parameters:

٢-٦-١- Iron Metabolism in Thalassemia:

Iron represents a paradox for living systems by being essential for a wide variety of metabolic processes, but it also has the potential to cause deleterious effects. Hemochromatosis is a disorder caused by excess iron deposition in parenchymal cells that leads to cellular damage and organ dysfunction. The disorder has two categories: genetic (or primary) hemochromatosis and secondary (or acquired) hemochromatosis. In primary hemochromatosis, an inherited autosomal recessive defect results in elevated intestinal iron absorption, despite normal iron intake. Secondary hemochromatosis results from iron overload due to elevated intake, classically in the form of repeated blood transfusions. Such a clinical scenario may be seen in the setting of ineffective erythropoiesis, for instance, in patients with thalassemia major, because it has completely dependent on a programmed of regular blood transfusion. Each transfusion of ٤٠٠ ml of blood carries with it approximately ٢٠٠ mg of iron which cannot be excreted (Haen, ١٩٩٥ and Hoffbrad *et al*., ٢٠٠٣).

The abnormal condition that ensues is similar in both primary and secondary hemochromatosis. When serum levels are elevated, iron is initially deposited in the reticuloendothelial cells; however, when their capacity is saturated (at about ١٠ g), the excessing iron is deposited in parenchymal cells

of the liver, spleen, and bone marrow in a crystalline form as ferritin and hemosiderin. Eventually, as iron stores continue to increase, there is deposition in the skin (patients usually have darkened skin), heart, gonads, and endocrine glands. Involvement of the pancreas, for instance, will cause parenchymal damage to the gland, which eventually will manifest itself as diabetes mellitus. Iron deposition has also been described in other endocrine glands, including the anterior lobe of the pituitary gland. As a number of patients affected by hemochromatosis eventually develop hypogonadism, it has been postulated that this may in part be secondary to iron-induced cellular damage to the gonadotrophs (Sparacia *et al.,* ٢٠٠٠).

In thalassemia, iron overload is the joint outcome of excessive iron absorption limited by a physiologic ceiling of a bout ٣ mg/day, plasma iron turnover in thalassemia may be ١٠ to ١٥ times normal, caused by the wasteful, ineffective erythropoiesis of an enormously expanded erythroid marrow (Hershko *et al*., ١٩٩٨).

٢-٦-١-١- Serum Ferritin:

A small fraction of body ferritin circulates in the serum, the concentration being related to tissue, particularly reticuloendothelial; iron stores (Hoffbrad *et al*., ٢٠٠٣). Ferritin is a water-soluble protein-iron complex; it is composed of an outer spherical apoprotein shell (Mol. Wt. ٤٨٠٠٠٠), enclosing a core of ferric hydroxyphosphate, containing about ٢٠٪ of iron by weight (٤٠٠٠-٥٠٠٠ iron atoms). Human ferritin is made up from ٢٤ subunits divided into two types: H subunits (Mol. Wt. ٢١٠٠٠) and L subunits (Mol. Wt.١٩٠٠٠). Internal cavity of the ferritin molecules communicates with the exterior by six channels through which ferrous iron may enter or leave (Haen, ١٩٩٥ and Hoffbrad *et al*., ٢٠٠٣).

The correlation between hepatic iron stores and plasma ferritin was highly significant in transfused patients with thalassemia major (Brittenham, ١٩٩٣).

Mazza *et al*., (١٩٩٥). Investigated that serum ferritin levels exhibit a tendency to be significantly correlated with the hemochromatosis in thalassemic patients. Fargion *et al.,* (١٩٨٢) found that even children with a limited number of transfusions had severe iron overload as indicated by the raised serum ferritin levels. There was a highly significant correlation between serum ferritin concentration and the amount of blood transfused in patients with iron overload and with homozygous β-thalassemia. Statistical analysis revealed serum ferritin as the most significant predictor of moderate to severe haemosiderosis, (Worwood *et al*., ١٩٨٠ and Li *et al.,* ٢٠٠٢). A high serum ferritin accompanied by a high percentage of saturation of a normal serum transferring usually indicates iron overload (Herbert *et al*., ١٩٩٧). In patients with α-thalassemia trait concentrations of ferritin were significantly lower than those associated with β-thalassemia trait (White *et al.,* ١٩٨٦).

Argyropoulou *et al*., (٢٠٠٠) found that the serum ferritin levels increased significantly in transfusion dependent β-thalassemia major. Very low birth weight infants had significantly higher serum ferritin after multiple blood transfusions and significantly associated with liver iron concentration. Body iron is stored within molecules of ferritin directly related to body iron stores of denatured ferritin (hemosidren) and appears to be in equilibrium with them (Herbart *et al*., ١٩٩٧). Serum ferritin levels were markedly elevated, ranging from ١,٤٨٥–١٦,٢١٥ μg/liter in the transfusion-dependent patients compared with ١٣٧–١٣٦٥ μg/liter in the transfusion-independent patients (Mahachoklertwattana *et al.,* ٢٠٠٣).

Pignatti *et al*., (١٩٩٨) observed that patients with thalassemia major who died had a serum ferritin level, measured the year before death, significantly higher than those who survived. There were no significant differences between boys and girls for serum ferritin levels and blood consumption (Asadi-pooya *et al*., ٢٠٠٤). Khider, (١٩٨٦) found that splenectomy raised the serum iron level, but serum ferritin level showed no significant difference between splenectomized and non-splenectomized patients. Salsaa & Zoumbos, (١٩٩٧) reported that there was a positive correlation between mean total number of transfusions and serum ferritin levels in multiple transfused thalassemic patients.

٢-٦-١-٢- Serum Iron and Total Iron Binding Capacity (TIBC):

The total iron binding capacity is made up by the serum iron and the unsaturated iron binding capacity (UIBC). The greater part of iron in the blood is transported bound to transferrin or siderophilin. About over ٣٠ mg iron is transported by this rout each day. It represents only about ٤ mg of body iron (Haen, ١٩٩٥).

Transferrin is a single chain glycoprotein, a globular protein with (M.w of ٨٠٠٠٠ Daltons). It is synthesized in the liver; it has a half-life of ٨ to ١٠ days, and it is capable of binding two iron atoms per molecule. Normally trasferrin is about ٣٥٪ saturated with iron (Rodak ١٩٩٥).

In thalassemia, the wasteful production of non-viable RBC stimulates iron absorption, but most of the iron released to the circulation is derived from RBC catabolism. This out pouring of catabolic iron exceeds the iron-binding capacity of transferrin (Hershko *et al*., ١٩٩٨). In the β -thalassemia group plasma iron

levels (١٧٠ ± ٧٨ mg/dL) are significantly higher than those of healthy controls (٧٨ ± ١٥ m g/dL), (p < .٠٠١) (Meral *et al.*, ٢٠٠٠).

Saraya *et al*., (١٩٨٥) found that the incidence of iron overload seen in beta-thalassemia patients was similar in transfused and non-transfused case and trasferrin saturation is not a good indicator of either iron depletion or overload. However, Ponka (١٩٩٩) showed that plasma can contain transferrin completely saturated with iron in patients with severe iron overload, associated with sever elevation of the serum iron.

In transfused patients with severe β-thalassemia, iron loading progresses the capacity of serum transferrin to bind and detoxify iron may be exceeded (Kushner *et al*., ٢٠٠١). There are significantly higher serum iron (P<٠.٠١) in very low birth weight infants after multiple blood transfusion (Ng *et al*., ٢٠٠١). A high serum ferritin accompanied by a high percentage of saturation of a normal serum transferrin usually indicates iron overload (Herbert *et al*., ١٩٩٧).

٢-٦-٢- Serum Transaminases and Serum Bilirubin:

Aminotransferases are normally intracellular enzymes, with the low levels found in the plasma; the presence of elevated plasma levels of aminotransferases indicates damage to cells rich in these enzymes. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are elevated in all liver diseases, but are high in viral hepatitis acquired by blood transfusion and/or liver toxicity can occur as a direct consequence of iron toxicity. ALT is more specific for liver disease than AST, but the latter is more sensitive because the liver contains larger amounts of AST. Elevated serum bilirubin results from hepatocellular damage that decreases the hepatic conjugation and excretion of bilirubin (Champe *et al.,* ٢٠٠٥).

Thalassemic children had significantly higher concentrations of serum ferritin, bilirubin and alanine amino-transferase activity (Soliman *et al.,* ١٩٩٨). No relationships were found between height deficiency and (AST) or (ALT) or IGF-١ levels (Cavallo *et al.,* ١٩٩٧). There are significant differences in the mean of serum glutamate-pyruvate transaminase (SGPT), serum glutamate-oxaloacetate transaminase (SGOT), and Serum bilirubin levels between splenectomized and non-splectomized patients (Jaafer, ١٩٨٩) and also showed a significant positive correlation between serum ferritin and SGPT, SGOT, and total bilirubin when compared with the control. Worwood *et al.,* (١٩٨٠) found a highly significant correlation between serum ferritin concentration and ALT activity. On the other hand, there was no correlation between serum ALT concentration and age, duration of transfusion and chelation, but serum ferritin was significantly associated with increased ALT concentrations (Li *et al.,* ٢٠٠٢). Hcv-seropositive subjects had higher ALT levels (Wanless *et al.,* ٢٠٠٢). There were poor correlations between serum AST and the serum ferritin ($r = ٠-٢٨٣$, $p = ٠.٠٦$). On the other hand, serum AST correlated well with degree of hepatitis, in patients with moderate or severe hepatitis had raised AST values ($p < ٠-٠١$) (Aldouri *et al.,* ١٩٨٧). Higher AST levels in HCV infected patients are most probably from liver and not from hemolysis because the time interval between transfusions was not different in positive and negative patients (Saberi *et al.,* ١٩٩٦).

In thalassemic patients the total serum ferritin and bound serum ferritin concentration are correlated positively with the AST, but free ferritin concentration did not correlate (Chapman *et al.,* ١٩٨٢). There was a significant increase in ALT value in beta-thalassemia patients with abnormal glucose tolerance than normal glucose tolerance test (Khalifa *et al.,* ٢٠٠٤). ٥٦٪ patients with homozygous thalassemia had high levels of AST and ALT

(Tchakurova *et al.,* ٢٠٠٣). In the majority of patients (٧٠٪) average GPT serum values were increased (٦٦.٣٣ +/- ٣٥.٤١ U/L) while only a few of the youngest age group exhibited normal values. The transaminase level showed a direct relationship with age, ferritin level and transfusional iron. Furthermore a direct correlation was found between iron and gamma globulin levels both being related to age (Gangemi *et al.,* ١٩٨٦). Serum ferritin had a significant positive correlation with ALT (r = ٠.٣١٥), but average hemoglobin maintained correlated negatively with serum ferritin levels (r = - ٠.٥٤٠), and AST (r = - ٠.٤١٧) (Naithani *et al.,* ٢٠٠٦).

٢-٦-٣- Serum Calcium ($Ca^{+٢}$), Phosphorus ($Po_{٤}^{=}$) and Alkaline Phosphates (ALP):

Lebanese beta-thalassemia children had high serum phosphorus value, low calcium levels, high alkaline phosphatase and low parathyroid hormones (PTH) values (Yazigi *et al.,* ٢٠٠٢). All the thalassemia patients reported who developed hypoparathyroidism and who were above the age of ١٠ year, had low mean serum calcium ١.٨٨mmol/L, high mean serum phosphate level was ١.٨٨mmol/L, also showed no consistent relation ship between serum ferritin levels and degree of hypocalcemia (Aleem *et al.,* ٢٠٠٠). Highest prevalence of hypoparathyroidism was seen in the age of ٢٠ years (Shamshiraz *et al.,* ٢٠٠٣). Serum calcium, phosphorus concentration and alkaline phosphatase activity were comparable with controls, ruling out the diagnosis of hypoparathyroidism in any patients (Soliman *et al.,* ١٩٩٨ and Angelopoulos *et al.,* ٢٠٠٦).

The levels of $Ca^{+٢}$, $Po^{=}_{٤}$, Ca-P related hormones and alkaline phosphates level were within normal limits in all patients, but inversely related to patients ferritin level (De-Verneioul *et al.,* ١٩٨٢; Chao and Hwang ١٩٩٦ and Mahachoklertwattana *et al.,* ٢٠٠٣). No significant difference was observed in mean serum $Ca^{+٢}$, phosphate, alkaline phosphotase and PTH before and ٢٤

months after bone marrow transplantation BMT (Khoshnyat *et al.,* ٢٠٠٣). Osteoporosis in female patients with thalassemia major was strongly associated with primary amenorrhea (*P* < .٠٠٠١), while in male patients with TM, hypogonadism was not significantly related to bone mineral density (BMD). Low BMD was also associated with diabetes mellitus (*P* < ٠.٠٠٠١), and increased ALT (*P* < ٠.٠١) (Origa *et al.,* ٢٠٠٥).

In thalassemics aged ١٠-١٣ years $Ca^{+٢}$ (P < ٠.٠٥), P (P < ٠.٠٥), PTH (P < ٠.٠٠١), calcitonin (CT) (P < ٠.٠٠١), Vit.D (P < ٠.٠٠١) levels were lower, whereas alkaline phosphatase (P < ٠.٠٠١) levels were higher than in controls (Zamboni *et al.*, ١٩٨٦). A ١٧-year-old girl with thalassemia major experienced tetany, the serum calcium level was ٥.٥ mg/dL, and the phosphorus level was ٦.٣ mg/dL. Serum levels of parathyroid hormone (PTH) and ٢٥-hydroxyvitamin D (٢٥-OHD) were subnormal at ١٢٥pg/mL and ٨.١ng/mL, respectively (Aloia *et al.,* ١٩٨٢).

