ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

The Effect of Iron Overload on the Function of Some Endocrine Glands in β-Thalassemia Major Patients.

Dr. Hussein Kadhem Abdul Hussein Al-Hakeim (PhD)*
Manal Farhan Mohsen Al-Hakany (BSc)*
(*): Department of Chemistry, College of Science, Al-Kufa University , Iraq.
Correspondence to: Dr. Hussein Kadhem Abdul Hussein, College of Science, Al-Kufa University, Iraq
(Mobile: 07801035399headm2000@yahoo.com)
E-mail:

Abstract

Thalassemia is a term that refers to a group of genetic disorders characterized by a defect in the synthesis of hemoglobin. It is sometimes called Mediterranean anemia. Many biochemical changes in the blood accompany this disease. In this research, some biochemical parameters were measured in thalassemic patients and compared with healthy control group. These parameters include Serum iron, TIBC, were measured Spectrophotometrically. Serum ferritin, Iisulin, GH, Testosterone, T3, T4, TSH, Cortisol, & Prolactine were measured by ELISA technique.

The results have revealed a moderate state of iron overload in the thalassemia patients. Furthermore, there is a decrease in the level of serum cortisol, T4, prolactine and pancreatic beta cell function percentage (HOMA%B) in thalassemic while serum TSH level is higher in thalassemic patients in comparing with control group. The changes in the hormone levels in addition to serum insulin, insulin/glucose ratio, and HOMA%B are correlated well with the degree of iron store in the body, concluded mainly from high ferritin level.Insulin resistance parameters showed a significant negative relation with Insulin sensitivity index. There are significant increases in PCV, Hb, T3, and testosterone in male patients in comparing with female thalassemic patients.

From the results of the present study, it can be concluded that iron overload state affects some endocrine glands secretions and there is a difference in the effects between male and females in prepubertal thalassemic patients. Therefore, it is important to estimate the endocrine function activity of thalassemia patients and treat the disorders if present as soon as possible.

Introduction

Thalassemia is a term that refers to a group of genetic disorders characterized by insufficient production of hemoglobin, wherein there is a defect in the synthesis of hemoglobin. It is sometimes called Mediterranean anemia. To understand how thalassemia affects the human body, it must first understand a little about how blood is made. If the body doesn't produce enough of alpha and a beta chains of globin, the red blood cells do not form properly and cannot carry sufficient oxygen. The result is anemia that begins in early childhood and lasts throughout life. Genes involved are those that control the production of alpha and beta globins contained in hemoglobin [1]. The two main types of thalassemia, alpha and beta, are named for the two protein chains that make up normal hemoglobin[2]

Thalassemia Major or Cooley's Anemia: Thalassemia major,is also called Mediterranean anemia or Cooley's anemia, named after the doctor who first described it in 1925, is the most severe form in which the defective gene is inherited from both parents[3]. Clinical presentation of Thalassemia major occurs between 6 and 24 months. Affected infants fail to thrive and become progressively pale. Feeding

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

problems, diarrhea, irritability, recurrent bouts of fever, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes (bossing of the skull, prominent malar eminence, depression of the bridge of the nose, tendency to a mongoloid slant of the eye, and hypertrophy of the maxillae, which tends to expose the upper teeth). If a regular transfusion program that maintains a minimum Hb concentration of 9.5 to 10.5 g/dL is initiated, growth and development tend to be normal up to 10 to 12 years [4].Patients with thalassemia major require lifelong transfusion therapy to survive, leading to toxic iron overload in the endocrine glands and heart. The pituitary gland is one of the most vulnerable targets, leading to irreversible hypogonadotropic hypogonadism in approximately half of patients. Improvements in magnetic resonance imaging (MRI) technology and understanding have allowed earlier recognition of preclinical iron deposition in the heart, pancreas, and liver; prior work also supports a similar role for the pituitary[5]. Transfused patients may develop complications related to iron overload. Complications of iron overload in children include growth retardation and failure or delay of sexual maturation. Later iron overloadrelated complications include involvement of the heart (dilated myocardiopathy or rarely arrythmias), liver(fibrosis and cirrhosis), and endocrine glands(diabetes mellitus, hypogonadism and insufficiency of the parathyroid, thyroid, pituitary, and, less commonly, adrenal glands) [6].

Iron Metabolism: Because there is no known regulated mechanism for iron excretion, and the amount of iron entering the body each day represents less than 0.1% of the total body iron endowment, most circulating iron must be derived from the recycling of iron within the system[7].

An attractive hypothesis proposed for central macrophages is that ferritin, synthesized by macrophages, secreted via exocytosis, and subsequently taken up by erythroblasts which could be a source of iron for heme synthesis[8] .It is not known whether central macrophages might release iron too by the ferroportin-dependent exporting pathway .therefore further studies are needed to clarify this matter.

The second pathway is the cycling of iron from hepatocytes to the blood and viceversa, which is the third is iron absorption through duodenal and upper jejunum that balances the 1–2 mg daily iron loss occurring through cellular exfoliation.

- Iron Absorption: Currently, there are two prevailing hypotheses explaining the mechanisms of this process; first, heme is taken up by receptor mediated endocytosis; secondly, the recent discovery of a heme transporter(PCFT/HCP1) may have the capability of transferrin heme from the small intestinal lumen directly into the cytoplasm[9]. One important criticism of the receptor mediated endocytosis hypothesis is that it assumes iron released from heme is transported out of the internalised vesicles in order to join the labile iron pool.
- Cells Regulating Body Iron Homeostasis: In addition to enterocytes,there are other cell lines, namely macrophages, hepatocytes in adults and placental cells during fetal life, have core functions within iron metabolism, that is to acquire iron from different sources (senescent erythrocytes and holotransferrin) and to deliver it to the rest of the body according to its needs. To do this important function these cells have a specialised mechanism for exporting iron to plasma, that is the iron exporter ferroportin [10]. Iron Storage: Following phagocytosis of old and damaged erythrocytes, tissue macrophages, particularly in the spleen, lyse cells and catabolyse hemoglobin presumably by heme oxygenase, to liberate iron. Some iron remains stored in macrophages, although some is exported to plasma

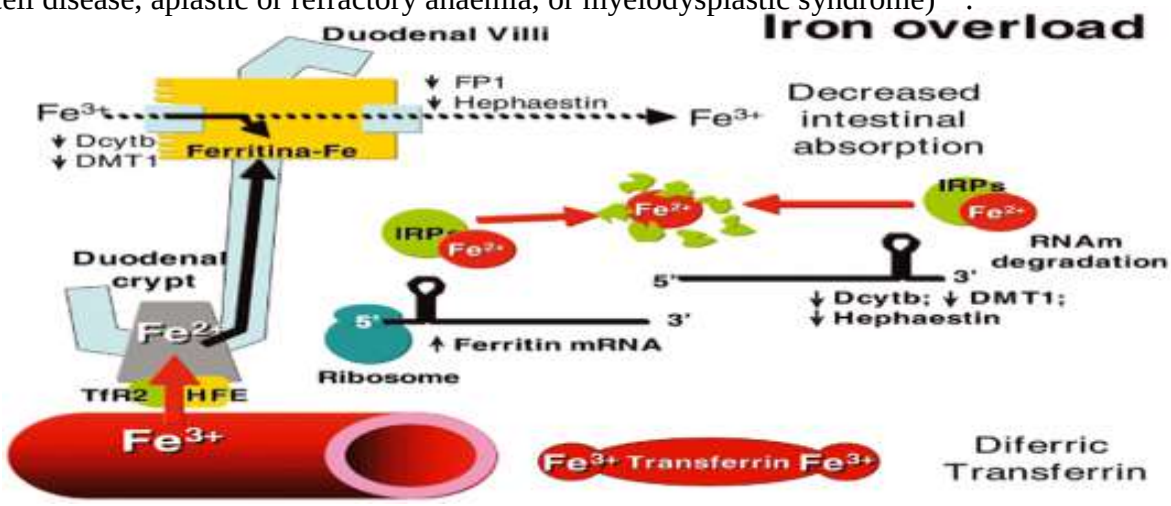
ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

transferrin. Ferroportin is critical for macrophage iron export and can be regulated to change the ratio between stored and released iron[10]. Hepatocytes represent the main depot for iron storage in normal conditions and in non-transfusional iron overload. Although the transferrin cycle may be involved in hepatocyte iron acquisition to some extent, non–transferrin-bound iron (NTBI) uptake pathways become particularly important when serum iron levels exceed transferrin binding capacity [11'12].

Iron Overload: The term haemosiderosis is generally used to indicate the pathological effect of iron accumulation in any given organ, which mainly occurs in the form of haemosiderin[13]. Haemosiderosis is haemochromatosis caused by excessive blood transfusions, that is, haemosiderosis is a form of secondary haemochromatosis[14]. Haemosiderosis is hemosiderin deposition within cells, while haemochromatosis is hemosiderin within cells and interstitial spaces[15]. Organs commonly are affected by haemochromatosis which they are the liver;heart ;and endocrine glands[12]. There are several methods available for diagnosing and monitoring hemosiderosis including: serum ferritin, liver biopsy, and MRI. Serum ferritin is a low cost, readily available, and minimally invasive method for assessing body iron stores. However, the major problem with using it as an indicator of hemosiderosis is that it can be elevated in a range of other medical conditions unrelated to iron levels including infection, inflammation, fever, liver disease, renal disease and cancer[16]. Low plasma hepcidin leads to high ferroportin levels, which allow increased iron uptake, hepatic iron overload, and low levels of iron stored in macrophages [17'19].

Under conditions of secondary iron overload due to chronic transfusion therapy(eg, in major thalassaemia, aplastic anaemia, etc), plasma hepcidin levels are elevated, resulting in degradation of ferroportin [18 '19].

Assessment of Iron Overload: The concentration and total amounts of iron in different tissues are critical parameters that determine clinical outcome in all forms of systemic iron overload, independent of whether the iron overload is caused by upregulated intestinal iron absorption(ie, hereditary haemochromatosis, thalassaemia intermedia, or iron-loading anaemia) or by blood transfusion (ie, thalassaemia major, sickle cell disease, aplastic or refractory anaemia, or myelodysplastic syndrome) [20].



ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

Figure (1): Iron overload state. (Biasiotto G, et al . 2003)

- Diagnosis of Systemic Iron-Overload Disorders:

The signs and symptoms of iron overload are insensitive and nonspecific. Thus, early diagnosis of iron overload requires to consider possibility when the physician is faced with such common findings as chronic fatigue, joint pain, impotence, osteoporosis, and diabetes. Scoring systems to identify patients at greatest risk for undetected systemic iron overload in the primary care setting are under development [21]. Screening laboratory tests include measurements of the serum ferritin level and transferrin saturation. Ferritin levels above 200 ng per milliliter(449 pmol per liter) in women or 300 ng per milliliter (674 pmol per liter) in men who have no signs of inflammatory disease and transferrin saturation above 45% in women or 50% in men warrant additional testing [22].

Accumulation of free iron in tissues characteristically occurs over decades in patients with hereditary disorders of iron metabolism, but it may take place within a few years in transfusion-dependent patients. The diagnosis of iron overload requires sequential steps. Clinical evaluation, biochemical testing, assessment of total body iron, and molecular tests concur to reach the correct diagnosis. Several comprehensive diagnosis and therapeutic algorithms have been recently proposed[19].

Materials and Methods:-

1-Patients:-

Only 60 patients (30 male and 30 female) were completed all biochemical analysis tests. Their ages ranged between 3-11 years old. Oral consent for the patients and control subjects were obtained from their parents. These patients were registered as thalassemic patients in "Thallassemia Center" at "Al-Zahra'a Teaching Hospital" in Najaf city-Iraq. The patients had β-thalassemia major as recorded in their files. Thirty apparently healthy children were selected as the control group. Their age ranges were comparable to that of patients. None of these subjects was anemic or has an obvious systemic disease.

2-Biochemical (measurements):-

Blood were aspirated from individuals in the morning and collected in plain tube for serum in order to estimate the following parameters.

Hemoglobin electrophoresis shows; Hgb A decreased, Hgb F increased, and Hgb A2 variable.

Serum Iron were estimated using colorimetric method, total Iron Binding Capacity were estimated using colorimetric method by the following method: An excess of iron is added to the serum iron to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation the iron in the supernatant is determined). Hemoglobin were estimated using colorimetric method. The Ferritin Quantitative Test , Serum ferritin, Iisulin, GH, Testosterone, T3, T4, TSH, Cortisol, & Prolactine were measured by enzyme-Linked immunosorbent assay (ELISA)technique. Transferrrin saturation percentage (TISP) was calculated by dividing serum iron concentration by TIBC[23].The percentage of transferrin saturation was calculated from the serum iron and transferrin concentrations, using the formula: serum iron (µmol/L)/transferrin (g/L) x 3.98. The formula is based on the maximal

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

binding of 2 mol Fe^{3+}/mol of transferrin and a molecular weight of 79,570 for transferrin [24]. From this formula

$$Transferrin(g/L) = \frac{S.Iron(umol/L)}{Transferrin.saturation\% * 3.98}$$

Biostatistical analysis:- The results were expressed the distribution types of the variables results were examined using Kolmogorov-Smirnov test. The results of the analysis divided the variables into two types according to the statistical distribution; the normally distributed variables and nonparametric variables.

For the variables that is normally distributed, the results were expressed as (mean±standard deviation). Pooled t-test has been used for the comparison between the control groups and among subdivided groups in the measured parameters. Pearson's correlation coefficients (r) were or used calculated to estimate the correlation between parameters.For nonparametric variables, that are not normally distributed, the results have been expressed as medians, in addition to (mean±standard deviation). Mann-Whitney U test was used for the comparison between the patients and control groups and among subdivided groups in the measured parameters. Spearman's correlation coefficients (ρ, rho) were calculated to estimate the correlation between parameters. The difference between groups is considered as statistically different when $p<0.05$). All statistical analysis were performed using SPSS Statistics version19.0.1 Multilingual program (2010), IBM-USA.

Results and Discussion:-

1 -Comparison Between Thalassemic Patients and Controls:-

1-1 Iron Status Parameters:-

The results in Table -1 showed an expected decrease ($p<0.05$) in hemoglobin concentration and PCV in thalassemic patients as compared with control group. These results are expected in thalassemia which is a variant of hemolytic anemia and hemoglobin and PCV decreases. These results in Table-1 showed a significantly high concentration of ferritin in thalassemic patients in comparing with control group. showed a significant increase ($p<0.05$) in serum iron concentration in thalassemic patients as compared with control group. These results indicated iron overload in those patients due to frequent transfusion and rapid hemolysis. found to be higher than control group ($p<0.05$). TS% in thalassemic patients is (36.49±14.08%) while in control group was (19.20±9.04%).

Table -1: Level of the measured parameters in thalassemic patients in comparison with control groupexpressed as mean±standard deviation. (Median in brackets).

Parameters	Thalassemia Group (n=60)	Control Group (n=30)	Significance p-Value
Hb (g/dL)	7.88±1.30	13.07±1.76	<0.0001
PCV %	24.65±3.88	40.20±5.288	<0.0001
Ferritin [a] (pmol/L)	510.98±251.65 (588.32)	177.971±162.17 (130.21)	<0.0001
EIBS [a] (mmol)	73.07±35.99 (84.13)	25.449±24.48 (18.6205)	<0.0001

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

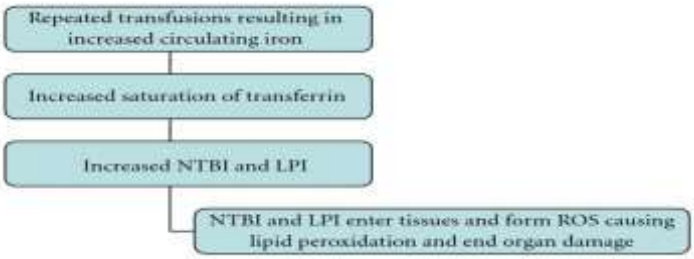
S.Iron (umol/L)	24.68±8.09	11.58±5.35	<0.0001
TIBC (umol/L)	69.44±14.76	61.00±11.45	<0.0001
TS %	36.49±14.08	19.20±9.04	<0.0001
Transferrin (g/L)	0.176±0.035	0.15±0.03	0.001
UIBC (umol/L)	45.50±16.22	49.42±11.15	N.S.

([a]):Using Mann-Witney test for nonparametric variables. While other p-values due to t-test.N.S.=p>0.05.