Hypoparathyroidism leads to decrease in plasma $Ca^{+٢}$ from the normal of ٩.٤ mg/dl to ٧ mg/l and blood phosphate concentration may double. Finally, the $Ca^{+٢}$ metabolism is affected by a number of hormones, such as PTH, thyroxine, oestrogen, cortisol, insulin, and calcitonin, as well as vitamin D, which are involved in the regulation of bone metabolism, effecting both progenitors and mature osteoblastic cells and osteoclasts. High levels of PTH, thyroxine, cortisol, and reduced levels of oestrogen, testosterone, vitamin D, calcitonin, and insulin accelerate bone loss, stimulate osteoblastic and osteoclastic activity (Griffin and Ojeda ٢٠٠٠; Ganong, ٢٠٠٥; Guyton and Hall, ٢٠٠٦). High alkaline phosphatase (ALP) usually means that the bone or liver has been damaged. If other liver tests such as bilirubin, aspartate aminotransferase (AST), or alanine aminotransferase (ALT) are also high, usually the ALP is coming from the liver. If calcium and phosphate measurements are abnormal, usually the ALP is coming from bone.

In patients with β-thalassemia Saberi *et al.,* (١٩٩٦) revealed that anti-HCV positivity has a significant correlation with higher aspartate aminotransferase (AST), but not with alanine aminotransferase (ALT), alkaline phosphatase and total bilirubin level. However, this correlation of liver function tests to anti-HCV status may not be exact because of other causes of abnormal liver function tests such as other viral infections (e.g., hepatitis B) and iron overload.

٢-٦-٤- Glucose Tolerance Test (GTT):

In normal, fasting person ingest ١ gm of glucose/kg of body weight, the blood glucose level rises from about ٩٠mg/١٠٠ ml to ١٢٠ to ١٤٠ mg /١٠٠ ml and falls back to bellow normal in about ٢ hours. In persons with diabetes, the fasting blood glucose concentration is always above ١١٠ to١٤٠ mg/١٠٠ ml and has abnormal GTT and the glucose level falls back to the control value only after ٤ to ٦ hours because the normal increase in insulin secretion after glucose ingestion does not occur, or there is decreased sensitivity to insulin. Plasma insulin is low in type I diabetes and increased in type II diabetes (Guyton and Hall, ٢٠٠٦).

Patients with beta-thalassemia major were kept alive by repeated blood transfusion and iron chelation. Chronic iron overload results in several endocrine abnormalities, especially the development of type ١ diabetes mellitus. Although insulin deficiency is secondary to pancreatic islet irons deposition, it is the main cause of the abnormalities in glucose metabolism in thalassemics (Fernandez-Real *et al.,* ٢٠٠٢). Iron stores are associated with insulin sensitivity, insulin secretion, and type II diabetes (Fig.٤) Approximately ١٠٪ of type II diabetes patients with high ferritin levels had trasferrin saturations greater than normal (٤٠٪) (Fernandez-Real *et al,* ٢٠٠٢).

Prevalence of diabetes and impaired glucose tolerance (IGT) were reported by Khalifa *et al.,* (٢٠٠٤) in transfused dependent Egyptian beta-thalassemic patients and also reported that the serum ferritin level was higher in patients who developed abnormal glucose tolerance. The fasting plasma glucose and ٢h post load plasma glucose showed positive correlation with serum ferritin level. On the other hand, serum concentrations of ferritin were negatively correlated with insulin sensitivity in subjects with hemosiderosis (Dmochowski *et al.,* ١٩٩٣) and also similar results were obtained by El-Hazmi *et al.,* (١٩٩٤); they reported the prevalence of diabetes mellitus and (IGT) and also mentioned either hypo or hyperinsulinaemia was encountered in the majority of these patients and those receiving regular desferal ٣.٠٨٪ had diabetes (Jassim, ١٩٨٩).

Fasting proinsulin ($p < ٠.٠٠٢$) and proinsulin-to-insulin ratio ($p < ٠.٠٢$) were significantly increased in patients with thalassemia irrespective of the degree of glucose tolerance. They correlated positively to serum ferritin, liver iron, patient age and serum AST ($p < ٠.٠٥$) this indicates early B-cell dysfunction due to siderosis (Cario *et al.,* ٢٠٠٣). Mean serum glucose level in oral glucose tolerance test in patients with thalassemia was not significantly differente before and ٢٤ months after bone marrow transplantation (Khoshnyat *et al.,* ٢٠٠٣).

After long-term high-blood transfusion, thalassemic children had significantly decreased serum insulin concentrations and low insulin/glucose ratios at ٦٠ and ١٢٠ min after an oral glucose load (١.٧٥ g/kg body weight) in comparison with values before therapy and those for controls. However, their serum glucose levels at ٦٠ and ١٢٠ min after the oral glucose load were significantly higher compared to control children (Soliman *et al.*, ١٩٩٦). ALT

and maximum ferritin levels were found significantly high in thalassemic patients with abnormal glucose metabolism (Canpolat *et al.,* ٢٠٠٤).

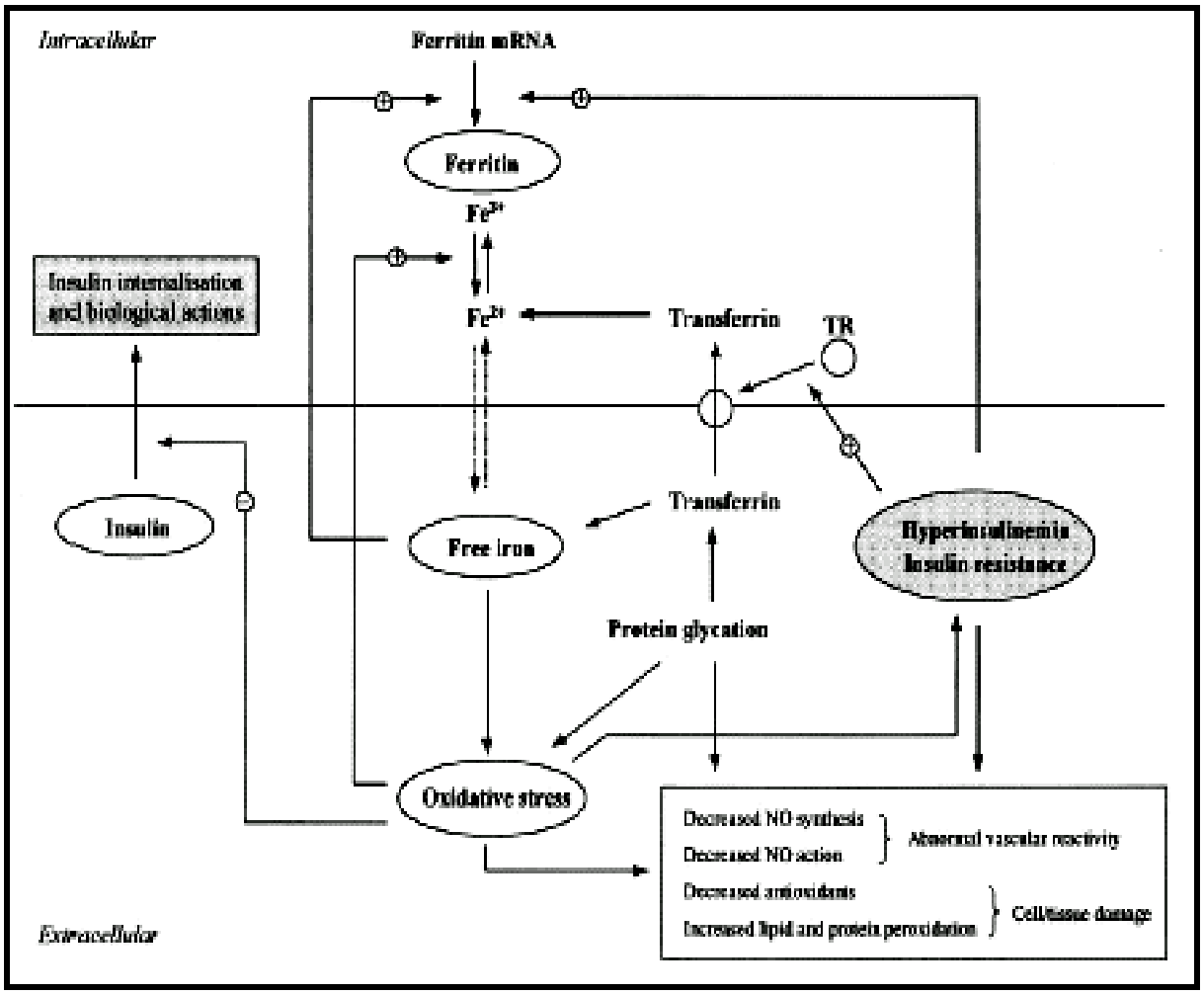

Figure (٤): Schematic representation of iron interactions with insulin resistance and oxidative stress (Fernandez-Real *et al.,* ٢٠٠٢).

٢-٧- The Splenomegaly Patient:

The spleen is an important blood filter that removes spherocytes and other abnormal red cells. It plays a significant role in the immune system, percolates through large numbers of phagocytes and lymphocytes. The

phagocytes remove bacteria and initiate immune responses. Abnormal red cells are removed if they are not as flexible as normal red cells and consequently are unable to squeeze through the slits between the endothelial cells that line the splenic sinuses (Vander *et al.*, ٢٠٠١ and Ganong, ٢٠٠٥)

Splenic enlargement is associated with anemia, leucopenia and thrombocytopenia caused by an enhanced capacity of the enlarged spleen for pooling, sequestering and destroying blood cells and also leading to an increased plasma volume (Hoffbrand *et al.*, ٢٠٠٣).

After splenectomy there is sharp reduction in blood requirement, but growth and sexual development are retarded, secondary sexual character developed but later than in normal individual. There is decrease in plasma volume in some patients and increase in life span of red cells in all patients but red cell mass did not rise indicating decrease in erythropoiesis in some patients (Bliendis *et al.*, ١٩٧٤). Splenectomy was significantly more common in the regularly transfused patients compared with the non transfused patients (Rees *et al.*, ١٩٩٩). Hypersplenism may be avoided by early and regular transfusion; many patients reaching adolescence in this decade have not required splenectomy. Because of the risk of post-splenectomy infection, splenectomy should generally be delayed until the age of ٥ years or later (Olivieri and Brittenham, ١٩٩٧**)**. In patients with β-thalassemia intermediate the operation remains the most important and definitive treatment since they rarely required transfusion after operation (Maniga *et al.,* ١٩٨٣).

٢-٨- Height and Weight:

Poly-transfused thalassemia patients grow normally until the age of ٥-٦ years when height deficiency occurs and remains constant until the

physiological pubertal age when it worsens because these patients lack the pubertal growth spurt (Cavallo *et al.,*١٩٩٧).In ٥٤٪ patients had height and weight below the ١٠th percentile, and was negatively correlated with the serum ferritin levels (Jaafer,١٩٨٩).

Among the short Italian patients with thalassemia, a majority (٧٧٪) of the patients had disproportionate short stature with short trunk but with less severe impairment of subischial leg length (Raiola *et al*., ٢٠٠٣).Disproportionate truncal shortening which is common especially among Chinese adolescents with thalassemia (Louis, ٢٠٠٥). Short trunk is not necessarily the cause of the short stature (Rodda *et al*., ١٩٩٥). Short stature was present in ٣١.١٪ of males and ٣٠.٥٪ of females, and the prevalence of growth hormone deficiency was ٧.٩٪ in males and ٨.٨٪ in females (De Sanctis *et al.,* ٢٠٠٤).

An abnormal upper to lower (U: L) ratio was commonly observed in patients with β-thalassemia major (Low *et al.,* ١٩٩٨), however short trunk despite normal stature is present in ٤٠٪ of patients (Nicoletti *et al*., ١٩٩٨). In pubertal age the short stature and short trunk are more evident (Nicoletti *et al*., ٢٠٠١). The height SDS showed a significant reduction with age and with elevated serum ferritin levels (Senanayake *et al.,* ١٩٩٩ and Shalitin *et al*., ٢٠٠٥). Weight for age in ٤٣ % of patients was lower than the ٥th percentile (Asadi-pooya *et al*., ٢٠٠٤).In ٤٤٪ of the patient's weight was below the third percentile for age (Karamifar *et al.,* ٢٠٠٢ and Karamifar *et al.,* ٢٠٠٥). In children with homozygous β-thalassemia height was significantly below the normal range (Madeddu *et al*., ١٩٧٨). The growth retardation in both height and weight was found to increase significantly with the increase in the age of the patients in Baghdad city (Jassim, ١٩٨٩).

MATERIALS AND METHODS

Seventy-four homozygous β-thalassemic patients ٤٠ males and ٣٤ females at different stages of the disease were examined at random as they were admitted to Erbil Thalassemic-haemophilic Center in Pediatric Hospital in Erbil, for regular blood transfusions, patients aged between ١٠-٣٨ years and with age means was (١٦.٣٢ – ١.٠٣) years old ; the study was performed from July ٢٠٠٥ to June ٢٠٠٦. To serve as a control group twenty-seven healthy individuals; nineteen males and eight females (age and sex matched) were included in this study. Normality refers to normal haemoglobin level, red cell indices, and red cell morphology and haemoglobin electrophoretic pattern.

Forty-five patients (twenty two male and twenty three females) had undergone splenectomy at ages of more than ٥ years. Most patients received multiple transfusions, which usually started in the first year of life and most of them began irregular therapy before the age of ١٠ years.

٣-١- Clinical Examination:

Every patient included in this study, was asked about the history of his illness, with special emphasis on the age of onset of disease, onset of first blood transfusion, the frequency of blood transfusions and, type of blood group, chelation therapy, Vit.C, folic acid (they receive regularly, irregularly, or no therapy), frequency, route of administration and duration of treatment. They were also asked about parent relativity, splenectomized or not, delayed puberty, and any female who had not begun menstruating by the age of ١٨ years was said to have primary amenorrhea (Karamifar *et al.,* ٢٠٠٥). Age, sex, weight and height were recorded in the patient's chart. The total body weight was taken before blood transfusion by using a digital balance and height was determined by using tape measurement.

٣-٢- Analytical Methods:

٣-٢-١- Blood Content Analysis:

٣-٢-١-١- Collection of Blood Samples:

Nine (٩) ml venous blood was obtained from each patient before blood transfusion by vein puncture, using a ten (١٠) ml disposable syringe and the

blood was divided into ٣ separate clean and sterile containers as follows: ٢ ml of blood into potassium-EDTA tube for the determination of the routine haematological parameters; ٢ ml of blood was put into potassium-EDTA tube for the preparation of hemolysate for haemoglobin-electrophoresis and the determination of the quantity of $HbA_٢$ and HbF. The remaining ٥ ml of blood into sterile, plain tubes to obtain the serum by centrifugation at ٣٠٠٠ rpm for ١٥ min. (at ٤C^o, using cooling centrifuge) to separate serum and divide it into two parts; one (١ml) was stored frozen at -٨٠^oC (Sony, Ultra low, Japan), for the latter estimation of serum iron and TIBC, the rest was used immediately for the estimation of hormones and other biochemical tests (Cheng, ٢٠٠٢).

٣-٢-١-٢- Hormonal Analysis:

Quantitative determination of hormones by Enzyme-linked Immunosorbent Assays (ELISA). All hormonal analysis was performed at Erbil Medical Laboratory. Automated method: Computer programs using cubic spline, ٤ PL (٤ Parameter Logistics) or Logit-Log, in a microtiter plate spectrophotometer reader (Jenway ٦٣٠٠) can generally give a good fit. The concentration of the samples can be read directly from this standard curve by using their average optical density.

٣-٢-١-٢-١- Determination of Growth Hormone (GH):

The GH Accu-Bind ELISA Micro-wells code; ١٧٢٥-٣٠٠ kit provides materials for the quantitative measurement of GH in serum (Henry, ١٩٩٦ and Tietz, ١٩٨٦). Principles; in this procedure, the immobilization takes place during the assay at the surface of a micro-plate well through the interaction of streptavidin coated on the well and exogenously added biotinylate monoclonal anti-GH antibody. Mix monoclonal biotinylated antibody, the enzyme-labeled

antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition, to form a soluble sandwich complex. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve is generated from which the antigen concentration of an unknown is ascertained (Henry, ١٩٩٦).

In this method, GH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies are added and the reactants mixed. Reaction between the various GH antibodies and native GH forms a sandwich complex that binds with the streptavidin coated to the well (Henry, ١٩٩٦).

After the completion of the required incubation period, the enzyme-growth hormone antibody bound conjugate is separated from the unbound enzyme-growth hormone antibody bound conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantities by reaction with a suitable substrate to produce color (Henry, ١٩٩٦).

The employment of several serum references of known GH levels permits the construction of a dose response curve of activity and concentration (standard curve). From comparison to the dose response curve, an unknown specimen's activity can be correlated with GH concentration (Henry, ١٩٩٦).

Normal range = ٠- ٥٥ µlU/ml

٣-٢-١-٢-٢- Determination of Follicle Stimulating Hormone (FSH):

The FSH EIA Kit (The PADTAN ELM EIA Kit) was used for quantitative determination of FSH levels in human serum and plasma (Sairam and Li, ١٩٧٣).

Summary of assay procedure; Pipette ٥٠ µl dilution buffer to each well, dispense ٥٠ µl of FSH standards, control sera or patients sample, incubate ٣٠min. at room temperature (RT), wash each well ٤ times with diluted wash solution, add ١٠٠ µl HRP-Anti-FSH Tracer, incubate ٣٠ min. at RT, wash ٤ times, dispense ١٠٠ µl of Tetra-Methyl Benzidine (TMB) for١٠ min, incubate ١٥ min at RT, add ٥٠ µl stop solution and mix ١٠ seconds and then read absorbance at ٤٥٠ nm.

Normal range

Adult male;	١.٤- ١٥.٤	mlU/ml
Adult female		
Follicular phase	١.٠- ١٠.٠	mlU/ml
Ovulatory peak phase	٦.٠- ١٧.٠	mlU/ml
Luteal phase	١.٠- ٩.٠	mlU/ml
Post-menopausal	١٩.٠- ١٠٠.٠	mlU/ml

٣-٢-١-٢-٣ Determination of Luteinizing Hormone (LH):

The LH Accu-Bind ELISA Micro-wells code; ٦٢٥-٣٠٠ (Monobind LH) kit provides as a micro-plate immuno-enzymo-metric assay for quantitative determination of LH concentration in human serum (Kosasa, ١٩٨١)

Expected values for the LH ELISA test (in mlU/ml)

Men	٠.٧٠- ٧.٤٠

Women

Follicular phase ٠.٥٠- ١٠.٥

Midcycle phase ١٨.٤- ٦١.٢

Luteal phase ٠.٥٠- ١٠.٥

Post-menopausal ٨.٢٠- ٤٠.٨

٣-٢-١-٢-٤- Determination of Testosterone:

The testosterone ELISA Kit (RE ٥٢١ ٥١) was used for quantitative determination of testosterone levels in human serum and plasma.

Normal range

Adult male; ٢.٤- ١٢ ng/ml

Adult female ٠.١- ١.٢ ng/ml

٣-٢-١-٥- Determination of ١٧ beta-Estradiol:

The ١٧ beta-Estradiol ELISA (IBL, RE ٥٢٠٤١) is enzyme immunoassay kit was used for the in-vitro-diagnostic quantitative determination of ١٧beta-Estradiol in human serum and plasma (Ratcliff *et al.,* ١٩٨٨).

Normal range

Adult male; ١٠- ٣٦ pg/ml

Adult female

Pre-menopausal ١٣- ١٩١ pg/ml

Post-menopausal ١١- ٦٥ pg/ml

٣-٢-١-٢-٦- Determination of Thyroid Stimulating Hormones (TSH):

The TSH ELISA Kit (Pishtazteb) was used for quantitative determination of TSH levels in human serum and plasma (Helenius and Tikanoja, ١٩٨٦)

Normal range ٠.٣٢- ٥.٢ mIU/L

٣-٢-١-٢-٧- Determination of Thyroxin ($T_٤$):

The $T_٤$ ELISA Kit (Pishtazteb) was used for quantitative determination of $T_٤$ levels in human serum and plasma (Liewendahl, ١٩٩٠).

Normal range ٤.٧- ١٢.٥ µg/dl

(µg/dl x ١٢.٨٧= nmol/L x ٠.٠٧٨= µg/dl)

٣-٢-١-٣ Hematological Analysis:

٣-٢-١-٣-١- Complete Blood Measurements:

Complete blood counts (CBC) of all blood samples were carried out at Erbil Medical Laboratory. The blood parameters included total white blood cells count (WBCs), red blood corpuscular count (RBCs), platelet count (PLTs), hemoglobin (Hb), packed cell volume (PCV), erythrocyte indices; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), RDW and platelet indices; MPV and PDW were measured by coulter counter (Sysmex K-١٠٠٠), TOA medical electronics CO., LTD.KOBE.JAPAN (Haen, ١٩٩٥ and Rodak, ١٩٩٥).

٣-٢-١-٣-٢- Differential Leukocyte Percentage and Count (DLC):

The leukocyte types were determined by preparation of blood films. A drop of (EDTA blood) was placed about ٢ to ٣ mm at one end of a clean slide. Place the pusher slide at ٣٠° to ٤٥° angle to the slide, and then move it back to make contact with the drop. The drop should spread along the edge of the pusher slide, and a film is made by a forward movement of the pusher slide. Blood films should be air – dried as soon as they were made. The blood films was fixed with undiluted stock Lei-shaman stain for ٢ to ٣ minutes, then the stain on film was diluted with distilled water, and left for ٥-٧ minutes, and then washed by a stream of tap water, the stained film was dried and examined under light microscope (Oil - immersion objective) employing a battlement counting technique, and at least (٢٠٠) consecutive leukocytes should be identified (Simmons, ١٩٧٦ and Evatt *et al*., ١٩٩٢).

٣-٢-١-٣-٣- Reticulocyte Count:

With a Pasteur pipette equal amounts of blood and (١٪) Brilliant Cresyl Blue (BCB) [BCB ١.٠ gm; NaCl ٠.٧ gm; Sodium citrate ٠.٦ gm and ١٠٠ ml DW] was added to a ١٢-x ٧٥-mm tube, and mixed well and stand at room temperature for ٣٠ minutes or incubate at ٣٧C° for ١٥ minutes. After incubation and mixing again expelled a small drop of the mixture on a slide and a thin film was made, then allowed to dry in the air. By using oil immersion, ١٠٠٠ erythrocytes counted and noted the number of reticulocyte encountered (Evatt *et al*., ١٩٩٢). The Reticulocyte percentage was calculated as follow**s:-**

$$\textbf{Reticulocyte (\%)} = \frac{\text{No. of reticulocyte counted}}{\text{No. of red blood cells counted (١٠٠٠)}} \times ١٠٠$$

٣-٢-١-٣-٤ Red Blood Corpuscular Morphology:

Freshly prepared blood films were stained using Leishmann's stain. By using oil immersion, ٥٠٠ erythrocytes counted and noted the number of hypochromic, microcytes, macrocytes, and target cells encountered (Evatt *et al*., ١٩٩٢).

٣-٢-١-٣-٥- Estimation of the Haemoglobinopathies:

٣-٢-١-٣-٥-١- Preparation of Haemolysates:

The anticoagulanted blood was washed three times in normal saline (٠.٩٪ NaCl) and then hemolysed by the addition of one volume of distilled water (DW) and mixed well by using vortex, and then one volume of carbon-tetrachloride (CCl_4) was added, after shaking in a mechanical agitator, centrifuge the mixture at ٣٠٠٠ rpm for ٣٠ min and pipette off the clear Hb solution into clean dry plastic tubes. Add a few drops of (٠.٣ mol/l) KCN to the haemolysate to stabilize the Hb as cyanmethaemoglobin (HiCN) (Dacie and Lewis, ١٩٨٤)

٣-٢-١-٣-٥-٢- Correction of the Hb Concentration of the Haemolysate;

The Hb concentration of the hemolysate was measured by the cyanmethaemoglobin method, using the (Jenway ٦٣٠٠) spectrophotometer. The absorbance was red at ٥٤٠ nm. The volume of the hemolysate was then measured. The Hb concentration of the hemolysate was corrected by the addition of the appropriate volume of DW in order to attend a concentration of ١٠ gm/dl according to the following formula (Tietz, ١٩٨٦).

$$\text{Required final volume} = \frac{\text{Volume of hemolysate X Hb. Concentration}}{\text{Required Hb. concentration}}$$

٣-٢-١-٣-٥-٣- Estimation of Hb $A_٢$:

The Marengo-Rowe (١٩٦٥) method used depends on the separation of $HbA_٢$ by electrophoresis on cellulose acetate membrane and ٠.١٣mol/l Tris-EDTA-borate, PH ٩.١ (Tris-hydroxymethyl- aminomethane ٩٢.٥g; EDTA (disodium salt) ٧.٨g; Boric acid ٤.٦g; water to ٥L) (Dacie and Lewis ١٩٨٤).

Procedure:

Two cellulose acetate strips per sample (Mylar supported Cellulosis) were soaked in buffer and blotted with filter paper, then strips were placed across the bridge of electrophoretic tank (LKB-٢٢١٩) secured with buffer soaked filter paper wicks. Approximately ١٠ µl of the hemolysate, leaving not less than a ٠.٥ cm gap at each side of the strip. A ٢٠٠ volt (approximately ١ mA/cm width of strip) was applied until clear separation of Hb $A_٢$ band, which occured in around ٦٠-٩٠ minutes. Then the zones of Hb A, and Hb $A_٢$ and a blank zone were cut and eluted into ٢٠ml, ٤ml, and ٤ ml respectively of DW for ٣٠ minutes in a plastic container. The absorbance was red in a spectrophotometer at a wave length of ٤١٣ nm and the percentage of Hb $A_٢$ calculated from the equation:

$$\text{Hb } A_٢ \% = \frac{A^{٤١٣}\ HbA_٢}{(A^{٤١٣}\ HbA \times ٥) + A^{٤١٣}\ HbA_٢} \times ١٠٠$$

Staining:

After the completion of separation, the strip was removed and stained for ١٥ minutes in ٠.٢٪ Panceau S (dissolving ٠.٢ g panceau S in ٣ g trichloroacetic acid and the volume corrected to ١٠٠ ml using DW). Then the excessive stain was removed with ٥-٧٪ acetic acid (Dacie and Lewis, ١٩٨٤). A known sample containing Hb F and Hb A is used as a control with each batch of samples.

٣-٢-١-٣-٥-٤- Estimation of Hb F by Alkali Denaturation:

The method used by Betke *et al.,* (١٩٥٩) depends on the resistance of Hb F to denaturation by alkaline solution (Dacie and Lewis, ١٩٨٤).

Procedure:

A cyanmethaemoglobin (HiCN) solution was prepared by adding ٠.٢ ml of hemolysate to ٤ ml Drabkin`s solution (Sodium bicarbonate ١.٠ gm; Potassium cyanide ٠.٠٥ gm; potassium ferric cyanide ٠.٢ gm and ١٠٠٠ ml DW) in a clean test tube, mixed well and to tube A containing ٢.٨ ml of the HiCN solution added ٠.٢ ml of ١.٢ mol/l NaoH; mixed well and after exactly ٢ min at room temperature ٢ ml of saturated ammonium sulphate solution was added, mixed thoroughly by using vortex, then allowed to stand for ١٠ minutes and then filtered into a dry clean test tube through a whatman No. ٤٢ filter paper (Dacie and Lewis ١٩٨٤).

Second tube (B) containing ٠.٧ ml of the HiCN solution added ٤.٣ ml of Drabkin`s solution and mixed well to make ٢٥٪ standard solution. The optimal

density of both tests was measured at a wave length of ٥٤٠ nm. The percentage of HbF was calculated as follows;

$$\text{Hb F \%} = \frac{A^{٥٤٠}\ \text{test} \times ٢٥}{A^{٥٤٠}\ \text{standard}}$$

٣-٢-١-٤ Biochemical Analysis:

٣-٢-١-٤-١ Iron Status Analysis:

٣-٢-١-٤-١-١-Determination of Serum Ferritin:

The Ferritin EIA Kit (The PADTAN ELM EIA Kit) was used for quantitative determination of Ferritin levels in human serum and plasma (Burtis and Ashwood, ١٩٩٩).