Some hematological and biochemical characteristics of our thalassemia patients are listed in Table-1 and compared with those of healthy controls. Iron indices, with the exception of TIBC and transferrin protein concentration, were markedly increased, and the mean concentration of serum ferritin was more than seven times higher than normal (Table-1). In states of iron overload or excess, the iron composition of ferritin increases [25'26] and this may be the most important cause for the elevation of serum ferritin. Unstable hemoglobin variants represent a rare etiology of congenital hemolytic anemia present particularly those with beta-thalassemia trait [27].These finding are in accordance with the results of other studies [28'29] found significant decrease in PCV% and Hb level in value in thalassemia patients in comparing with controls. These results indicate iron overload in the patients group due to the fact that serum ferritin is used as a marker for iron overload disorders, such as hemochromatosis and hemosiderosis i.e., abnormally raised **ferritin** levels may reflect **iron** overload [30]. Since ferritin is also an acute-phase reactant, it is often elevated in the infection and inflammation [31 '32]. Since the thalassemic patients group under study have no obvious inflammatory diseases or infection, the increase in serum ferritin indicates iron overload that due to the frequent transfusions to the patients. Furthermore, ferritin can be elevated during periods of acute malnourishment[33]that may be accompanied with the anemia of thalassemic patients. Plasma ferritin is considered the best single indicator of total body iron [31]. Patients with thalassemia major have inevitably suffered from complications of the disease, due to iron overload. The major causes of death in thalassemia patients are congestive heart failure and fatal cardiac tachyarrhythmia leading to sudden cardiac death. The free radical-mediated pathway is the principal mechanism of iron toxicity. The consequent series of events caused by iron overload lead to catastrophic cardiac effects [34].Estimated iron body store (EIBS) was calculated for patients and control group from serum ferritin level. The results in (Table-1) indicated a significant higher iron store in thalassemia patients bodies in comparise with control group. Since 1979, chronic therapy with subcutaneous deferoxamine has had a dramatic impact on survival in secondary iron overload from β-thalassemia [35'36] .Patients with thalassemia major accumulate body iron over time as a consequence of continuous RBCs transfusions which cause hepatic, endocrine, and cardiac complications [37]. These results are further confirmed the accumulation of high amount of iron in the tissues leading to all subsequent complications of iron overload state. Iron overload and subsequent tissue iron deposition can be seen in some disorders such as idiopathic hemochromatosis [38] and transfusion-related siderosis [39]. Thalassemia major is a prototypical secondary iron overload state associated with ineffective erythropoiesis and shortened RBCs survival [40]. Although there is considerable variation in severity of the disorder, most patients who are homozygous for the disorder become dependent on transfusions. Large amounts of iron from transfused erythrocytes, hemolyzed red blood cells and dietary iron, which is hyperabsorbed, accumulate and are incorporated in the heart as ferritin or hemosiderin. Prior to the use of iron chelation therapy with deferoxamine, the cardiac manifestations of iron overload usually led to death by the end of the second decade of life [40] .Iron released from heme and ferritin favors

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

different processes of oxidation including LDL-C oxidation [41] .The general effect of catalytic iron is to convert poorly reactive free radicals such as H_2O_2 into highly reactive ones, such as the hydroxyl radical. In vitro addition of oxygen-derived free radical scavengers or antioxidants can reverse defective endothelium-dependent relaxation [42'43] .Thalassemic patients in the present study suffering from iron overload and they are vulnerable to the complications of this state. Excess iron tends to deposit in the hepatic parenchyma, in endocrine organs, and in cardiac myocytes, causing end organ damage by ROS-mediated lipid peroxidation[44 ' 45] . (Figure -2).



(Figure -2): Mechanism of end organ damage in iron overload.

In patients with β-thalassemia major (TM), long-term transfusion causes iron overload that results in cardiac damage, liver fibrosis, gonadal dysfunction, and growth retardation. Cardiac iron overload still remains the main cause of mortality in TM despite chelation therapy [36 ' 46].Serum iron levels increase with hemolytic anemias, especially thalassemia, while decreased serum iron levels are associated with iron deficiency anemia. Transferrin increases in iron deficiency anemia and decreases in microcytic anemias caused by chronic disease. Transferrin saturation levels increase in aplastic anemia and thalassemia. Decreased transferrin saturation levels are associated with iron deficiency anemias. Elevated TIBC levels are found in iron deficiency anemia. Decreased TIBC levels are seen with hemolytic anemia, pernicious anemia, sickle cell anemia and thalassemia [47].

In one study, the TIBC level decreased significantly in the beta thalassema group compared with those in the other control group and even other types of thalassemia [48]. Furthermore, they found that serum iron and TIBC are better indices for monitoring iron loading in children with thalassema in comparing with transferrin level. They used the increasing in serum iron level and decreased TIBC level in the diagnosis of beta thalassema in children with anemia [48].

Transferrin saturation values in excess of 60 percent may be indicative of hemochromatosis or iron overload (WHO 2001). This high percentage in thalassemia patients indicated iron overload state that due mainly to the frequent blood transfusion to correct the deficiency in Hb% and PCV level in those patients [49.] TS% can be elevated by increased iron stores and a variety of other conditions [38]. Elevated transferrin saturation reflects increased iron stores[50]. These results confirmed by the increase in serum ferritin level in thalassemic patients in comparing with control group as noticed in (Table-1). In one study, patients with elevated TS% had a concurrently elevated serum ferritin level [50]. The same results obtained in the present study. Transferrin concentration in thalassemic patients is lower ($p<0.05$) than the control group (0.15±0.03, 0.176±0.035g/l), respectively. Transferrin levels are typically elevated at times of iron depletion (low ferritin stimulates the release of transferrin from the liver) and vice versa [51]. When iron level increase, as in thalassemia patients, some iron will not bind to transferrin and bound with other blood components leading to formation of plasma non-transferrin bound iron (NTBI) which is potentially toxic and contributes to the generation of reactive oxygen species

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

(ROS), consequently leading to tissue damage and organ dysfunction [52]. Non-transferrin bound iron (NTBI) is detectable in plasma of beta-thalassemia patients and participates in free-radical formation and oxidative tissue damage. Iron overload is responsible for the most damaging effects of the thalassemias, making iron chelation a focal point of the management of these diseases. In addition, as iron accumulates in the organs, dysfunction of the liver, endocrine glands, and heart become the main factors in limiting the survival of patients with β-thalassemia [53].

1-2-Hormones:-

The results in Table -2 tabulated the hormones level in thalassemic and control groups in addition to p-values of the comparison between both groups. From the table, it can be seen that there is a significant decrease ($p<0.05$) in serum cortisol, T4, and prolactine in thalassemic groups in comparing with healthy control. While serum TSH level is higher ($p<0.05$) in thalassemic patients as compared with healthy control group.

Table -2: Hormones level in thalassemic and control groups. (Median in brackets)

Parameters	Thalassemia Group (n=60)	Control Group (n=30)	Significance p-Value
GH [a] (ng/ml)	7.15±7.95 (3.88)	6.37±7.93 (3.10)	N.S.
Testosterone [a] (ng/ml)	0.91±2.67 (0.23)	1.22±0.66 (0.98)	N.S.
T3 [a] (ng/ml)	0.85±0.75 (1.074)	1.06±0.76 (0.79)	N.S.
T4 (ng/ml)	6.12±2.79	7.73±1.81	0.001
TSH (ng/ml)	5.48±1.86	4.36±1.36	0.002
Cortisol [a] (µg/dl)	4.58±5.11 (2.90)	8.73±6.98 (6.55)	0.006
PRL [a] (ng/ml)	5.27±4.04 (3.94)	3.95±2.13 (4.06)	0.044

These results may refer to the decline in the function of thyroid gland and subsequent increase in the secretion of TSH from pituitary gland to compensate that reduction in thalassemic patients group. Furthermore, these results indicated involvement of adrenal cortex and prolactine secreting cells in the pituitary gland.

The significant decline in thyroid hormones was in keeping with published results from other studies[54,55]. The mechanism for thyroid dysfunction can be either primary thyroid damage from iron infiltration or secondary to pituitary dysfunction because of haemosiderosis of thyrotroph cells. As in diabetes, the strongest predictor for development of hypothyroidism has been shown to be duration of transfusion therapy[54,55].

Endocrine abnormalities occur in most types of thalassemia but with greater frequency in TM. Iron toxicity may also indirectly affect heart function by damaging other organs in varying degrees. The endocrine abnormalities hypothyroidism and

diabetes mellitus can have a significant impact on cardiac function [56]. In one study, frequent blood transfusions can lead to iron overload which may result in several endocrine complications, short stature was seen in 36.0% of TM patients. In our cohort, 12 patients had delayed puberty (male 70.0% and female 33.3%). Prevalence of osteoporosis was 36.0%. Hypogonadism was noted in 40.0% of males and 46.7% of females, 53.4% of the female population had menstrual abnormalities with prevalence of primary and secondary endocrinopathies was much lower. 8.0% had diabetes mellitus and only one patient had hypocortisolism. Iron chelation appeared insufficient in our study population. The high frequency of endocrine complications noted in our study supports the rationale for regular follow up of transfusion dependent thalassaemic patients to ensure early detection and timely treatment of associated complications[57].