Normal values

٦ mon-١٥ year	٧- ١٤٠	ng/ml
Adult; Men	٢٠- ٢٥٠	ng/ml
Women	١٠- ١٢٠	ng/ml

٣-٢-١-٤-١-٢ Determination of Serum Iron:

Spectrophotometric method is used for serum iron determination. The kit was purchased from (Pars Azmun Company –Iran) (Thomas, ١٩٩٨). It

was based on comparing the color that develops, when the ferric iron released from transferrine protein is reduced to the ferrous state by the action of acid diluents and subsequent coupling of the reduced iron in serum with chromogen reagent (Nitro-PAPS) to form a colored complex and then absorbance of the latter compound is read in spectrophotometer at ٥٩٥ nm, setting the blank at zero, with that which develops from a standard solution (Muntzel, ١٩٩٢). The serum iron was calculated as follows;

$$\text{Serum iron } (\mu g/dl) = \frac{A^{\circ 95} \text{ test}}{A^{\circ 95} \text{ standard}} \text{ X standard conc. } (١٠٠\ \mu g/dl)$$

Normal values

٣-١٥ year	٢٢- ١٣٥	µg/dl
Adult; Men	٥٠- ١٤٠	µg/dl
Women	٤٠- ١٤٠	µg/dl

٣-٢-١-٤-١-٣- Determination of Serum TIBC:

Spectrophotometric method (Magnesium Carbonate Precipitating Method) is used for serum TIBC determination. The kit was purchased from (Darman kave Company –Iran) (Muntzel, ١٩٩٢). The serum iron was calculated as follows;

TIBC (µg/dl) = Serum iron (µg/dl) X ٣

UIBC = TIBC – serum iron

Normal values = ٢٣٠- ٤٤٠ µg/dl

٣-٢-١-٤-٢ Liver Function Test:

٣-٢-١-٤-٢-١ Determination of Serum Aspartate Transaminase (AST or GOT).

International Federation of clinical chemistry (IFCC) method is used for serum AST (GOT) determination. The kit was purchased from (Pars Azmun Company –Iran) (Bergmeyer *et al.,* ١٩٨٥).

Normal values

Adult; men	٥ - ٣٧	IU/L
Women	٥ - ٣١	IU/L

٣-٢-١-٤-٢-٢- Determination of Serum Alanine Transaminase (ALT or GPT):

International Federation of clinical chemistry (IFCC) method is used for serum ALT (GPT) determination. The kit was purchased from (Pars Azmun Company –Iran) (Bergmeyer *et al.,* ١٩٨٥).

Normal values

Adult; men	٥- ٤١	IU/L
Women	٥- ٣١	IU/L

٣-٢-١-٤-٢-٣- Determination of Serum Alkaline Phosphates (ALP):

P-Nitrophenyl phosphate method was used for serum alkaline phosphatase determination [EC٣.١.٣.١ (ALP), DGKC method] determination. The kit was purchased from (Pars Azmun Company –Iran).

Normal values

Adult; men	٨٠- ٣٠٦ IU/L
Women	٦٤- ٣٠٦ IU/L
٥-١٥ year	١٨٠- ١٣٠٠ IU/L

٣-٢-١-٤-٢-٤- Determination of Serum Total Bilirubin:

Dichloroanylin (DCA) method was used for serum total bilirubin estimation (Thomas ١٩٩٨). The kit was purchased from (Pars Azmun Company –Iran), in which albumin conjugated bilirubin reacts with DCA, to form a colored compound.

Normal values ٠.١- ١.٣ mg/dl

٣-٢-١-٤-٣ Determination of Serum Calcium ($Ca^{+٢}$):

Cresolphthalein complexon method was used for Serum Calcium determination (Burtis & Ashwood, ١٩٩٩ and Thomas, ١٩٩٨). This Kit was purchased from (Pars Azmun Company –Iran).

$$Ca^{+٢} + \text{O.Cresolphthalein} \xrightarrow[\text{Medium}]{\text{Alkaline}} \text{Ca-O. Cresolphthalein (Purple)}$$

Normal values ٨.٦- ١٠.٣ mg/dl

٣-٢-١-٤-٤-Determination of Serum Phosphorus:

A molybdate method was used for Serum phosphorus (phosphate) estimation (Annino and Giese, ١٩٧٧). This Kit was purchased from (Pars Azmun Company –Iran). Serum phosphorus reacts with molybdate by acid diluents to form colored complex.

Normal values ٢.٥- ٥ mg/dl

٣-٢-١-٤-٥ Determination of Glucose Tolerance Test (GTT):

The enzymatic or colorimetric methods, [Glucose Oxidase, (GOD-PAP methods)] were used for Serum glucose estimation (Thomas, ١٩٩٨). This Kit was purchased from (Pars Azmun Company –Iran).

Control and patients were fasted overnight for ١٢ hours; Blood samples were collected from them by intravenous method and analyzed for glucose level employing the glucose oxidase method. One gram of glucose per kilogram of body weight is given to each person. Then five blood draws are usually taken. One draw is in the fasting state, and then the next draws are at ٣٠ minute, ٦٠ minute, ٩٠ minute and ١٢٠ minute after drinking the glucola in order to measure the blood glucose values and to get curve. The form of the curve tells us a lot about the body's sugar metabolism (Thomas, ١٩٩٨).

٣-٣- Statistical Analysis:

All data are expressed as Means ± Standard Error Means (M ± SEM), statistical analysis of the obtained data was done according to independent-samples t-test, one way ANOVA, and Least Significant Difference Test (LSD) was

used to compare the means of no. of blood transfusion groups in the study and also correlation coefficient tests were used according to Daniel (١٩٨٣). The statistical analysis was carried out using statistically available software (SPSS version ١١.٥).

Patient's Chart (١)

Name;

Age; Sex;

Onset of blood transfusion;

Blood transfusion interval;

Frequency of blood transfusion;

No. affected in the family;

Parent relative; Yes No

Splenectomy; Yes No

Weight (kg);

Total Height (cm);

Spinal trunk height (cm);

Receiving chelation therapy; Yes ☐ No ☐ Irregularly ☐

Receiving Vit.C Yes ☐ No ☐ Irregularly ☐

Receiving Vit.D Yes ☐ No ☐ Irregularly ☐

Menstruation; Yes ☐ No ☐ Irregularly ☐

Cycle phase;

Follicular phase; ☐

Midcycle; ☐

Luteal phase; ☐

Haematological parameters;

Hormonal parameters;

Biochemical parameters;

RESULTS

٤-١- Blood Content:

٤-١-١- Hormonal Parameters:

The hormonal results are shown in Table (١). There is evidence of primary hypothyroidism as defined by a high baseline TSH (١٤.٣٤٢ ± ٣.٧٨٤ mIU/ml in male & ٦.٩٧٨ ± ١.٣٢٩ mIU/ml in female) and a low or normal $T_٤$ (٥.٨١٥ ±

٠.٣٩٢٩ ng/ml in male) in children with multi-transfused iron loaded thalassemia in comparison with those of the controls.

Independent-samples t-test analysis indicates that FSH value increases significantly (p<٠.٠٥) and testosterone level falls (p<٠.٠٠٠). In contrast, LH level changed no significantly in male thalassemic patients compared with those values in normal pre-pubertal and pubertal controls.

In girls with primary amenorrhea, statistical analysis estimate a significant decrease in LH level (p<٠.٠١) and estrogen level (p<٠.٠٥); However, FSH level remains within normal ranges.

Thalassemic children had growth retardation or short stature defined by significantly lower GH concentration (١.١٨ ± ٠.٢١٢١ µg/L) in male & (١.٤٢٦٣ ± ٠.٢٩٠٢ µg/L) in female than those for the controls (٢.٨٢ ± ٠.٣٢٧٧ µg/L) in male & (٢.٤٢ ± ٠.٢٩٣٩ µg/L) in female and also defined by height & weight below the normal subjects.

Correlations between circulating hormonal and serum ferritin concentrations for all the beta-thalassemic patients and healthy subjects are presented in Table (٢). Serum ferritin concentration correlated negatively with testosterone (p<٠.٠٥) in male, estrogen (p<٠.٠٥) in female and GH (p<٠.٠٥) in both sexes.

٤-١-٢- Haematological parameters:

The haematological results obtained from this study are summarized in Tables (٣, ٤, ٥a, ٥b, ٦a, ٦b).

٤-١-٢-١- Haemoglobin level

As the results show, a significant difference was found in the mean Hb level between the control group and the transfused thalassemic patients ($p<٠.٠٠٠$). No significant difference was found in the mean Hb level between splenectomized and non-splenectomized patients.

HbF and $HbA_٢$ levels were increased significantly ($p<٠.٠٠$) in both sexes of patients, while there is no significant effect of splenectomy on HbF and $HbA_٢$ level.

٤-١-٢-٢- Red Corpuscular parameters:

The results indicate that red cell count ($p<٠.٠٠٠$), haematcrite value or PCV ($p<٠.٠٠٠$), MCH ($p<٠.٠٠٠$) in female & ($p<٠.٠١$)in male and MCHC ($p<٠.٠٠$) were significantly decreased and RDW-CV increased ($p<٠.٠٠$), while MCV values were not changed significantly in thalassemic patients compared with control. On the other hand, no significant differences were found in the mean values of RBCs, HCT, RDW-CV, MCV, MCH and MCHC (in female)

between the splenectomized and non-splenectomized patients. The same change in male with exception that the MCHC is significantly decreased.

٤-١-٢-٣- Total and Differential Leucocytes Count:

It is seen in Table ٣,٤,٥a,٦a that disease significantly increased WBCs ($p<٠.٠٠$), lymphocyte % ($p<٠.٠٠٠$), lymphocyte absolute value ($p<٠.٠٠١$ in female and $p<٠.٠٢٣$ in male), eosinophil % ($p<٠.٠٠٨$ in male & $p<٠.٠١$ in female), eosinophil absolute ($p<٠.٠٠٠$ in female & $p<٠.٠٢٢$ in male) and monocyte absolute in female ($p<٠.٠١$) and significantly reduced neutrophil % ($p<٠.٠٠٠$), while monocyte % and neutrophil absolute value in both sexes and monocyte absolute in males were not changed significantly.

White blood cell counts ($p<٠.٠٠١$), neutrophil absolute value ($p<٠.٠١٣$ in males & $p<٠.٠٥$ in female), in male lymphocyte % ($p<٠.٠٢٥$), lymphocyte

absolute value ($p<٠.٠٢١$), monocyte absolute($p<٠.٠١$) and eosinophil absolute value ($p<٠.٠٠٤$) were increased and neutrophil % ($p<٠.٠٠٣$ in male) decreased significantly among splenectomized thalassemic patients, however the monocyte %, eosinophil %, in both sexes and neutrophil %, lymphocyte %, lymphocyte absolute, monocyte absolute value in female showed no significant changes.

٤-١-٢-٤- RBC Morphology:

The red blood cells showed the classical morphology of thalassemia major, like striking anisopoikilocytosis with many target cells and hypochromic cells table (٥b & ٦b).

Examination of blood smears of the control and thalassemic patient's shows a significant increase in the target cell %, microcytes % and hypochromic % (٠.٠٠٠) in both sexes. An appreciable number of patient's blood smears had shown macrocytosis. The red cell morphology was greatly disturbed after splenectomy with increasing target cell % (p<٠.٠٤٩ in male and p<٠.٠٠٠ in females), microcytes % (p<٠.٠٢٦ in female and hypochromic % (p<٠.٠٤٩ in females) while microcytes % and hypochromic % in male were not changed significantly between the splenectomized and non-splenectomized patients.

٤-١-٢-٥- Platelet Count:

The platelet counts in the whole blood of both splenectomized and non-splenectomized patients (p<٠.٠٠) were higher than those of healthy subjects. Patients with thalassemia showed significantly increased PDW (p<٠.٠١ in males and p<٠.٠٥ in female), MPV (p<٠.٠٣٧ in male) and P-LCR percentage (p<٠.٠٠١ in male and p<٠.٠٤ in female) compared with healthy subjects.

The splenectomized patients showed significant increase PLT count in both sexes and P-LCR (p<٠.٠٠٨) only in male, however, the PDW, MPV in both sexes and P-LCR in female was not significantly different from the non-splenectomized group.

٤-١-٢-٦- Reticulocyte Percentage:

As shown in Table (٥b & ٦b), there was a significant increase in reticulocyte (%) between multiple transfused thalassemic patients and control group, whereas reticulocytes (%) had no significant difference between the splenectomized and non-splenectomized patients.

٤-١-٣- Biochemical Parameters:

The results of some biochemical investigations are shown in Tables (٧a, ٧b, ٨a & ٨b) and figure (٥, ٦, ٧, and ٨)

٤-١-٣-١- Glucose Tolerance Test (GTT):

The fasting glucose was significantly higher in female, ($p<٠.٠١$) and slightly greater in male beta-thalassemia patients compared to controls. The oral glucose tolerance value was higher in female after ٣٠ min ($p<٠.٠٤٢$), ٦٠ min ($p<٠.٠٠١$), and ٩٠ min ($p<٠.٠٢١$), while in male only after ٦٠ min ($p<٠.٠١$) and ٩٠ min ($p<٠.٠٤٢$) with no significant changes in both sexes after ١٢٠ minute.

In males, splenectomy had no effect on fasting glucose and GTT, whereas female had significantly reduced in GTT value as shown in (Figures ٧ and ٨).

٤-١-٣-٢- Liver Function Test:

The results show that thalassemic children had significantly higher ($p<٠.٠٠٠$) concentrations of serum GPT, GOT, total bilirubin and alkaline phosphates ($p<٠.٠٠٣$ in females) compared with healthy subjects.

A significant difference was found in the mean SGOT, S. bilirubin and female SGPT and no difference was found in the mean alkaline phosphates and SGPT levels (in male) between the splenectomized and non-splenectomized patients.

٤-١-٣-٣- Serum Calcium and Phosphorous Concentration:

Patients with beta-thalassemia had significantly lower serum calcium ($p<٠.٠٤٤$ in male & $p<٠.٠١$ in female) and higher serum phosphorus concentration compared with healthy subjects. Splenectomy had no effect on serum $Ca^{+٢}$ & phosphorous.