The change in cortisol level between thalassemic and control group revealed a unique results about the involvement of adrenal cortex. However, stimulating tests in addition to large scale study are necessary to confirm this involvement. In one study, it is noticed that no evidence of adrenocortical hypofunction. The morning cortisol/ACTH levels were all within the reference interval[58-60]. Adrenal function is routinely determined by measuring serum total cortisol, which may not adequately reflect the biologically active free cortisol. In healthy individuals, 90% of measured serum total cortisol is bound to cortisol binding globulin (CBG) with small fractions of albumin-bound and active free forms [61]; The serum glucose concentration was insignificantly higher in the patients than in the controls ($P>0.05$). Pancreatic beta-cells function may be impaired during adolescence or later on. Its impairment ranges from hyperinsulinemia, secondary to insulin resistance, with normal glucose tolerance to beta-cells failure with insulin-dependent diabetes mellitus. Primary hypothyroidism may affect thalassemic patients from the second decade of life. They concluded that, despite the improvement of the treatment, the involvement of the endocrine system still burdens the life of these patients. Further therapeutic improvement would reasonably reduce morbidity and, hopefully, mortality of thalassemic patients and make the endocrine disorders easier to treat[62]. There was no significant difference between the serum insulin level of cases and controls. Other insulin resistance indices were significantly higher in cases compared to controls.

The results indicated that the most significant positive correlations were present inserum TSH with Iron, ferritin, and EIBC. While T4 hormone was correlated with ferritin and EIBS only. The other results showed different patterns of correlations between hormones and iron parameters but statistically they were not significant ($p>0.05$). These fact may not completely excluded the correlations because the number of patients were relatively low and they all under treatment in the medical centers under expert staff. However, these results grossely indicated that the most sensitive gland to the iron overload state, indicated mainly by ferritin, is the thyroid gland. This is comparable to another cohort study which reported low prevalence of hypothyroidism of 4.0% in beta-thalassaemia, although 26.9% of their patients with normal thyroid function showed an exaggerated TSH response to TRH test[63]. However, in a recent survey, a high prevalence of primary overt hypothyroidism was present in 16% of beta-thalassaemic patients[68]. The disorder is mostly due to iron deposition in the thyroids. However, the thyroid pituitary axis seems to be less sensitive to iron deposition damage than gonadal and GH axis. Hence, secondary hypothyroidism is rare in thalassaemic patients, which was not observed in our series, as also in other reports[63'64]. In studies with low prevalence of overt hypothyroidism, mild thyroid dysfunctions were more common as reported in 12.5% of patients[63].

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

Thyroid dysfunction has been reported in 9% to 60% of patients with thalassemia,19,20 but its severity is variable in different series. [65]. Hypothyroidism was found in 11.8% of our patients. A high prevalence of endocrine abnormalities in beta thalassemia major patients is reported by several authors[66'67].Some studies have reported a relationship between the level of ferritin and the development of endocrinopathies [68].Our data suggests that increasing serum ferritin levels show increased incidence of some endocrinopathies.

Diabetes mellitus was observed in 8 (26.7%) patients, of whom 6 patients (5 male, 1 female) had severe hyperglycaemia and were treated by insulin. Among the mechanisms involved for precipitating diabetes mellitus, iron overload in the pancreatic beta-cells leading to pancreatic dysfunction is the most likely one[69].

Studies had reported prevalence of diabetes mellitus in beta-thalassaemic patients to range up to 24%[70]. Diabetes appears to be unusual in beta-thalassaemics of less than 16 years old[71]. In our thalassaemics, a state of hypocortisolism was not noted and there was no clinical suspicion for the involvement of ACTH or adrenal gland. Reports have indicated that iron deposition may occur in the adrenals particularly in the zona glomeruloza and only when this is severe, it will interfere with its function[72]. Finally, in the thalassaemic patients reviewed, no relationship between serum ferritin level and development of endocrinopathy was observed. There was no significant difference in mean ferritin in thalassaemic patients with or without endocrinopathy, as well as in patients without or with one or more endocrinopathy There is contradiction between the different studies, with some revealing a positive relationship[70 ' 71]. While others did not[63 '64].

Among cases the mean T3 was comparable to that of controls. The mean T4 of cases was significantly lower ($p<0.001$) than that of controls. The mean TSH level was significantly higher ($p<0.01$) in cases as compared to controls. Among thalassemia cases 70% were euthyroid, 18% were compensated hypothyroid, 12% were uncompensated hypothyroid and none was overt hypothyroid or hyperthyroid. After 1 year we found no significant change in their proportions (REF). As pharmacological treatment for hypothyroidism is readily available, it is important to monitor thyroid function in these patients and institute prompt therapy when indicated.The basal growth hormone level in the present study was not significantly different between thalassemic and control group and not correlated with any parameter of iron state (Tables-2&3). In other studies growth failure was a commonly observed phenomenon with one-third had significant growth impairment with height below 3rd percentile of predicted. Patients with TM often experience profound growth retardation partly reflecting both the diversion of caloric resources to erythropoiesis and the effects of anaemia (as chronic transfusion support frequently improves childhood growth rates [59 '73]. However, unless intensive chelation therapy is instituted early in life, the adolescent growth spurt is often delayed, even in children who are hypertransfused[55'59]. GH therapy has been used in some centres internationally at the discretion of individual clinicians[74].

In comparing with previous study [75], carried out in the same city, Najaf, GH in that study thalassemic is lower in thalassemia patients as compared to healthy subjects while in the present study there is no significant difference between the two groups. In that study, most of the thalassemia patients were significantly shorter than the healthy subjects in our study. The insignificant difference in the current study in GH do not necesseraly reflects the normal growth or normal stature due to the low number of patients included in the study and the inclusion of females in the present study while the previous study included only males. These results indicating the need for vigilant

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

monitoring of patients with thalassemia in order to treat endocrine dysfunction at an appropriate age[76]. The prevalence of endocrine complications shows a difference between centers, particularly for GH deficiency[77]. Reduced GH secretion in thalassemia patients are related to a neurosecretory dysfunction as a result of iron overload rather than to liver damage [76].

1-3- Insulin Resistance Parameters:-

The percentage of beta cell function (HOMA%B) measured using HOMA calculator was the only parameter of insulin resistance state parameters that showed a significant difference (p=0.037) between thalassemic and control group (Table-3).

Table -3: Insulin Resistance parameters in Thalassemic and Control Groups. (Median in brackets)

Parameters	Thalassemia Group (n=60)	Control Group (n=30)	Significance p-Value
Insulin [a] (µlU/ml)	10.78±11.88 (5.52)	9.67±9.21 (5.86)	N.S.
Glucose (mmol/l)	6.07±1.82	5.65±1.13	N.S.
Ins/Glu [a]	1.88±2.19 (1.02)	1.71±1.62 (0.94)	N.S.
HOMA2IR [a]	1.36±1.30 (0.81)	1.27±1.20 (0.80)	N.S.
HOMA%B [a]	124.10±67.87 (70.80)	131.48±73.06 (66.65)	0.037
HOMA%S	86.45±58.89	84.09±54.13	N.S.

The serum glucose concentration was insignificantly higher in the patients than in the controls ($P>0.05$). Pancreatic beta-cells function may be impaired during adolescence or later on. Its impairment ranges from hyperinsulinemia, secondary to insulin resistance, with normal glucose tolerance to beta-cells failure with insulin-dependent diabetes mellitus.

Insulin resistance is common in hemoglobinopathy including thalassemias. Excessive iron deposition in the liver is associated with a high prevalence of glucose intolerance in patients with hemoglobinopathy requiring repeated blood transfusion [78].

In one study, normal glucose-tolerant individuals with thalassemia minor had higher fasting insulin concentrations and lower HDL cholesterol than control subjects. Thalassemia minor subjects were more insulin resistant, according to HOMA2IR and HOMA2IR values correlated with and alanine aminotransferase in subjects with thalassemia minor only. Individuals with thalassemia minor had insulin resistance,. Normal glucose tolerance was maintained in these individuals by hypersecretion of insulin. The fasting hyperinsulinemia and exaggerated insulin response during the OGTT imply that both the liver and muscle are resistant to insulin action. The strong correlation between hepatic enzymes and HOMA2IR as well as elevated hsCRP in subjects with thalassemia suggests that low-grade hepatic inflammation is closely

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

correlated with insulin resistance. An increased iron turnover from low-grade hemolysis of microcytic erythrocytes may lead to hepatic damage and increased oxidative stress, both of which can contribute to insulin resistance[79].

2- Correlation Between Hormones and Iron Status:-

The results in Table-4 presented the correlation coefficients and p-values for the relations between every hormone and each parameter of iron status in thalassemic patients. Endocrine and metabolic abnormalities are quite common in thalassemia major, attributable, at least in part, to chronic iron overload. Defective insulin production is a complication that occurs only during the late stages of development of iron overload.

Table -4: Correlation between hormones and Iron status. Pearson r-values for patients

Parameters		GH*	Test*	T3*	T4	TSH	Cortisol*	PRL*
PCV%	R-value	0.133	-0.030	-0.027	0.065	-0.098	-0.081	0.087
	P-value	(0.312)	(0.821)	(0.840)	(0.624)	(0.457)	(0.539)	(0.510)
Hb(g/L)	R-value	0.133	-0.030	-0.027	0.065	-0.098	-0.081	0.087
	P-value	(0.312)	(0.821)	(0.840)	(0.624)	(0.457)	(0.539)	(0.510)
Iron	R-value	-0.106	-0.211	-0.048	-0.076	0.254	-0.175	0.148
	P-value	(0.419)	(0.105)	(0.717)	(0.566)	(0.050)	(0.182)	(0.260)
TIBC	R-value	-0.043	0.063	0.103	0.097	0.082	-0.069	0.060
	P-value	(0.745)	(0.632)	(0.435)	(0.459)	(0.533)	(0.600)	(0.649)
Ferritin*	R-value	-0.011	-0.069	0.025	0.430**	0.305*	-0.003	-0.124
	P-value	(0.900)	(0.598)	(0.849)	(0.001)	(0.018)	(0.984)	(0.343)
EIBS*	R-value	-0.011	-0.069	0.025	0.430**	0.305*	-0.003	-0.124
	P-value	(0.931)	(0.598)	(0.849)	(0.001)	(0.018)	(0.984)	(0.343)
UIBC	R-value	-0.030	0.054	0.087	0.146	-0.063	0.021	-0.007
	P-value	(0.823)	(0.684)	(0.510)	(0.266)	(0.633)	(0.876)	(0.957)
TS%	R-value	-0.022	-0.155	-0.081	-0.132	0.162	-0.147	0.122
	P-value	(0.869)	(0.241)	(0.544)	(0.319)	(0.221)	(0.267)	(0.359)
Transferrin	R-value	-0.171	0.021	0.133	0.059	-0.009	-0.001	-0.052
	P-value	(0.194)	(0.872)	(0.314)	(0.657)	(0.946)	(0.997)	(0.698)

*Correlation is significant at the 0.05 level (2-tailed).

The results indicated that the most significant positive correlations were present inserum TSH with Iron, ferritin, and EIBC. While T4 hormone was correlated with ferritin and EIBS only. The other results showed different patterns of correlations between hormones and iron parameters but statistically they were not significant ($p>0.05$). These fact may not completely excluded the correlations because the number of patients were relatively low and they all under treatment in the medical centers under expert staff. However, these results grossely indicated that the most sensitive gland to the iron overload state, indicated mainly by ferritin, is the thyroid gland.

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

3-Correlation Between Insulin Resistance Parameters and Iron Status Parameters:-

The results in Table-5 presented the correlation coefficients and p-values for the relations between insulin resistance state parameters and each parameter of iron status in thalassemic patients.

Table -5: Correlation between Pearson r-values for patients between Insulin Resistance and Iron status.

Parameters		Insulin [a]	Glucose	Ins/Glu	HOMA2IR	HOMA%S	HOMA%B
PCV%	R-value	-0.137	0.122	-0.039	0.002	0.037	0.001
	P-value	(0.298)	(0.354)	(0.772)	(0.987)	(0.781)	(0.993)
Hb(g/L)	R-value	-0.137	0.122	-0.039	0.002	0.037	0.001
	P-value	(0.298)	(0.354)	(0.772)	(0.987)	(0.781)	(0.993)
Iron	R-value	0.188	0.162	0.097	0.220	-0.153	0.138
	P-value	(0.150)	(0.217)	(0.465)	(0.091)	(0.243)	(0.294)
TIBC	R-value	0.092	0.189	0.214	0.238	-0.243*	0.219
	P-value	(0.483)	(0.149)	(0.104)	(0.067)	(0.055)	(0.092)
Ferritin*	R-value	0.263*	-0.226	0.265*	0.107	-0.230	0.266
	P-value	(0.042)	(0.083)	(0.042)	(0.415)	(0.078)	(0.041)
EIBS*	R-value	0.263*	-0.226	0.265*	0.107	-0.230	0.266
	P-value	(0.042)	(0.083)	(0.042)	(0.415)	(0.078)	(0.041)
UIBC	R-value	0.222*	0.081	0.128	0.103	-0.122	0.143
	P-value	(0.064)	(0.539)	(0.336)	(0.432)	(0.353)	(0.277)
TS%	R-value	0.166	0.099	-0.045	0.044	-0.002	-0.042
	P-value	(0.205)	(0.455)	(0.734)	(0.740)	(0.989)	(0.754)
Transferrin	R-value	-0.073	-0.075	0.195	0.207	-0.194	0.220
	P-value	(0.585)	(0.570)	(0.140)	(0.115)	(0.142)	(0.094)

The results in Table-5 are very interesting. Insulin resistance parameters showed a significant negative relation with HOMA%S (r=-0.255, p=0.049). Furthermore, ferritin and subsequently EIBS, showed a significant correlation with each; insulin

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

(r=0.263, p=0.042), insulin/glucose ratio (r=0.265, p=0.042), and HOMA%B (r=0.266, p=0.041). Up today, numerous reports on iron overload and endocrine problems, especially diabetes mellitus in thalassemic patients, have been published, and the reported incidence of impaired glucose tolerance and diabetes mellitus in TM are 0%–26%[71'80]. In our study 17.9% of patients had abnormal serum glucose: 5.1% with overt diabetes, 5.1% with suspicions of diabetes, and the remaining with IFG. Most of the studies suggest that impaired glucose metabolism in the thalassemic patient is a result of insulin resistance[71'80]. According to these studies, iron overload causes insulin resistance directly or through hepatic dysfunction. In our study, 62% of diabetic and pre-diabetic patients had a positive family history of diabetes, and 50% had elevated transaminases. An increase in fasting serum glucose and IR index accompanied with normoinsulinemia suggests some degree of IR and relative pancreatic failure, because normally the islet cells should produce more insulin to overcome hyperglycemia. It is likely that an elevated level of iron and ferritin cause iron toxicity in the liver and pancreas and insulin dysregulation, due to hepatic and pancreatic dysfunction, which is most likely the cause of impaired glucose metabolism in our patients. Lipid abnormality has been frequently reported in thalassemia, but its pathophysiology is not totally clear[82 '83].

4- Comparison Between Male & Female Patients:-

The Comparison of the measured parameters between male and female thalassemic patients are presented in Table-6. There are significant increases ($p<0.05$) in PCV, Hb, T3, and testosterone in male patients in comparing with female thalassemic patients.

Table -6: Comparison between Male and Female thalassemic patients. (Median in brackets)

Parameters		Male Patients (n=30)	Female Patients (n=30)	Significance P-value
PCV	%	25.90±4.318	23.40±2.978	0.012
Hb	(g/L)	8.30±1.44	7.46±0.99	0.012
Iron	(umol/L)	23.40±7.03	25.95±8.96	N.S.
Ferritin*	(pmol/L)	549.02±208.95 (601.622)	472.95±286.69 (582.395)	N.S.
EIBS*	(mmol)	78.509±29.879 (86.032)	67.631±40.996 (83.282)	N.S.
TIBC	(umol/L)	66.34±13.83	72.53±15.22	N.S.
UIBC	(umol/l)	44.41±15.09	46.58±17.45	N.S.
TS	%	35.86±12.95	37.10±15.32	N.S.
Transferrin	(g/L)	0.17±0.03	0.18±0.04	N.S.
GH [a]	(ng/ml)	7.53±6.36 (3.67)	7.43±8.12 (4.30)	N.S.
Testosterone [a]	(ng/ml)	1.36±3.68 (0.64)	0.52±0.45 (0.32)	0.004
T3[a]	(ng/ml)	1.09±0.94	0.61±0.36	0.002
T4	(ng/ml)	6.15±2.86	6.08±2.77	N.S.
TSH	(ng/ml)	5.04±1.80	5.92±1.83	N.S.

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

Cortisol [a]	(µg/dl)	5.38±5.04 (2.77)	4.77±5.25 (3.16)	N.S.
PRL [a]	(ng/ml)	5.53±3.66 (4.52)	5.02±4.44 (3.76)	N.S.
Insulin [a]	(µlU/ ml)	13.92±12.65 (6.38)	9.26±8.91 (4.954)	N.S.
Glucose	(mg/dl)	107.23±35.94	111.42±29.64	N.S.
Ins/Glu [a]		2.61±2.01 (1.16)	1.68 ±1.62 (0.90)	N.S.
HOMA2IR [a]		1.40±1.31 (0.81)	1.21±1.17 (0.62)	N.S.
HOMA B [a]	%	84.81±55.86 (67.40)	87.37±60.32 (72.65)	N.S.
HOMA S	%	136.82±71.58	111.39±62.55	N.S.

([a]): Using Mann-Witney test for nonparametric variables. While other p-values due to t-test.N.S.is p>0.05.