٤-١-٣-٤- Iron Status:

In our study patients, serum ferritin and serum iron level increased (p<٠.٠٠٠) and TIBC reduced (p<٠.٠٠٠) significantly. On the other hand, only serum iron level changed significantly in splenectomized patients.

٤-١-٤- Clinical Parameters:

The results are summarized in Tables (٩ & ١٠). In boys, mean values for body weight were (٣٣.٥٢ ± ٢.٢٦١ Kg), total height was (١٤٤.٣١ ± ٣.٢٥ Cm) and spinal trunk height (٧١.١٥ ± ٢.٠١ Cm) and in girls weight (٣٩.٥٧ ± ٢.٨٢ Kg), height was (١٤٥.٧٨ ± ٢.٠٩ Cm) and spinal trunk height (٧٢.٢٦ ± ١.٠٩ Cm) significantly reduced in multi transfused patients compared with control group.

No significant difference was observed in body weight, total height in males, spinal trunk height and number of blood transfusion; however the transfusion interval per day increased and height in females decreased significantly in the splenectomized patients.

٤-٢- The correlation between serum ferritin and blood parameters:

٤-٢-١- The correlation between serum ferritin and haematological parameters:

The results of correlation between serum ferritin and haematological parameters are shown in Tables (١١, ١٢, ١٣ & ١٤).

٤-٢-١-١- Correlation with Haemoglobin Level:

In the thalassemic patients the serum ferritin concentration was correlated significantly (negatively) with the Hb, positively with male HbF and HbA_2, but no correlation was observed with HbF and HbA_2 in females.

A significant positive correlation was seen between s. ferritin level and Hb ($r = ٠.٥٢٦$, $p<٠.٠٥$) in splenectomized females & HbA_2 ($r = ٠.٦٨٢$, $p<٠.٠٥$) in non-splenectomized male patients.

٤-٢-١-٢- Correlation with RBCs Parameters:

There was negative correlation between s. ferritin and RBCs counts, HCT value, MCH value, MCHC value in both sexes and MCV value (in female) and correlated positively with RDW-CV only in male poly-transfused patients.

The s. ferritin was correlated significantly with RBCs count in splenectomized ($p<٠.٠٥$) and non-splenectomized ($p<٠.٠١$) females and with HCT ($p<٠.٠٥$) only in splenectomized females.

٤-٢-١-٣- Correlation with White Blood Cell Parameters:

In male patients with beta-thalassemia major, the s. ferritin correlated positively with lymphocyte % ($p<٠.٠١$), eosinophil % ($p<٠.٠٥$), absolute lymphocyte ($p<٠.٠٥$), absolute eosinophil ($p<٠.٠٥$) count and absolute monocytes ($p<٠.٠٥$) in both sexes; however, neutrophil % negatively

correlated with s. ferritin. On the other hand, s. ferritin was not correlated with WBCs count.

The s. ferritin correlated positively with eosinophil % ($p<٠.٠٥$) in male splenectomized patients and with monocyte% ($p<٠.٠٥$) in non-splenectomized females.

٤-٢-١-٤- Correlation with RBC Morphology:

In present study patients, serum ferritin concentration correlated positively with microcytes %, hypochromic % and target cell % (only in male). There was no correlation between s. ferritin and RBC morphology in splenectomized and non-splenectomized patients, except target cell % ($p<٠.٠٥$) in non-splenectomized female.

٤-٢-١-٥- Correlation with Platelet Parameters:

The results indicate that platelet count ($p<٠.٠١$), PDW ($p<٠.٠١$), MPV ($p<٠.٠١$) and P-LCR % ($p<٠.٠١$) were correlated positively with s. ferritin in male beta-thalassemia patients. There was only positive correlation between s. ferritin and PDW in non-splenectomized female patients.

٤-٢-١-٦- Correlation with Reticulocyte Count:

A significant positive correlation was seen between s. ferritin level and reticulocyte count ($r = ٠.٥٥٢$, $p<٠.٠١$ in males and $r = ٠.٤٢٧$, $p<٠.٠٥$ in females). On the other hand, s. ferritin was not correlated with reticulocyte count in splenectomized and non-splenectomized patients.

٤-٢-٢- The Correlation between Serum Ferritin and Biochemical Parameters:

Tables (١٥a, ١٥b, ١٦a and ١٦b) give the degree of correlation and their level of significance between s. ferritin and biochemical parameters.

٤-٢-٢-١- Correlation with Glucose Tolerance Test (GTT):

In females, serum ferritin levels have shown a significant positive correlation with the s. fasting glucose ($r = ٠.٨٠١$, $p<٠.٠٠١$), and with GTT after ٣٠ min., after ٦٠ min., after ٩٠ min. & ١٢٠ min. all at the level ($p<٠.٠١$) significance and in male, only after ٦٠ min. ($p<٠.٠٥$) correlated with s. ferritin.

In splenectomized female, s. fasting glucose ($p<٠.٠١$), GTT after ٣٠ min. ($p<٠.٠١$), after ٦٠ min. ($p<٠.٠٥$), after ٩٠ min. ($p<٠.٠١$) & ١٢٠ min. ($p<٠.٠١$) are correlate positively with s. ferritin while in male there is no correlation.

٤-٢-٢-٢- Correlation with Liver Function Test:

As shown in Tables (١٥a & ١٦a) some liver function tests affected with high serum ferritin concentration. In male, s. ferritin correlate positively with SGPT ($p<٠.٠١$), SGOT ($p<٠.٠١$) and s. total bilirubin ($p<٠.٠١$) but alkaline phosphatase did not correlate with s. ferritin and in male no positive correlation in splenectomized patients.

Results indicate that there was no significant correlation between s. ferritin and most liver function tests in splenectomized and non-splenectomized patients.

٤-٢-٣- Correlation with Serum Calcium and Phosphorus:

The present results show that s. ferritin in female beta-thalassemic children did not correlated with serum calcium and phosphorus value, but in male s. phosphorus ($p<٠.٠١$) correlated positively with s. ferritin, and there is no correlation in splenectomized and non-splenectomized patients.

٤-٢-٤- Correlation with Serum Iron Status:

The present study revealed that β-thalassemia major patients, had high s. ferritin level correlated positively with s. iron ($r = ٠.٩٢٢$ in male & $r = ٠.٤٠٤$ in female) and negatively with TIBC levels ($r = -٠.٨٥٧$ in male & $r = -٠.٦٣٦$ in female).

Tables (١٦a) show no correlation between s. ferritin and TIBC value, but there is positive correlation with s. iron in splenectomized ($r = ٠.٦٦٩$ in males & $r = ٠.٥٩٩$ in female) and non-splenectomized($r = ٠.٧٠٣$) in male patients.

٤-٢-٣- The Correlation between Serum Ferritin and Clinical Parameters:

The results of the present study are presented in Tables (١٧ & ١٨). The results indicated that s. ferritin correlated negatively with total weight ($p<٠.٠١$), total height ($p<٠.٠٥$) and spinal trunk height ($p<٠.٠٥$) in male, but

in female s. ferritin correlated negatively only with spinal trunk height (p<٠.٠٥).

Serum ferritin correlated significantly with weight (r = ٠.٥٢٤) and height (r = ٠.٥٢٩) in splenectomized females but in non-splenectomized males serum ferritin correlated positively only with weight (r = ٠.٧٠٤, p<٠.٠٥).

٤-٣-١- The Correlation between Height Spinal Trunk and Blood Parameters:

The results shown in Table (١٩) indicate that height spinal trunk in male beta-thalassemia major patients had a positive correlation with RBCs count (p< ٠.٠٥), Hb concentration (p<٠.٠١), HCT value (p<٠.٠١) and negative correlation with PDW value. On the other hand, height spinal trunk in female had a positive correlation with WBCs count (p<٠.٠١) RBCs count (p< ٠.٠١), Hb concentration (p<٠.٠١), HCT value (p<٠.٠١), MCH value (p<٠.٠٥), MCHC (p<٠.٠٥), in contrast, female had a negative correlation with HbA_2 (p<٠.٠١) and PLT count (p<٠.٠١).

٤-٣-٢- The Correlation between Height Spinal Trunk and Clinical Parameters:

The results shown in Table (٢٠) indicate that there are highly positive significant correlation between height spinal trunk and total weight (r = ٠.٧٩٧ in male & r = ٠.٨٦٢ in female) and height (r = ٠.٨٧٥ in male & r = ٠.٩٠٦ in female) in patients with beta-thalassemia major.

٤-٤- Effect of No. of Blood Transfusion on Iron Status:

The results shown in Table (٢١) indicate that in multi-transfused beta-thalassemic patient's s. ferritin level & s. iron concentration (only in males) increased significantly by increasing unit of blood transfusion {is divided into three groups (G١<٢٠٠, G٢=٢٠٠-٣٠٠, & G٣ >٣٠٠ unit)}. On the other hand, there were changes in s. iron (in female) and TIBC in both sexes but not significantly. Test of L.S.D showed significant difference between the effects of amount of blood transfusion groups on s. ferritin {group ٣ in male ($p<٠.٠٥$) and group ٢ in female ($p<٠.٠٠١$)} and on males s. iron in the third group ($p<٠.٠٠١$). Moreover there were significant differences between effects of two groups (G٢ & G٣) at s. ferritin level in both sexes and on s. iron only in males.

DISCUSSION

٥-١- Endocrine Complication:

Endocrine evaluation of ٣٨ patients (١٩ males & ١٩ females) with homozygous transfusion-dependent beta-thalassemia reveals that most patients have an endocrine abnormality of some form, including: Pubertal delay, growth retardation, short stature, primary hypothyroidism and diabetes, and suggests that these abnormalities are sufficiently common and that they should be suspected in all patients.

٥-١-١- Growth Complication:

The present results which are recorded in (table ١) demonstrate growth retardation, short statures and delayed pubertal development in patients with multi-transfused dependent beta-thalassemia major in Kurdish peoples. Growth retardation was evaluated according to the growth hormone deficiency, significantly reduced total body weight, total height and spinal trunk height compared with healthy subject. These results support the findings of Wonke *et al.,* (١٩٩٨); Shalitin *et al.,* (٢٠٠٥) and Rosa *et al.,* (٢٠٠٥) showing significant decrease in the GH level in patients with iron-overloaded beta-thalassemia major; Olivieri and Brittenham (١٩٩٧) found that poor pubertal growth had been observed in well-transfused patients and also Wu *et al.,* (٢٠٠٣) recorded GH deficiency, lower IGF-١ & IGFBP-٣ in β-thalassemic patients and the prevalence of GH deficiency was observed in ٧.٩٪ male and ٨.٨٪ female by (De Sanctis *et al.,* ٢٠٠٤). However, this study was in contrast

with the finding of Cavallo *et al.,* (١٩٩٧) who reported that growth retardation in poly-transfused beta-thalassemia patients is not GH dependent.

On the other hand, in this study, body weight, height, spinal trunk height are significantly reduced in patients compared with control group are shown in

(table ٨ & ٩) and this is in agreement with Jaafer (١٩٨٩); Nicoletti *et al.,* (٢٠٠١); Raiola *et al.,* (٢٠٠٣) and Louis (٢٠٠٥) and who have showed that the majority of patients had disproportionate short height with short trunk. Our findings differ somewhat from those of Rodda *et al.,* (١٩٩٥); Nicotti *et al.,* (١٩٩٨) and Low *et al.,* (١٩٩٨) who reported that short spinal trunk not correlated with short stature in blood transfused patients. Senanayake *et al.,* (١٩٩٩) and Shalitin *et al.,* (٢٠٠٥) indicated that height significantly decreased with elevated s. ferritin and with age and also in Baghdad city, Jassim (١٩٨٩) found that growth retarded with increase in the age of the patients. Whereas similar results were reported by Karamifar *et al.,* (٢٠٠٢); Asadi-Pooya *et al.,* (٢٠٠٤) and Karamifar *et al.,* (٢٠٠٥) observing a significant decrease in the weight with increasing the age of the children with homozygous β-thalassemia.

The causes of growth retardation of children with beta-thalassemia are multi-factorial; thus, one of these factors may be growth hormones deficiency which may play a large role in the growth failure resulting short stature of these patients (Oerter *et al.,* ١٩٩٣) mentioned that Patients with beta-thalassemia have a defective GH-IGF١-IGFBP-٣ axis that might be secondary to haemosiderosis of the pituitary gland, liver and pancreas. This is in agreement with the results of present study in finding a negative correlation between s. ferritin and GH levels and steroids can influence growth through the modulation of IGF-١ induced cellular response and their deficiency causes growth delay and osteoporosis of thalassemic children. This may explain the

relatively short upper segment and vertebral changes observed in our thalassemic patients (Soliman *et al.,* ١٩٩٨; Cavallo *et al.,* ١٩٩٩ and Louis, ٢٠٠٥).

Growth retardation could also be due to toxic effects of desferoxamine through uncontrolled serum ferritin levels causing damage to hypothalamic-pituitary axis (Arcasoy *et al.,* ١٩٩٩ and Saxena, ٢٠٠٣). The mechanism by which iron interferes osteoid maturation and mineralization may be explained by the incorporation of iron into crystals of calcium hydroxyapatite, which consequently affects the growth of calcium hydroxyapatite crystals and increases osteoid in bone tissue, these changes lead to ineffective haemopoiesis with progressive marrow expansion, and additional genetic factors, such as the COLlA١ gene polymorphism, which seem to play an important role in the development of low bone mass in these patients. The diminished osteoblast function is accompanied by a comparable increase in osteoclast activity, leading to bone loss and osteoporosis; on the other hand testosterone has a direct stimulatory effect on osteoblast proliferation and differentiation (Mahachoklertwattana *et al.,* ٢٠٠٣; Voskaridou and Terpos ٢٠٠٤ and Shalitin *et al.,* ٢٠٠٥).

Other factors such as chronic anemia caused stunting effect on both long and trabecular bone and hence body disproportion and increased the amount of circulating free haemoglobin may inhibit cartilage growth, chronic liver disease and hypersplenism and also malnutrition can inhibit growth through inhibition of IGF-١ and IGFBP-٣ synthesis and insulin release (Saxena ٢٠٠٣ and Karamifar *et al.,* ٢٠٠٥).