The change in Hb and PCV% can be easly explained by the genetic changes between male and female; males have higher Hb than females in normal subjects and the present finding indicating the same profile of change. Storage iron in adult women has less storage iron, depending on the extent of menses, pregnancies, deliveries, lactation, and iron intake [84]. Total body iron content in normal adults is the result of the balance between iron losses and iron absorbed from the diet. Increased absorption of dietary iron, or iron from multiple transfusions will ultimately result in iron overload. As the body content of iron increases, the saturation of circulating transferrin with iron increases, resulting in the production of increased amounts of non-transferrin-bound iron[85'86] .Transfusion and iron-chelation therapy have prolonged and improved the quality of life in patients with this disease. The improvement is mainly due to the decrease in mortality from heart failure. Such a treatment, however, leads to chronic iron overload and frequently to endocrine complications[6]. Primary and secondary characteristics of sexual development are usually delayed for both boys and girls[87].Boys frequently do not develop, or have sparse, facial and body hair and tend to have decreased libido, even if sperm production does occur. There is increasing evidence that hypogonadism may be primarily due to iron overload [87'59].

Hormonal replacement is indicated for residual endocrine insufficiency [88]. Growth hormone therapy has had variable success [89]. Hypogonadotropic hypogonadism impairs fertility but it can be corrected with the use of hormonal replacement in male patients. A small number of female patients, including those with thalassemia major or thalassemia intermedia, have been able to become pregnant, either spontaneously (if they have received adequate chelation therapy) or with assisted reproductive techniques [89] . Pregnancy generally appears safe if baseline cardiac function is good [90].

5-Effect of Number of Transfusion:-

The correlation study between the number of transfusion units with all the measured parameters is presented in Table-7. The results in general showed a positive correlation between the number of blood transfusion and iron overload parameters. These parameters indicating increase in iron store and iron overload that increased with increasing times of blood transfusion.

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

Table -7: Correlation coefficient (r) of the comparisons between transfusion numbers with every measured parameters.

Iron indices		r-Value	p-Value
Hb	(g/L)	-0.068	N.S.
PCV	%	-0.068	N.S.
Ferritin	(pmol/L)	0.411	0.007
EIBS	(mmol)	0.411	0.007
Iron	(umol/L)	0.373	0.018
TIBC	(umol/L)	-0.321	0.029
TS	%	0.117	N.S.
Transferrin	(g/L)	-0.245	N.S.
UIBC	(umol/L)	-0.198	N.S.
GH [a]	(ng/ml)	0.084	N.S.
Testosterone [a]	(ng/ml)	0.123	N.S.
T3[a]	(ng/ml)	-0.334	0.042
T4	(ng/ml)	-0.163	N.S.
TSH	(ng/ml)	-0.133	N.S.
Cortisol [a]	(µg/dl)	-0.353	0.035
PRL [a]	(ng/ml)	0.035	N.S.
Insulin [a]	(µlU/ ml)	-0.211	N.S.
Glucose	(mg/dl)	0.035	N.S.
Ins/Glu [a]		-0.228	N.S.
HOMA2IR [a]		-0.216	N.S.
HOMA B [a]	%	-0.256	0.048
HOMA S	%	0.184	N.S.

The results in Table-7 showed also a negative correlation between each of the following parameters;TIBC, serum T3, cortisol, and beta cell function index with the transfusion times in major thalassemia patients. In thalassemia major, iron overload is the joint outcome of multiple blood transfusions and an inappropriately increased iron absorption associated with ineffective erythropoiesis. Erythrocyte transfusion therapy is associated with iron overload and possible iron-induced organ damage[91].

Treatment with transfusion programs and chelating therapy has considerably prolonged survival in thalassemic patients. However, as a result of hyper transfusion therapy and increased longevity, iron tissue toxicity has become more common, and contributes significantly to morbidity in these patients [91] The results in Table-7 showed also a negative correlation between each of the following parameters;TIBC, serum T3, cortisol, and beta cell function index with the transfusion times in major thalassemia patients. These resuls are further confirmed the effect of iron overload, caused by increase blood transfusion times, on the function of some endocrine glands [92].

Conclusion:- Our study should be qualified for its relatively small sample size (n =60) and its cross-sectional design. While causality cannot usually be detected in cross-sectional studies, associations can be examined. Moreover,there is accumulation of iron in tissues including the endocrine glands causes problems in the normal functions of the glands concluded from the decrease in the level of serum cortisol, T4, prolactine and pancreatic beta cell function percentage (HOMA%B) in thalassemic while serum TSH level is higher in thalassemic patients in comparing with control group. Insulin resistance parameters showed a significant negative relation with

Insulin sensitivity index. there are significant increases in PCV%, Hb, T3, and testosterone in male patients in comparing with female thalassemic patients . These aspects are of importance and the targets of our future works.

References:-

1- Dogherty P, Einarson T., Koren G., Sher G.. The effectiveness of deferiprone in thalassemia. 1997. Blood 90: 894.
2-
Mayoclinic.http://www.mayoclinic.com/health/thalassemia/DS00905/DSECTION=complications. Retrieved 2011.
3-Parks R. Medical Review: E. Gregory Thompson, Internal Medicine & Brian Leber-Hematology 12th ed;2009;vol. 1: 1083–131.
4-http:// www.thalassemia.org.cy].
5-Wood JC, Noetzl L, Hyderi A, Joukar M, Coates T, Mittelman S. Predicting pituitary iron and endocrine dysfunction. Ann N Y Acad Sci. 2010; 1202:123-8.
6- Borgna-Pignatti C, Galanello R: Thalassemias and related disorders: quantitative disorders of hemoglobin synthesis. In Wintrobe's Clinical Hematology Volume 42. 11th edition. Lippincott Williams & Wilkins. Philadelphia; 2004:1319-65.
7- De Domenico I, McVey Ward D, Kaplan J. Regulation of iron acquisition and storage: consequences for iron-linked disorders. *Nat Rev Mol Cell Biol.* 2008;9:72–81.
8- Leimberg MJ, Prus E, Konijn AM, Fibach E. Macrophages function as a ferritin iron source for cultured human erythroid precursors. *J Cell Biochem.* 2008;103:1211–8
9- West AR, Oates PS. Mechanisms of heme iron absorption: current questions and controversies. World J Gastroenterol. 2008;14:4101-10
10- Ganz T. Cellular iron: ferroportin is the only way out. Cell Metab. 2005;1:155-157.
11- Barisani D, Berg CL, Wessling-Resnick M, Gollan JL. Evidence for a low Km transporter for non-transferrin-bound iron in isolated rat hepatocytes. Am J Physiol. 1995;269:G570-576.
12- Andrews NC. "Disorders of iron metabolism". N. Engl. J. Med. (1999);341 (26): 1986–95.
13-Merriam-Webster's Medical Dictionary 2009
14 -Joffe S, eMedicine Specialties>Radiology>Gastrointestinal>Hemochromatosis. 2009
15-Charles E., Kahn. Notecards on radiology gamuts, diseases, anatomy. Medical College of Wisconsin.2002.
16- Pierre St. Non-invasive measurement and imaging of liver iron concentrations using proton magnetic resonance. Blood. (2005)105: 855–61.
17- FoesterJ, RodgersGM eds. Wintrobe's Clinical Hematology. 12th edn. Philadelphia:Wolters Kluwer LWW, 2009:857–80.
18-Lee DH, Zhou LJ, Zhou Z, Xie JX, Jung JU, Liu Y,et al. Neogenin inhibits HJV secretion and regulates BMP induced hepcidin expression and iron homeostasis. Blood. 2010; 19(1): 90–102
19- Pietrangelo A. Hereditary hemochromatosis: pathogeneis, diagnosis, and treatment. Gastroenterology .2010;139:393–408.
20-Shander A, Sazama K. Clinical consequences of iron overload from chronic red blood cell transfusions, its diagnosis, and its management by chelation therapy. Transfusion 2010;50:1144–55.

21- Mainous AG III, Diaz VA, Everett CJ, Wilson PWF,Tilley BC ,Koopman RJ, et al. Iron overload screeNing tool (IRON): development of a tool to guide screening in primary care. Am J Hematol. 2011;86:733-7.
22- McLaren CE, Barton JC, Adams PC, Harris, E.L., Acton, R.T., Press,et al. Hemochromatosis and Iron Overload Screening (HEIRS) study design for an evaluation of 100,000 primary care-based adults. Am J Med Sci .2003;325:53-62.
23- Richer, Stuart; Rudy, David *; Statkute, Laisvyde; Karofty, Kurt; Frankowski, Jim Serum Iron, Transferrin Saturation, Ferritin, and Dietary Data in Age-Related Macular Degeneration. American Journal of Therapeutics. 2002;9(1):25-28.
24- Morgan EH. Transferrin. Haeberli A eds. Human protein data 1992 VCH Weinheim.
25-Moura, E., Noordermeer, M.A., Verhoeven, N., Verheul, A.F., and Marx, J.J. 1998. Blood 92:2511–2519.
26-Christian Thomas[1] and Lothar Thomas[a1]. Clinical Chemistry. 2002;48:1066-1076.
27 -Amber M.,Nicole A. , Kristina S., Hyde PNP, Jane S., MS,Russell E. Ware. The diagnostic dilemma of congenital unstable hemoglobinopathies. Article first published online: 2010.
28-Khin Ei Han, Aung Myo Han, Kyaw Win, Thein Thein Myint. Thalassemia in the outpatient department of the Yangon Children's Hospital in Myanmar: basic hematological values of thalassemia traits. Southeast Asian J Trop Med Public Health. 1992;23(2):264-8.
29 -Akram H & MahboobRed T Cell Na –K-ATP Pase Activity and Electrolyte Homeostasis in Thalassemia.J.Med.sci, 2004,4(1):19-23
30-National Kidney Foundation 2001.
31- Baer DM, Tekawa IS, Hurley LB. Iron stores are not associated with acute myocardial infarction. Circulation. 1994; 89: 2915–2918 .
32- Kalender B, Mutlu B, Ersoz M, Kalkan A, Yilmaz A. The effects of acute phase proteins on **serum** albumin, transferrin and haemoglobin in haemodialysis patients. Int J Clin Pract ,2002;56(7):505-8
33- Kennedy A, Kohn M, Lammi A, Clarke S. Iron status and haematological changes in adolescent female inpatients with anorexia nervosa. J.Paediatr Child Health. (2004); 40(8):430–2
34-Lekawanvijit S& Chattipakorn N..Iron overload thalassemic cardiomyopathy: Iron status assessment and mechanisms of mechanical and electrical disturbance due to iron toxicity. Can J Cardiol. 2009 ; 25(4):213-8.
35- Wolfe L, Olivieri N, Sallan D ,Colan S, Rose V, Propper R, et al. Prevention of cardiac disease by subcutaneous deferoxamine in patients with thalassemia major. N Engl J Med 1985; 312: 1600-3.
36 -Olivieri NF, Brittenham GM. Iron-chelating therapy and the treatment of thalassemia,Blood 1997;89(3):739-61.
37-Galanello R, Agus A, Campus S, Danjou F, Giardina PJ, Grady RW.Combined iron chelation therapy. Ann N Y Acad Sci. 2010;1202:79-86
38- Olson LJ, Edwards WD, McCall JT, Ilstrup DM, Gersh BJ. Cardiac iron deposition in idiopathic hemochromatosis: histologic and analytic assessment of 14 hearts from autopsy J Am Coll Cardiol 1987;10:1239–43
39- Robert E. Fleming, M.D., and Prem Ponka, N Engl J Med, Iron Overload in Human Disease; 2012; 366:348-59
40- Dover GJ, Valle D. Therapy for β-thalassemia? a paradigm for the treatment of genetic disorders. N Engl J Med 1994; 331: 609-10

41- Abdalla DSP, Campa A, Monteiro MP: Low density lipoprotein oxidation by stimulated neutrophils and ferritin. *Atherosclerosis* 1992;97:149-159
42- Rosen P, Ballhausen T, Bloch W, Addicks K. Endothelial relaxation is disturbed byoxidative stress in the diabetic rat heart influence of tocopherol as antioxidant.Diabetologia 1995; 38: 1157-11
43- Ting HH, Timimi FK, Boles KS, Creager SJ, Ganz P, Creager MA. Vitamin C improves endothelium-dependent vasodilation in patients with non-insulin-dependent diabetes mellitus. J Clin Invest. 1996 ; 1;97(1):22–8
44- Goddard J. G. and Sweeney G. D., Ferric nitrilotriacetate: a potent stimulant of in vivo lipid peroxidation in mice,Biochemical Pharmacology, 1983:32(24): 3879–82.
45- Hershko C., Link G., Konijn A. M., and Cabantchik Z. I.,Objectives and mechanism of iron chelation therapy,Annals of the New York Academy of Sciences,2005 ;vol. 1054: 124–35.
46-Evstatiev R, Dejaco C, Gasche C, Kulnigg-Dabsch. S, Effect of iron therapy on platelet counts in patients with inflammatory bowel disease-associated anemia. 2012;7(4):e34520
47 -Marie A. Moisio, and Elmer W. Moisio. Delmar Understanding Laboratory and Diagnostic Tests, Publishers 1998. Ch.3:82
48-Huang YJ, Wu SG, Ou XB, Zhang L. Changes of iron metabolism indices in children with various genotypes of thalassema. Zhongguo Dang Dai Er Ke Za Zhi. 2010;12(2):85-8. English Abstract.
49-Hassan MK, Taha JY, Al-Naama LM, Widad NM, Jasim SN. Frequency of haemoglobinopathies and glucose-6-phosphate dehydrogenaseDeficiency in basra F.East Mediterr HealthJ.2003;9(1-2):45-54
50- Looker AC, Johnson CL. Prevalence of elevated serum transferrin saturation in adults in the United States. Ann Intern Med. 1998;1;129(11):940-5
51- Vujić Spasić M, Kiss J, Herrmann T, Galy B, Martinache S, Stolte J, et al. Hfe acts in hepatocytes to prevent hemochromatosis. Cell Metab. (2008);7 (2): 173–8.
52-Ounjaijean S, Thephinlap C, Khansuwan U, Phisalapong C, Fucharoen S, Porter JB, et al. Effect of green tea on iron status and oxidative stress in iron-loaded rats. Med Chem. 2008 ;4(4):365-70
53 -Fung EB, Harmatz P, Milet M, Ballas SK, De Castro L, Hagar W, et al . Morbidity and mortality in chronically transfused subjects with thalassemia and sickle cell disease: A report from the multi-center study of iron overload. *Am J Hematol.* 2007;82:255–65
54 -Ferrara M, Matarese SMR, Francese M, Borrelli B, Coppola A, Coppola L et al. Effect of VDR polymorphism on growth and bone mineral density in homozygous beta Thalassaemia. Br J Haematol. 2001; 117: 436–40.
55 -Rund D, Rachmilewitz E. Medical progress, b-Thalassemia. N Engl J Med 2005; 353: 1135–46
56- Tsironi M, Korovesis K, Farmakis D, Deftereos S, Aessopos A. Hypocalcemic heart failure in thalassemic patients. *Int J Hematol.* 2006;83:314–7.
57- Ong C.K., Lim S.L., Tan W.C., Ong E.E., Goh A.S., *Endocrine Complications in Transfusion Dependent Thalassaemia in Penang Hospital.* Medical Journal of Malaysia, (2008); 63 (2):109-12.
58- Olivieri NF. Medical Progress, the b-Thalassaemia. N Engl J Med.1999; 341: 99–109.
59 -Fung EB, Harmatz PR, Lee PDK, Milet M, Bellevue R, Jeng MR et al ; The Multi-Centre Study of Iron Overload Research Group. Increased prevalence of iron-

overload associated endocrinopathy in thalassemia versus sickle cell disease. Br J Haematol 2006; 135:574–82.;
60 -Italian Working Group on Endocrine Complications in Non-endocrine Diseases. Multicentre study on prevalence of endocrine complications in thalassemia major. Clin Endocrinol.1995; 42: 581–6.
61 -Mueller UW, Potter JM Binding of cortisol to human albumin and serum: the effect of protein concentration. Biochem Pharmacol. 1981;30:727–33.
62-Delvecchio M, Cavallo L. Growth and endocrine function in thalassemia major in childhood and adolescence. J Endocrinol Invest. 2010 ;33(1):61-8
63- Zervas A, Katopodi A, Protonotariou A, Livadas S, Karagiorga M, Politis C, et al. Assessment of thyroid function in two hundred patients with beta-thalassemia major. *Thyroid* 2002;12(2):151-4
64- Filosa A, Di Maio S, Aloj G, Acampora C.Longitudinal study on thyroid function in patients with thalassemia major. *J Pediatr Endocrinol Metab* 2006;19(12):1397-404
65- Pantelakis S. Thyroid disorders and diabetes mellitus as complications of thalassaemia major. Acta Paediatr Suppl 1994; 406: 111-3
66 -De Sanctis V, Vullo C, Katz M, Wonke B, Hoffbrand VA, Di Palma A, et al. Endocrine complications in thalassaemia major. Prog Clin Biol Res 1989; 309: 77-83.
67- Canale VC, Steinherz P, New M, Erlandson M. Endocrine function in thalassemia major [Abstract]. Ann NY Acad Sci 1974; 232: 333-45
68- Costin G, Kogut MD, Hyman CB, Ortega JA. Endocrine abnormalities in thalassemia major. Am J Dis Child 1979; 133: 497-502
69-Tuomainen TP, Nyyssönen K, Salonen R, Tervahauta A, Korpela H, Lakka T, et al. Body iron stores are associated with serum insulin and blood glucose concentrations. Population study in 1,013 eastern Finnish men. *Diabetes Care*. 1997;20(3):426-8.
70- Chern JP, Lin KH, Lu MY, Lin DT, Lin KS, Chen JD, et al. Abnormal glucose tolerance in transfusion-dependent beta-thalassemic patients. *Diabetes Care* 2001;24(5):850-4
71 -Suvarna J, Ingle H, Deshmukh CT.Insulin resistance and beta cell function in chronically transfused patients of thalassemia major. *Indian Pediatr* 2006;43(5):393-400.
72- Sklar CA, Lew LQ, Yoon DJ, David R.Adrenal function in thalassemia major following long-term treatment with multiple transfusions and chelation therapy. Evidence for dissociation of cortisol and adrenal androgen secretion. *Am J Dis Child* 1987;141(3):327-30
73- Skordis N, Michaelidou M, Savva SC, Ioannou Y, Rousounides A, Kleanthous M et al. The impact of genotype on endocrine complications in Thalassaemia major. Eur J Haematol. 2006; 77: 150–6
74- Grundy RG, Woods KA, Savage MO, Evans JP. Relationship of endocrinopathy to iron chelation status in young patients with thalassaemia major. Arch Dis Child. 1994;71:128–32.
75- Abdulzahraa M. S., Al-Hakeim HK, aaRidha MM. Study of the effect of iron overload on the function of endocrine glands in male thalassemia patients: Asian J.Trans.Sci. 2011;5(2): 127-31
76- Moayeri H, Oloomi Z.Prevalence of growth and puberty failure with respect to growth hormone and gonadotropins secretion in beta-thalassemia major. Arch Iran Med .2006;9(4):329-334