٥-١-٢- Gonads Complication:

In the present study gonadotropin level and sex hormones levels were appropriate for the delayed pubertal stage in almost all patients. This prospective study confirms the previous case reports of s. steroid hormones level deficiency in multi-transfused patients (De Sanctis *et al.,* ١٩٨٨; El-Hazmi *et al.,* ١٩٩٤ and Yazigi *et al.,* ٢٠٠٢) who show a decrease in LH, testosterone and estrogen levels in iron overload thalassemic patients and also supports findings of Soliman *et al.,* (١٩٩٨) observed consequent deficiency of sex steroid hormones of the patients.

On the other hand, male patients had slightly high FSH levels which suggest early gonadal failure and finding in accordance only with Oerter *et al.,* (١٩٩٣). In contrast, the findings differ somewhat from those of De Sanctis *et al.,* (١٩٨٨) and Yazigi *et al.,* (٢٠٠٢) indicating a decrease in FSH level and LH level in thalassemic patients.

The data demonstrate that s.ferritin concentration correlated negatively with steroid hormones, whereas there is no correlation with FSH and LH levels. This result supports the findings of El-Hazmi *et al.,* (١٩٩٤); Shamshiraz *et al.,* (٢٠٠٣) and Shalitin *et al.,* (٢٠٠٥) they showed testosterone, estrogen and LH negatively correlated with s.ferritin concentration in poly-transfused patients compared with normal subjects.

The delay in the onset of puberty in patients with multi-transfused dependent could be the result of iron deposition in either the hypothalamic-pituitary portion of the reproductive axis, the gonads, or both. Gonadotrophins are the most sensitive hormones to iron toxicity and there was evidence of iron deposition as defined by elevation of total iron state level in serum (Landau *et al.,* ١٩٧٨).

The precise mechanism whereby iron overload causes tissue damage is evidence of free radical formation and lipid peroxidation resulting in mitochondrial lysosomal damage (Karamifar *et al.,* ٢٠٠٥), and generation of ROS from iron overload is likely to be the most logical explanation for the etiology of gland damage (Perera *et al.,* ٢٠٠٢). Owing to the selective abnormality with deficiency restricted to the gonadotrophins, it is difficult to explain our findings solely on the basis of iron deposition in the pituitary gland or hypothalamus. More than likely other factors are responsible for organ damage, such as chronic anemia, malnutrition, hepatic dysfunction, zinc deficiency and increased collagen deposition secondary to increased activity of iron-dependant protocollagen proline hydroxylase enzyme (Landau *et al.,* (١٩٧٨) and Karamifar *et al.,* (٢٠٠٥). Diminished amounts of antioxidants vit.E and vit.C in the serum of patients with thalassemia syndrome have been reported by De Sanctis *et al.,* (١٩٨٨) and genotoxic drugs may cause DNA damage Perera *et al.,* (٢٠٠٢).

٥-١-٣- Thyroid Complication:

There was evidence of subclinical hypothyroidism as defined by a high baseline TSH and a normal or low T٤ in Kurdish patients with poly-transfused thalassemia major in comparison with those of the control group are shown in (table ٢) and on the other hand, s. ferritin correlated positively with TSH level in the present study. The other investigators found similar results Agarwal *et al.,* (١٩٩٢); Al-Hader *et al.,* (١٩٩٣); Oerter *et al.,* (١٩٩٣) and Shalitin *et al.,* (٢٠٠٥) who showed that the TSH level was increased obviously but mean concentration of T٤ showed normal value in iron overload thalassemia patients and in contrast to the findings of Yazigi *et al.,* (٢٠٠٢); Zervas *et al.,* (٢٠٠٢) and Shamshiraz *et al.,* (٢٠٠٣) they observed that there was no evidence of

hypothyroidism and no correlation between s. ferritin levels and thyroid status in mild iron overload children.

It has been proposed that iron overload, chronic anemia, and tissue damage by hypoxia may be detrimental to the thyroid tissue and cytokines, especially interleukin-٦ (IL-٦), which has been reported to be produced into the thyroid gland by the thyroid epithelial cells, and has various effects on thyroid function, are potential candidates in β-thalassemia (Bartalena *et al.,* ١٩٩٥).

٥-٢- Haematological Parameters:

These parameters were much affected by repeated blood transfusion, since most of the transfused patients had their bone marrow suppressed and had disrupted normal mechanism for control of erythropoiesis and it seems that bone marrow doesn't respond to erythropoietin, therefore, haematological data did not reflect the true haematologic picture of the patients (Weatherall, ١٩٩٧).

٥-٢-١- Hb Levels:

The results showed that thalassemia, leads to moderate to low Hb level are shown in (table ٣). These results were in agreement with other findings Jaafer, (١٩٨٩); Rashid, (١٩٩٨); Clarke and Higgins (٢٠٠٠) who showed significant decrease in Hb level in B-thalassemia patients. This abnormality may be due to point mutation in β-chain gene which are responsible for decreased or absent β-globin synthesis, which result the production inadequate amounts of HbA. As a result, a vast excess of α-globin accumulates and usually associates with heme to form hemoglobin.

Possessing no stable molecular configuration, α-hemoglobin aggregates and precipitates in early hemoglobin-producing in the bone marrow, which leads to apoptosis of these cells and ineffective erythropoiesis (Clarke and Higgins, ٢٠٠٠ and Bank, ٢٠٠٥) and also Khider, (١٩٨٦) who reported no difference in Hb levels between males and females.

Splenectomy has not affected the total Hb level, neither on the HbF % and Hb A_2, since splenectomy is lower the transfused requirements of transfusion dependent thalassemics (Alan *et al.,* ١٩٨٠).

On the other hand, the results indicate a significant increase in Hb F and HbA_2 level in our patients, these are similar to previous findings Khider, (١٩٨٦); Rashid, (١٩٩٨) and Rees *et al.,* (١٩٩٩) they found obvious increase in HbF and HbA_2 levels especially in non-transfused than transfused patients, multi-transfusion inhibit erythropoiesis activity. The elevated HbF and HbA_2 level due to excess production of γ and δ chain compensates for beta-chain loss (Powers, ١٩٨٩).

In addition, there is negative correlation between s. ferritin concentration and Hb levels and positive correlation with HbF and HbA_2 in males and no correlation in females. This result differs from those obtained by Khider, (١٩٨٦); and Jaafer, (١٩٨٩) who showed no correlation between s. ferritin and Hb level, HbF % and HbA_2 % in Mosul and Baghdad respectively, the lower levels of HbF % and HbA_2 % may suggest that multiple transfusions from an early age lowers HbF % and HbA_2 % levels. Also there are two possible interpretations of this finding; first, it is possible that the regular blood transfusion itselves leads to a relative reduction in γ-globin synthesis via reducing serum Transferrin Receptor (sTfR) and erythropoietin levels and inducing erythroid suppression (Rees *et al.,* ١٩٩٩). The second, possibility is

that the repeated blood transfusions result in iron overload. Therefore, excess iron accumulates in reticuloendothelial cells and paranchymal cells of bone marrow and causes tissue damage by generating free radicals that destroy intracellular organelles, DNA and cellular membranes (Ng *et al.,* ٢٠٠٥).

٥-٢-٢- RBCs Indices and Morphology:

The present results show that RBCs count, PCV%, MCH, and MCHC value are reduced significantly and RDW-CV value is increased. This is in agreement with the results of other studies in β-thalassemia children (Rashid, ١٩٩٨) who showed a decrease in RBCs count and PCV% and also (Akar and Gokge, ٢٠٠٢ and Meral *et al.,* ٢٠٠٢) who reported reduction in MCHC, MCH, PCV% and high RDW values. The red cells that reach the peripheral blood also contain excess α-globin; this causes the formation of inclusion bodies and an increase in reactive oxygen species (ROS) levels, which leads to membrane damage and causes these cells to be preferentially hemolyzed (Olivieri, ١٩٩٩ and Bank, ٢٠٠٥). The increase in RDW-CV value was incontrast to that reported by (Clarke and Higgins, ٢٠٠٠) who showing no change in RDW value which may be due to produce a uniform microcytic red cell population without a concomitant increase in RDW.

On the other hand, splenectomy had no affect on the mean values of RBC, PCV %, MCV, MCH and RDW. This is similar to the finding of Jaafer, (١٩٨٩) who investigated no change in RBC indices, RBC counts and PCV % between splenectomized and non-splenectomized patients, although splenectomy produces alteration in the morphology of the red cells.

The results showed a negative correlation between s. ferritin and RBCs count, PCV %, RBC indices and positive correlation with RDW values and with

red cell morphology abnormality. As iron loading progresses, the capacity of serum transferring, to bind and detoxify iron may be exceeded and a non-transferrin-bound fraction of plasma iron may promote the generation of free hydroxyl radicals, propagators of oxygen-related damage (Olivieri, ١٩٩٩).

The morphological abnormalities of the red cells were classical are shown in (table ٥b); they show a significant increase in microcytic percentage (%), hypochromic cell %, target cell % and adequate increase in macrocytic cell due to inadequate folate and Vit.B١٢ supply, as the needs for the latter are increased in these patients due to continued haemolysis (Vatanavicharn *et al.,* ١٩٧٩ and Weatherall, ١٩٩٧). Similar results were also obtained by Weatherall and Clegg, (١٩٧٢) and Sears *et al.,* (٢٠٠٣).

Effects of splenectomy on red cells morphology in splenectomized patients markedly increased target cell %, hypochromic cell %, microcytic % and other types of anisopoikilocytes are shown in (table ٦b) and this was in accordance with previous studies Tillmann & Schroter, (١٩٧٩) and Khider, (١٩٨٦). These changes are attributed to the loss of the pitting mechanism of the spleen which is responsible for removed of inclusions in the red cells during their temporary sequestration in the spleen or by decreasing deformability of erythrocytes via the presence of inclusions bodies as well as by irregular cell shape, decreased flexibility of membranes and diminished intracellular haemoglobin fluidity, which result in rigidity of erythrocyte in splenectomized patients (Tillmann & Schroter, ١٩٧٩).

٥-٢-٣- Reticulocyte:

A significant increase in reticulocyte% in multi-transfused patients was in accordance with other reports by Dacie, (١٩٨٨) that showed moderate

reticulocytosis (٢-٧ %) indicating that the endogenous bone marrow production of red cells is not suppressed and reticulocyte reaching the peripheral circulation. But, in contrast with the findings of Rees *et al.,* (١٩٩٩) and Tancabelic *et al.,* (١٩٩٩) indicating that erythropoiesis was more completely suppressed.

There were no changes in reticulocyte% between splenectomized and non-splenectomized patients; this finding was in contrast to those reported by other workers (Sood, ١٩٨٥ and Jaafer, ١٩٨٩) that showed a significant increase in reticulocyte% in splenectomized patients. This result may be due to the decrease in sequestration and destruction of the patient young red cells in the splenic pulps and sinuses.

There is a positive correlation between s. ferritin level and reticulocyte count and was in contrast to Khider, (١٩٨٦) who reported no relation, indicating that the bone marrow was not affected by iron overload induced from repeated blood transfusion.

٥-٢-٤- White Blood Cells:

A leukocytosis, agranulocytosis and neutropenia were observed in the β-thalassemia patients compared with the control group and this was in accordance with other reports (Bouroncle and Doan, ١٩٦٤) who showed neutropenia might result from hypersplenism. Sood, (١٩٨٥) and Speer *et al.,* (١٩٩٠) showed lymphocytosis. This phenomenon reflects a non-specific stimulation of the antibody-producing cells by repeated blood transfusions. And it was in contrast to the findings of (Rashid, ١٩٩٨) reporting that no increase in WBC count in patients.

In splenectomized patients, increase in WBC count, lymphocytosis and neutropenia was in agreement with other results (Piga *et al.,* ٢٠٠٥ and) showing development of neutropenia in splenectomized patients and (Cohen *et al.,* ٢٠٠٣ and Hodge *et al.,* ١٩٩٩) who demonstrated that there was highly significant absolute lymphocytosis in splenectomized patient compared with non-splenectomized. They suggest that splenectomy is not the sole determinant of lymphocytosis. The changes in lymphocytes may reflect the effect of cytokines produced during blood storage together with stimulation by minor incompatibility red cell antigens.

On the other hand, s. ferritin is not correlated with WBC count but correlated positively with lymphocyte, eosinophil indices, and absolute monocytes and negatively correlated with neutrophil%. These changes indicate that the ineffective erythropoiesis present in β-thalassemia is the result of an increased rate of apoptosis of the marrow erythroid cells. Transfusion leads to iron overload and also to immune derangements, both of which exert a negative effect on the functional integrity of the immune system. There are a large number of immune abnormalities in thalassemia, namely defective function of polymorphonuclear neutrophils and monocytes, decrease of $CD_{٤}^{+}$ cells and increase of $CD_{٨}^{+}$ cells, diminished mitogen responses and low natural killer cell activity. It is well established that the production of cytokines from cells of the immune system {IL-١, tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ)}, plays a major role in the generation of cardinal manifestations of acute infections, like fever and anaemia (Salsaa and Zoumbos, ١٩٩٧).

٥-٢-٥- Platelet Counts:

The platelet counts in the whole blood of our thalassemic patients were higher than those in the blood of healthy subjects. This finding is in agreement with the results obtained by Unchern *et al.,* (٢٠٠٣) they found higher PLT counts and MPV in thalassemic patients. This result reflects a weak response of thalassemic platelets to aggregation. This defects may be restricted to Vit.C and Vit.E deficiency, or membrane abnormality, iron overload, presence of iso-antibodies in patients' plasma, resulting from previous repeated blood transfusion or/and enhanced platelet consumption which in turn stimulates erythropoiesis (Elder, ١٩٧٨ and Hussain *et al.,* ١٩٧٩). The significant increase in the number of PLT count and P-LCR value in splenectomized patients was in accordance with previous studies such as Coacci *et al.,* (١٩٧٦); Khider, (١٩٨٦); Elder and Rachmilewitz, (٢٠٠٢) and Canatan *et al.,* (٢٠٠١) who also reported moderate to high platelet counts in splenectomized β-thalassemic patients due to release storage platelet into circular system.