77- De Sanctis V, Eleftheriou A, Malaventura C; Thalassaemia International Federation Study Group on Growth and Endocrine Complications in Thalassaemia. Prevalence of endocrine complications and short stature in patients with thalassaemia major: A multicenter study by the Thalassaemia International Federation (TIF). Pediatr Endocrinol Rev 2004;2(Suppl 2):249-55.
78-Merkel PA, Simonson DC, Amiel SA, Plewe G, Sherwin RS, Pearson HA, et al. Insulin resistance and hyperinsulinemia in patients with thalassemia major treated by hypertransfusion. N Engl J Med .1988;318(13):809-814
79-Maddux BA, See W, Lawrence JC, Goldfine AL, Goldfine ID, Evans JL: Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by mircomolar concentrations of alpha-lipoic acid. Diabetes , 2001;50:404–410.
80-Khalifa AS, Salem M, Mounir E, El-Tawil MM, El-Sawy M, Abd Al-Aziz MM.,et al. Abnormal glucose tolerance in Egyptian β-thalassemic patients: Possible association with genotyping. *Pediatr Diabetes.* 2004;**5**:126-*32*
81-Shamshirsaz AA, Bekheirnia MR, Kamgar M, Pourzahedgilani N, Bouzari N, Habibzadeh M, et al. Metabolic and endocrinologic complications in beta-thalassemia major: a multicenter study in Tehran. BMC Endocr Disord 2003;;3(1):4.
82-Calandra S, Bertolini S, Pes GM, Deiana L, Tarugi P, Pisciotta L, et al. Beta-thalassemia is a modifying factor of the clinical expression of familial hypercholesterolemia. *Semin Vasc Med.* 2004;**4**:271–8.
83-Amendola G, Danise P, Todisco N, D'urzo G., DipalmaA. and DI Concilio,. Lipid profile in β-thalassemia intermedia patients: Correlation with erythroid bone marrow activity. *Int J Lab Hematol.* 2007;**29**:172–176.).
84-Cook JD, Flowers, CH Skikne BS. The quantitative assessment of body iron. Blood 2003;101:3359.
85-Sahlstedt L, Ebeling F, Von Bonsdorff L , Parkkinen J, Ruutu T. Non-transferrin-bound iron during allogeneic stem cell transplantation. Br J Haematol. 2001;113:836.
86- Esposito,BP, Breuer W, Sirankapracha P ,Pootrakul P, Hershko C, Cabantchik ZI.,et al . Labile plasma iron in iron overload: redox activity and susceptibility to chelation,Blood .2003;102:2670.
87-Chern JP, Lin KH, Tsai WY, Wang SC, Lu MY, Lin DT, et al. Hypogonadotropic hypogonadism and hematologic phenotype in patients with transfusion dependent betathalassemia. J Pediatr Hematol Oncol 2003; 25: 880-4.
88- Raiola G, Galati MC, De Sanctis V, Caruso NM, Pintor C, De Simone M, et al. Growth and puberty in thalassemia major. J Pediatr Endocrinol Metab. 2003;16 Suppl 2:259-66.
89- De Sanctis V.Growth and puberty and its management in thalassaemia. *Horm Res* 2002;58(Suppl 1):72-79.
90-Cohen AR, Galanello R, Pennell DJ, Cunningham MJ, Vichinsky E. Thalassemia. Hematology (Am Soc Hematol Educ Program) 2004;1:14-34.
91-Jonathan W, Pine Jr. The thalassemia and related disorderds. In: Lee GR, Foerster J, Lukens J, Paraskevas F, Greer JP, Rodgers GM, et al, editors. Wintrobe's clinical hematology. 10th ed. Baltimore: Williams & Wilkins 1999. p. 1405-49.
92-Mula-Abed WA, Al Hashmi H, Al Muslahi M, Al Muslahi H, Al Lamki M.Prevalence of Endocrinopathies in Patients with Beta-Thalassaemia Major - A Cross-Sectional Study in Oman ,Najafipour F ; Evaluation of Endocrine Disorders in Patients with Thalassemia Major. Intern J Endocr. Met Group: 6 (2), 2008;23(4):257-62.

ISSN: 2073-8854
Magazin of Al-Kufa University for Biology / VOL.5 / NO.2 / year : 2013

تأثير تراكم الحديد على وظائف بعض الغدد الصم عند مرضى فقر دم البحر الابيض المتوسط نوع بيتا.علاقة خاصة للهبسيدين

الباحثون: منال فرحان محسن
د. حسين كاظم الحكيم
جامعة الكوفة-كلية العلوم-قسم الكيمياء
المراسلات: د. حسين كاظم الحكيم- جامعة الكوفة-كلية العلوم-قسم الكيمياء
E-mail: headm2010@yahoo.com

الخلاصة

يطلق مصطلح الثالاسيميا على مجموعة من الاضطرابات الوراثية التي تؤثر في عملية تصنيع الهيموكلوبين ويسمى هذا المرض أحيانا بفقر دم البحر الأبيض المتوسط. يرافق هذا المرض الكثير من التغيرات الكيموحياتية في الدم. درست في هذا البحث تم قياس بعض المتغيرات الكيموحياتية المهمة في المرضى وقورنت نتائجهم بمجموعة السيطرة. شملت القياسات العملية المتغيرات التالية: تركيز الحديد الكلي و TIBC بالطريقة الفوتومترية. كما تم قياس الفرتين والانسولين وهرمون النمو والشحمون الخصوي والثايروكسين ثلاثي الجزيئة والثايروكسين والهرمون المحفز للدرقية و الكورتزول والبرولاكتين بتقنية الاليزا .

وأظهرت النتائج حالة متوسطة من حالة تراكم الحديد في الجسم لد ى مرضى الثلاسيميا. وعلاوة على ذلك، هناك انخفاض في مستوى هرمون الكورتيزول ، الثايروكسين و البرولاكتين في الدم والنسبة المئوية لوظائف خلايا بيتا في البنكرياس(HOMA B٪) في الثلاسيميا، بينما مستوى مصل الهرمون المحفز للدرقية TSH أعلى لدى المرضى الذين يعانون الثلاسيميا مقارنة مع مجموعة الاصحاء.

أظهرت النتائج ارتباط التغيرات في مستويات الهرمونات بالإضافة إلى الأنسولين ، ونسبة الانسولين / الجلوكوز و(HOMA B٪) مع درجة تراكم الحديد في الجسم، والذي تم الحصول عليه واستنتاجه من الارتفاع في مستوى الفيريتين.

أظهرت عوامل مقاومة الانسولين وجود علاقة سلبية مع مؤشر حساسية الانسولين. وهناك زيادات معنوية في حجم كريات الدم الحمرPCV، خضاب الدم Hb ، هرمون الثايروكسين ثلاثي الجزيئة T3 وهرمون الشحمون الخصوي لدى المرضى الذكور مقارنة مع مرضى الثلاسيميا الإناث. ومن نتائج هذه الدراسة، يمكن الاستنتاج بأن حالة تراكم الحديد تؤثر على بعض افرازات الغدد الصماء، وهناك فرق في التأثيرات بين المرضى الذكور والإناث الذين يعانون الثلاسيميا ماقبل البلوغ. ولذلك فمن المهم تقدير فعالية وظائف الغدد الصماء لمرضى الثلاسيميا وعلاج الاضطرابات إذا كانت موجودة في أسرع وقت ممكن.