Serum ferritin in males correlated positively with PLT counts, MPV, PDW and P-LCR values and only with PDW in females, since iron accumulation as a result of repeated blood transfusion leads to bone marrow complication.

٥-٣- Biochemical Parameters:

٥-٣-١- Fasting Serum Glucose and Oral Glucose Tolerance Test (OGTT):

In this study, the fasting s. glucose and mean s. glucose level in OGTT were significantly different in β-thalassemia patients in comparison with controls and this was in agreement with other studies dealing with thalassemic patients (El-Hazmi *et al.,* ١٩٩٤ and Khalifa *et al.,* ٢٠٠٢) who reported that the prevalence of diabetes and IGT in the majority of these patients was due to direct impairment of insulin excretory function by the chronic iron overload.

Dmochwski *et al.,* (١٩٩٣) and Monge *et al.,* (٢٠٠١) demonstrated an evidence of immune system activation against pancreatic beta-cells. They proposed that pancreatic iron deposition may, though oxidative damage, act as an environmental factor that triggers the autoimmune response which, in turn, contributes to selective beta-cell damage. Labropoulou-Karataza *et al.,* (١٩٩٩) suggested that the interplay of iron burden and hepatitis C may be the determining factor in the development of abnormal GTT, because iron-induced hepatic damage is exacerbated by infection with the hepatitis C virus; hepatic dysfunction may be the most important cause for the development of insulin resistance and abnormal glucose tolerance.

In the present study, the serum ferritin level was higher in patients who developed abnormal GT and at the same time, the fasting plasma glucose and ٢h post load plasma glucose showed positive correlation with serum ferritin levels in females and in males at ٦٠ & ٩٠ min. after the oral glucose administration was in accordance with previous studies (Dmochowski *et al.,* ١٩٩٣; El-Hazmi *et al.,* ١٩٩٤; Khoshnyat *et al.,* ٢٠٠٣ and Khalifa *et al.,* ٢٠٠٤) they demonstrated that fasting s. glucose and GTT showed positive correlation with serum ferritin level in multi-transfused patients. Fernandez- Real *et al.,* (٢٠٠٢) and Jehn *et al.,* (٢٠٠٤) they suggested that the iron influences glucose metabolism. Iron in a potent pro-oxidant that increases the cell oxidative stress, causing inhibition of insulin internalization and actions, results in hyper-insulinemia and insulin resistance. Free iron also exerts a positive feedback on ferritin synthesis, while oxidative stress increases the release of iron from ferritin. Increase of oxidative stress and insulin resistance cause endothelial and tissue damage. Protein glycation, as seen in diabetes, further amplifies these abnormalities stimulating iron released from transferring, increasing the cell oxidative stress and directly causing endothelial and tissue damage.

٥-٣-٢- Liver Function Test:

In this study we report that thalassemic children had higher concentration of serum GPT, GOT, total bilirubin and alkaline phosphatase (in females) in comparison with the healthy subjects. Our results are consistent with the previous studies (Gangemi *et al.,* ١٩٨٦; Soliman *et al.,* ١٩٩٨ and Tchakurova *et al.,* ٢٠٠٣) who have shown a high level of serum transaminases and serum total bilirubin in poly-transfused patients and also (Champe *et al.,* ٢٠٠٥) suggested that the elevation of s. total bilirubin which results from hepatocellular damage may be due to iron overload causes hepatocyte necrosis and fibrosis and multiple transfusion caused repeated episodes of hepatitis that tend to become chronic or/and lead to decrease the hepatic conjugation and excretion of bilirubin, therefore chronic liver disease reflect in our patients. Other researchers (Saberi *et al.,* ١٩٩٦ and Wanless *et al.,* ٢٠٠٢) had shown that chronic hepatitis occurred very frequently in children with transfusion dependent thalassemia in the other area.

In the present study, we observed a positive association between elevated s. ferritin and s. transaminases levels and s. total bilirubin. This finding was in agreement with the investigating of other reports (Worwood *et al.,* ١٩٨٠ and Jaafer, ١٩٨٩) who showed that there was a significant correlation between s. ferritin and s. aminotransferase and s. total bilirubin in homozygous β-thalassemia; and also similar findings were reported by Chapmen *et al.,* (١٩٨٢).

There was evidence of iron overload in thalassemic patients. This relationship is probably due to the increasing release of stored intracellular ferritin with increased iron stores and increasing severe liver damage, but may reflect diminished uptake of circulating ferritin by damaged hepatocytes, as

the liver is normally responsible for the clearance of s. ferritin (Champe *et al.,* ٢٠٠٥). In acute and chronic liver disease, s. ferritin correlated with liver function, the circulating level depends on both the activity of hepatocellular damage and on the paranchymal iron stores.

A highly significant value for SGOT, SGPT (in females) and s. bilirubin were found in the splenectomized patients compared to the non-splenectomized ones. This may represent more liver damage in the splenectomized patients due to more iron deposition in hepatocytes with consequent necrosis and fibrosis. These findings had been documented by other workers who showed that after splenectomy more iron derived from transfusions will be stored in the kupffer cells in the liver. These cells will, therefore, become more rapidly overloaded, so that any additional iron will be added to the serum ion compartment, and larger amounts of iron will now be deposited in the hepatocytes, leading to liver cell damage and eventually to cirrhosis (Hershko *et al.,* ١٩٧٣ and Okon *et al.,* ١٩٧٦).

٥-٣-٣- Serum Calcium and Phosphorous Concentration:

Patients with homozygous β-thalassemia had low s. calcium, higher phosphorous and high alkaline phosphatase in comparison to control. These findings were in agreement with the findings of the previous studies on calcium homeostasis in β-thalassemia patients (Yazigi *et al.,* ٢٠٠٢) in Lebanese-children (Aleem *et al.,* ٢٠٠٠) in Saudia Arabia (Soliman *et al.,* ١٩٩٨) in Egypt children. The $Ca^{+٢}$ metabolism is affected by a number of hormones, such as PTH, thyroxine, oestrogen, cortisol, insulin, and calcitonin, as well as vitamin D; all these are involved in the regulation of bone metabolism, affecting both progenitors and mature osteoblastic cells and osteoclasts. High levels of PTH, thyroxine, cortisol, and reduced levels of oestrogen, testosterone, vitamin D,

calcitonin, and insulin accelerate bone loss, stimulating osteoblastic and osteoclastic activity (Griffin and Ojeda ٢٠٠٠; Ganong, ٢٠٠٥; Guyton and Hall, ٢٠٠٦) and a high deferoxamine dosage has been indicated as the main factor of cartilage and ione homeostasis alteration (Hatori *et al.*, ١٩٩٥).

In present study, there was no obvious correlation between s. ferritin and calcium metabolism. Similar results were reported by other workers; (Aleem *et al.,* ٢٠٠٠) showed no association between s. ferritin levels and degree of hypocalcaemia. Multi-center study (١٩٩٤) found that thalassemic patients had endocrine complications, with a low s. ferritin level. Thus it is very reasonable to believe that there are other possible factors as well responsible for organ damage, including individual sensitivity to iron damage, increased collagen deposition secondary to increased activity of the iron-dependent protocollagen proline hydroxylase enzyme, with subsequent disturbed microcirculation in the parathyroid, pancreas and chronic anemia.

٥-٣-٤- Iron Status:

In the multi-transfused patients, serum ferritin, s. iron level increased and TIBC decreased significantly compared to the normal children and these are similar to other reports (Saraya *et al.*, ١٩٨٥; Herbert *et al.,* ١٩٩٧; Argyropoulou *et al.*, ٢٠٠٠; Meral *et al.*, ٢٠٠٠ and Mahachoklertwattana *et al.,* ٢٠٠٣) They indicated that the probability of iron overload observed in beta-thalassemia was similar in transfused and non-transfused case. Iron overload develops when there is an intrinsic defect in the regulation of iron absorption such as in hereditary haemochromatosis, but commonly, it is due to medical interventions, such as hemolytic anaemia. However, repeated blood transfusions result in iron overload because the human does not have the

physiological means of excreting iron, resulting in an increase in the total iron stores (Hollan, ١٩٩٧).

On the other hand, Hershko *et al.,* (١٩٨٨) and Hershko *et al.,* (١٩٩٨) had shown that iron loading progresses the capacity of s. transferrin, to bind and detoxify iron may be exceeded and a non-transferrin bound fraction of plasma iron may promote the generation of free hydroxyl radicals, propagators of oxygen-related damage.

There was a strong positive correlation between the level of serum ferritin and the number of blood transferrins received, s. iron and transferring saturation. The only well-defined function of ferritin is the storage and detoxification of interacellular non functional iron (Ponka, ١٩٩٩). These finding are in agreement with the findings of other researchers, Worwood *et al.,* (١٩٨٠); Herbart *et al*., (١٩٩٧); Meral *et al.*, (٢٠٠٠) and Li *et al.,* (٢٠٠٢) who have shown that similar results present in homozygous beta-thalassemia, and also Ponka, (١٩٩٩) had shown that plasma contains transferrin completely saturated with iron in patients with severe iron overload. It is obvious that thalassemic patients in our locality are iron overloaded and that this load increases with transfusion and age and since iron overload is the major limiting factor for the survival of these patients, chelation therapy is an important factor in the management of iron overload. In this study most of them had received chelation therapy irregularly.

Serum iron level in splenectomized thalassemia increased significantly. This result was in agreement with Khider's, (١٩٨٦) finding who have shown that iron load increased after splenectomy. It may be gives a partial protection against transfusional siderosis by providing a relatively safe site for storage of excessive iron.

٥-٤- Splenectomy in β-thalassemia Patients:

Patients had been splenectomized at various ages in our studies. The main inductions for splenectomy were progressive increase in transfusion requirement and the sheer size of the organ causing mechanical discomfort. These represent the same indications for splenectomy in thalassemic patients reported elsewhere Hoffbrand *et al.,* (٢٠٠٣) and Rees *et al.,* (١٩٩٩) who have shown that splenic enlargement is associated with anemia and iron overload induced by blood transfusion by an enhanced capacity of the enlarged spleen for pooling, sequestering and destroying blood cells and also leading to an increased plasma volume.

Therefore, the main causes for the enlargement of both liver and spleen in the patients understudy were probably iron overload from both external and internal sources, as well as the extramedullary hemopoiesis which progresses with years (Pearson and Brien, ١٩٧٥).

Table (١٧) Correlation between serum ferritin and clinical parameters in patients with β thalassemia major and control group

Clinical Parameters			Weight kg	Height cm	Height Spinal trunk cm	No. of blood transfusion ٤٥٠ ml	Transfu Interv day
erum rritin ng/ml	Non splenectomy n=١٠	r	٠.٧٠٤	٠.٥٥٣	٠.٦١٨	٠.٣٨٦	٠.٠١
		P	.٠٥ ٢٧	N.S	٢٧N.S	N.S	٢٧ N.S
	Splenectomy n=٩	r	٠.٥٨٥ ٠.٠١	٠.٣٦٧	٠.٣٣٦ ٠.٠٥	- ٠.٤٨٨	- ٠.٢ ٠.٠٥
		P	N.S	N.S	N.S	N.S	N.S
Serum ferritin ng/ml			- ٠.١٩٧	- ٠.١٢٤		- ٠.١٨١	
	n		٢٧	٢٧		٢٧	
P-value			N.S	N.S		٠.٠٥	

erum rritin ng/ml	Non splenectomy n=٣	r	- ٠.٤٣٤	٠.٤٣٤	-٠.٥٦٣	- ٠. ٣٤١	- ٠.٤
		P	N.S	N.S	N.S	N.S	N.S
	Splenectomy n= ١٦	r	٠.٥٢٤	٠ .٥٢٩	٠.٣٧٥	-٠.١١٤	- ٠.٣
		p	٠.٠٥	٠.٠٥	N.S	N.S	N.S

Table (١٨). Correlation between serum ferritin and some clinical parameters in splenectomized and non-splenectomized patients with β thalassemia major

CONCLUSIONS

The results of present study show that Kurdish beta-thalassemic children are shorter and less sexually mature than their counterparts and have multiple hormonal deficiencies.

١- Some hormonal deficiencies have been described in thalassemic children, as a result of hemochromatosis, the most common being abnormalities of the hypothalamic-pituitary-gonadal axis, resulting in

a- A significant number of children with probable hypogonadotrophic hypo-gonadism, caused by gonadotrophic dysfunction (Low LH level and slightly high in FSH values) and low testosterone level in boys and low estradiol level in girls.

b- Depressed GH values in both sexes.

c- Evidence of primary hypothyroidism, characterized mostly by high levels of TSH and a decrease in thyroid hormones concentration.

d- High serum ferritin concentration was significantly correlated with low testosterone levels in boys, low estradiol in girls, low GH and high level TSH in multi-transfused patients.

e- Patients with thalassemia are at risk for osteoporosis. That is indicated by low serum calcium, phosphorous and with evidence of endocrinopathy.

f- Spinal growth impairment was associated with short stature in thalassemic patients and also correlated with total body weight and with some other haematological parameters such as Hb, RBC, and HCT.

٢- Abnormal glucose tolerance is common in multi-transfused thalassemics, indicated by high level of fasting serum glucose concentration and ٢hr OGTT in patients compared to normal subjects.

٣- Sever anaemia was characteristically hypochromic, microcytic with marked anisopoikilocytosis and some normoblastaemia. An appreciable number of patients had shown macrocytosis; which may be due to inadequate folate supply.

٤- Haemoglobin F and $HbA_{٢}$ levels were high in both sexes patients; it could be measured by alkaline denaturation technique and electrophoretic cellulose acetate paper, respectively.

٥- All patients were iron loaded as indicated by the high serum ferritin levels.

٦- Serum ferritin and s. iron were increased and TIBC was decreased in these patients. Positive correlation was also found between s. ferritin levels and s. iron levels or negatively with TIBC.

٧- In multi-transfused beta-thalassemic patient's s. ferritin level increased significantly by increasing the unit of blood transfusion.

٨- Splenectomy

a- Has increased the white blood cell and the platelet counts; it has also increased red cell distortion and normoblastaemia.

b- Lowered the transfusional requirements.

c- Raised the serum iron levels, but serum ferritin level showed no significant difference between splenectomized and non-splenectomized patients.

RECOMMENDATIONS

١- The results suggest that all patients with thalassemic and growth failure need periodic careful evaluations; it was important for us to evaluate their hormones status, in order to offer them an optimal treatment at any age when body constitutes their capital for a healthy growth development in the future.

٢- The routine protocol management of the patients has to be improved. This includes the following points:

a- Increase blood transfusion and desferal should be given in proper dose to the patients, with s.c infusion pump and Vit.C, in order to decrease tissue haemochromatosis and hypoxia.

b- Hepatitis B. vaccine should be given to those patients who are negative for HBs. Ag.

c- Splenectomy and use pneumoccocal vaccines in patients who scheduled for splenectomy.

d- The regular use of folic acid, Vit.$B_{١٢}$ and Vit.D.

e- Bone marrow transplantation.

f- The introduction of super-transfusion with neo-cytes (young red cells), might be effective in decreasing the rate of iron accumulation in homozygous beta-thalassemia.

٣- Prevention of the disease should be the sole aim through :

a- Prenatal diagnosis is very important to the couples at risk of disease and to provide choices for abortion for the couples.

b- Premarital examination should be done routinely especially for relative parents.

c- The evolution of β-globin mutations in the Iraqi populations, especially in the Kurdish region, by using polymerase chain reaction (PCR) which is very important. Since, when the range of molecular defects within an affected population was known, it became possible to combine this approach with first trimester prenatal diagnosis. Such programs have greatly reduced the incidence of β-thalassemia in various populations (Alter, ١٩٩٠).

d- The periodic determination of serum ferritin to evaluate the iron status in our patients.

٤- Health education of these families in order to increase their knowledge about the disease and explain to them that the disease is incurable and it is better not to have another child with the disease.

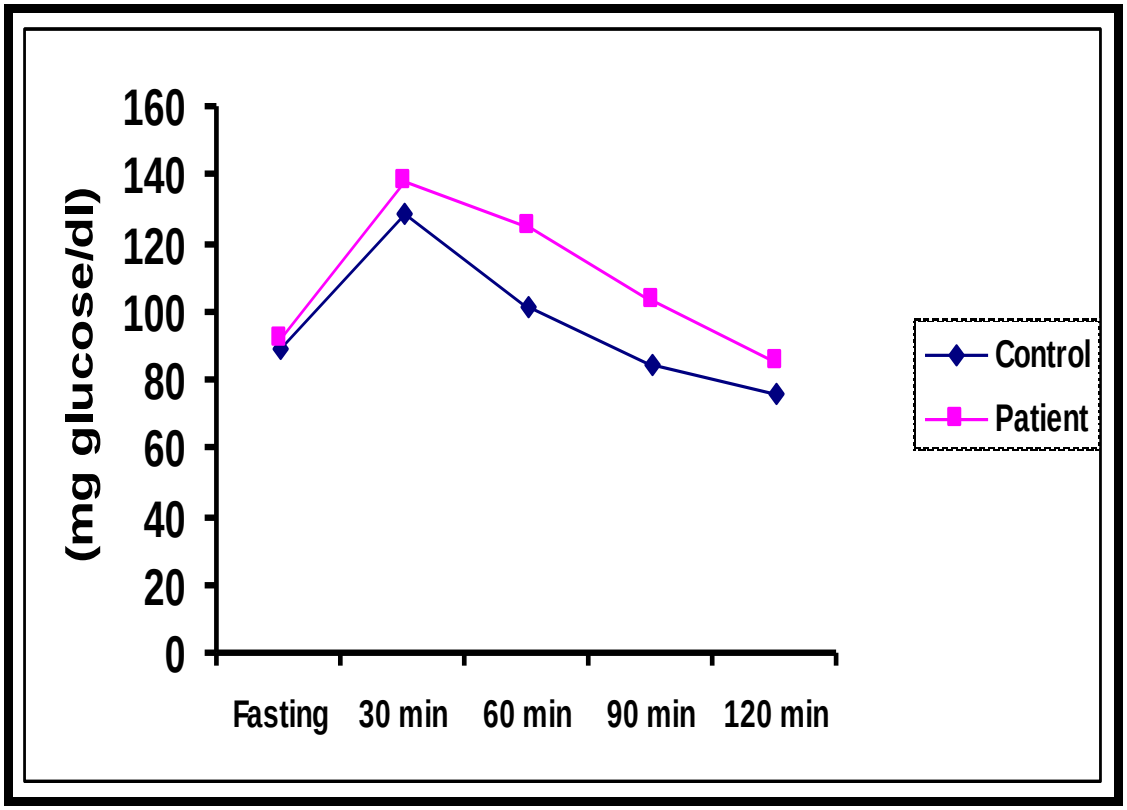

Figure (٥): Glucose Tolerance Test in male patients with B-thalassemia major and control group.

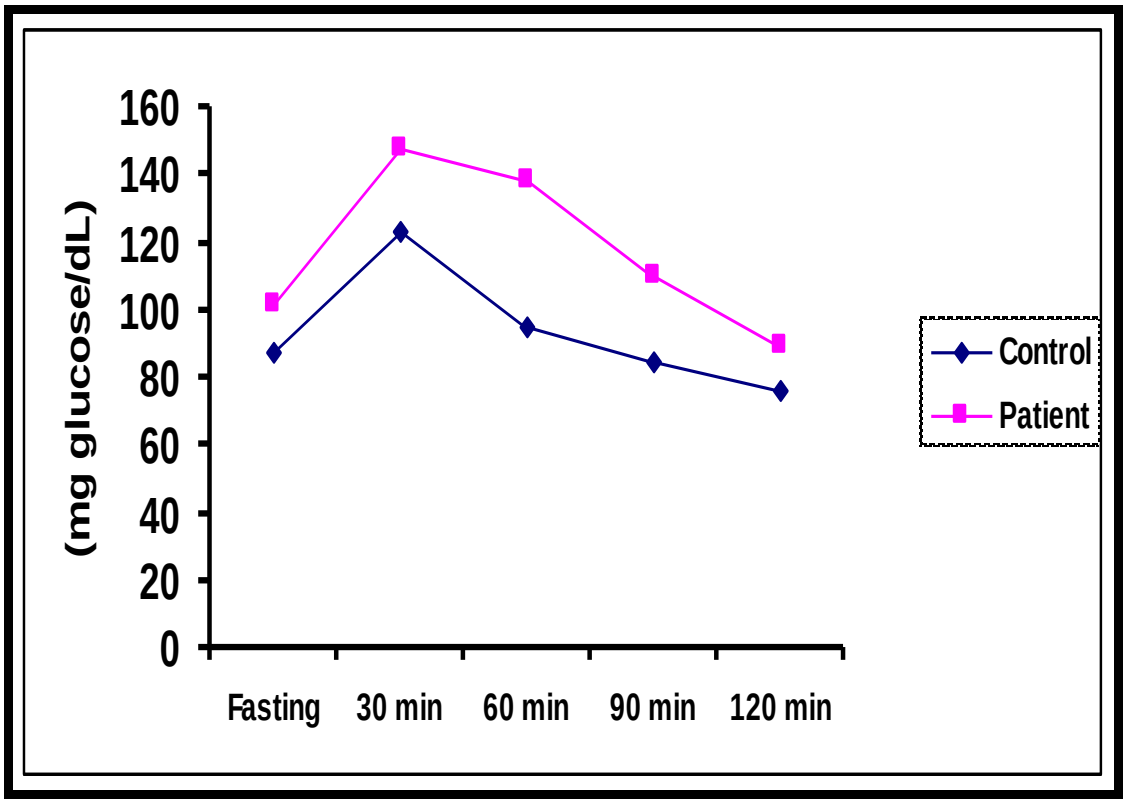

Figure (٦): Glucose Tolerance Test in female patients with B-thalassemia major and control group.

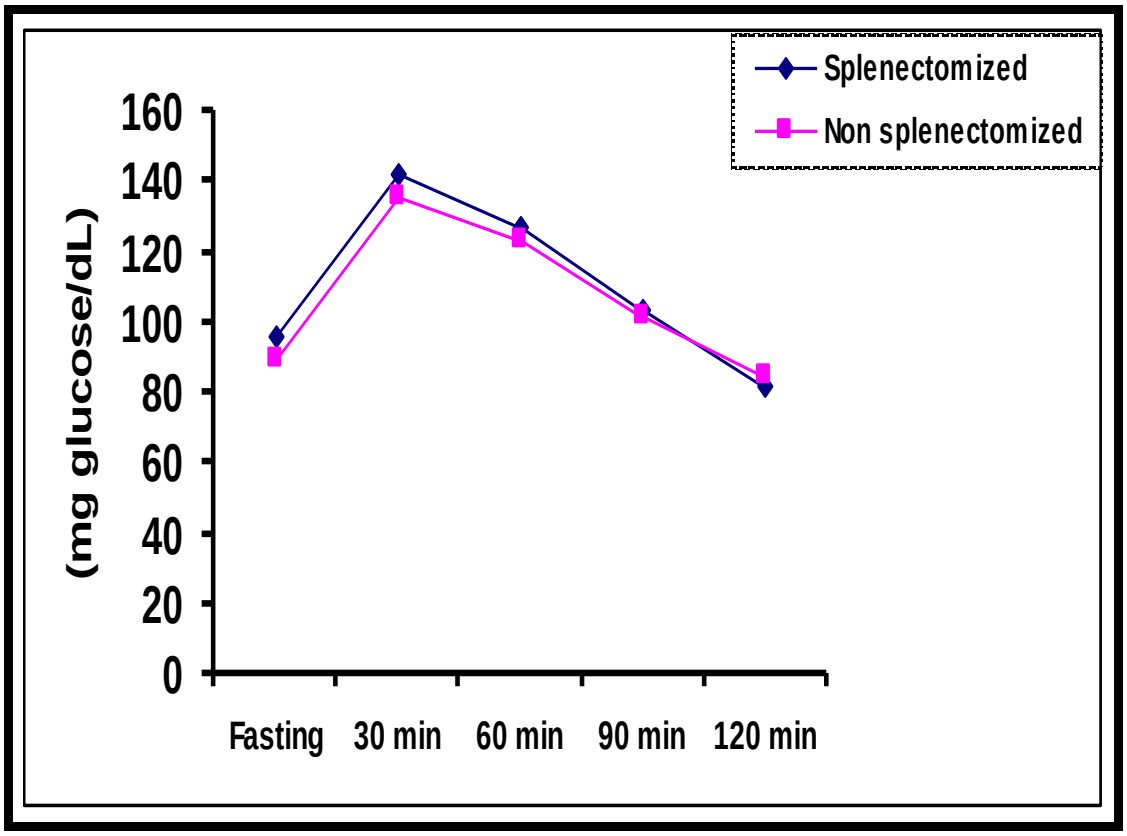

Figure (٧): Glucose Tolerance Test in splenectomized and non splenectomized male patients with B-thalassemia major.

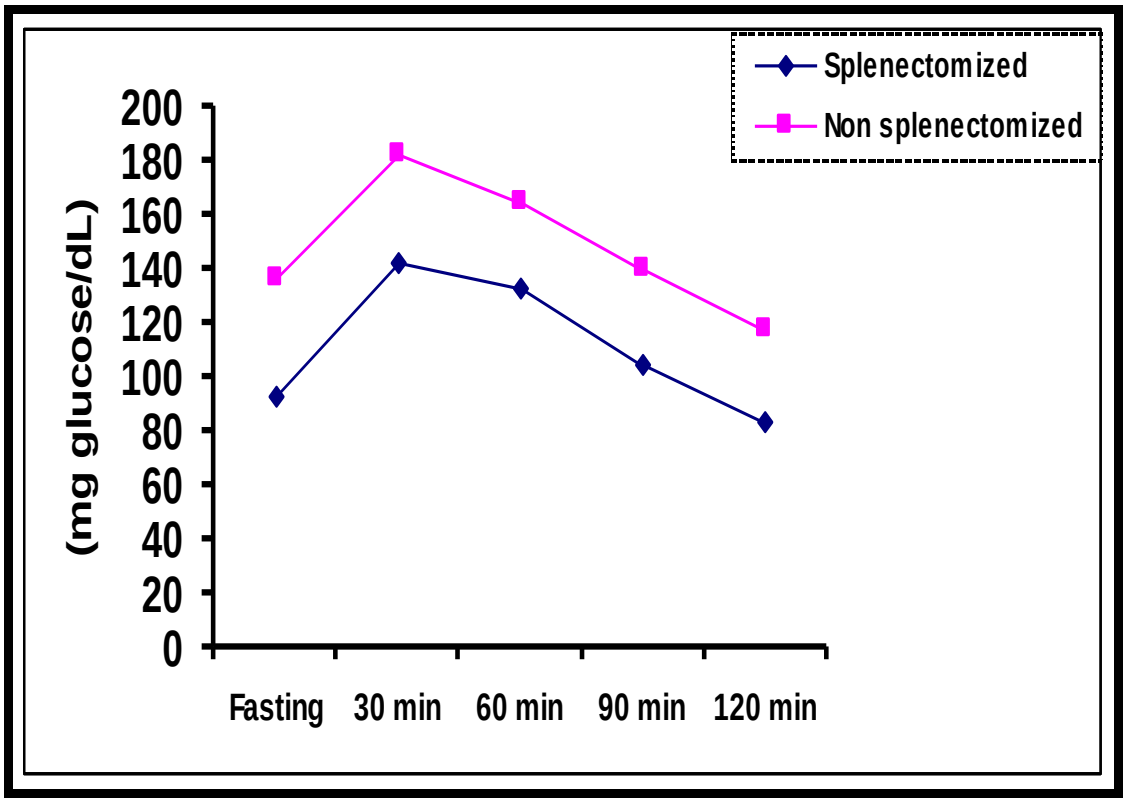

Figure (٨): Glucose Tolerance Test in splenectomized and non splenectomized female patients with B-thalassemia major.

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