Correlation Between Serum Ferritin And Liver Function in Thalassemia Patients

Zainab Qasim Muhammad Al-Yasiri

Assistant Lect., Biology Department / College of Education for Girls / Dhi Qar University / Iraq

Abstract

Thalassemia ,is one of the most important hemolytic genetic, diseases that break down red blood cells, Children with thalassemia cannot produce. enough hemoglobin because; the bone marrow cannot produce enough red blood cells to meet their needs and the red blood cells produced ,by them are almost devoid of hemoglobin, and the disease is divided into alpha, and Beta thalassemia.

The current study was designed with the aim of .investigating the relationship between serum ferritin and hepatic enzymes in thalassemia. patients, iron is stored primarily in the form of ,ferritin in liver cells. The study included 60 patients with thalassemia in; Dhi Qar governorate, distributed between. the two gender during the, research period, their ages ranged between (2 -26) years old, blood samples were taken in order to testing of. hemoglobin, ferritin, and liver enzymes (ATP, GOT, GPT) , and the same, tests were performed on healthy people 40 person ;as they are a control group.

The results recorded; a high percentage of ,thalassemia incidence in males and a decrease in females. The study also showed; that the incidence of thalassemia reached its peak in the age. group (2-6) years and decreased with age, as it reached its lowest levels in the age, group (22-26) years.

The study revealed .a significant decrease (0.05> P) in hemoglobin concentration in thalassemia, patients compared with the ;control group, and the results recorded a significant; increase (0.05 P <) in the concentration of ferritin in thalassemia, patients compared with the control group.

The current study; also showed a significant increase (P <0.05) in GPT enzyme concentration in thalassemia. patients compared with the control group. There was a significant increase (P <0.05) of GOT enzyme; concentration in patients compared to the ,control group.

The results indicated. that there was a significant increase (P <0.05) in the concentration of the ALP enzyme in the patients. compared with the control group.

Keywords: *thalassemia, ferritin, liver*

Introduction

Thalassemia :It is one of the most important hereditary hemolytic diseases and the most prevalent in the, world, Thalassemia is a Greek word in origin that means anemia in the Mediterranean region, The disease. is widely known in this region and .is also known as Mediterranean anemia.In the United States of America, it was known as Cooley's anemia, due to the. disease's discoverer who discovered it ,in 1925 [(1)]

Thalassemia is. a genetic disease that affects the manufacture of protein chains in hemoglobin., Incomplete chains are synthesized in red blood cells, which makes hemoglobin unable to transport oxygen to the various ;organs of the body [(2)]. This imbalance leads to a lack of, maturation of red blood cells This causes early .decomposition and breakdown of the cells after a short period of their production in the bone. marrow, which causes chronic anemia for children in the early stages of their. life as a result of receiving defective genes, one from the father ;and the other from the

mother[3].

Thalassemia is often diagnosed; in the first six months of a newborn's life, and it may be fatal if the patient does not receive, appropriate treatment, Children with thalassemia need a blood transfusion, every 3-4 weeks, depending on the patient's age and the degree of. hemoglobin deficiency in the blood in order to be able to .survive [4].

Thalassemia major disease does not affect. the fetus in the uterus, because fetuses have a special type of ,hemoglobin called fetal hemoglobin and differs from adult hemoglobin, and when. a fetus is born most of the hemoglobin in its body remains the hemoglobin of ;fetuses in the first six months of life, but the problem of thalassemia .lies in the inability of children To generate sufficient amounts of hemoglobin, so thalassemia, major symptoms begin to appear in children in the first year of. life [5].

The lifespan of red blood, cells in thalassemia patients is very short, so these pellets are gradually eaten, causing severe anemia, and so patients. undergo periodic blood ,transfusions to keep the red blood cells close to the required level, which improves, the patient's condition and ensures the transfer. of oxygen to the tissues and the. normal,. growth of the body, Anemia worsens and the child's growth stops, As a result, the bone ;marrow expands and becomes unable to produce sufficient; amounts of red blood cells, and as a result, the shape of the child's ,forehead and bones changes [6].

Types of Thalassemia :

1-Alpha-thalassemia:

It is uncommon. and has been found periodically in different parts of the world. There are four alpha globin genes, so the ,disease symptoms appear according to the number of .defective genes:

- **Silent alpha thalassemia:**

It occurs if the .mutation affects only one gene, then the person does not have any health .problem.

- Alpha thalassemia trait carrier :

If the mutation affects. only two of the genes and it does not accompany any health problem, but children may be born to him with health, problems in hemoglobin if .his wife is a carrier of the same trait [7].

- Hemoglobin H-disease

If the mutation ;affects three of the four genes, where the child has an abnormal hemoglobin and is of low quantity, and after a few ;months the child develops anemia of the moderate type, children do not need a blood .transfusion, but adults may need, a blood transfusion.

- **severe hydropsy fetalis**:

If all four genes .are damaged, the fetus will develop severe dropsy in the womb of its mother. as a result of severe anemia and heart failure as a secondary result of ;Hb deficiency in the blood.[8].

1- Beta-thalassemia:

It is the most common. genetic disorder worldwide, there are two beta globin genes, so the symptoms of the, disease appear according to the number of defective. genes:

- **Thalassemia minor (carriers of the disease):**

When there. is a defect in one of the beta. globin genes, this condition is called a carrier of the disease, and does not suffer from any health ,problem, except from a slight anemia that does not require. a blood transfusion, He is able to transmit the disease to his. children [9].

- **Medium thalassemia:**

It occurs when there; is a defect (mutation) in both of the beta globin genes, resulting in a moderate decrease in the amount of beta globin. produced in the body, leading to a moderately .severe decrease in the hemoglobin level in the blood, and the patient does, not need a periodic transfusion [10].

- **Thalassemia major:**

It occurs when. there is a defect (mutation) in both of the beta globin genes, but the type of damage in the beta gene this time is more .severe ,This results in a severe decrease in the proportion of; beta-globin, thus reducing hemoglobin as a result of breaking down the. abnormal red blood cells before the end of their life span, The patient needs periodic blood; transfusions every 3-4 weeks to maintain a high level of Hb in order for the

body, to grow healthy [11].

Aim of Study:

In light of the foregoing, the present ,study suggested in order to investigate the effect of thalassemia infection on liver function by studying. the following criteria:

1- Measuring. the levels of liver enzymes represented by GOT, GPT and ALT.

2- Measurement. of serum ferritin level (iron-storing protein).

3- Measurement of .hemoglobin level in the blood.

4- To identify the largest; infection rate among different age groups.

5- Comparing the infection. rate between the two gender and, investigating the causes.

Materials and methods :

1-Blood samples:

Blood; samples were drawn from thalassemia. patients during their visit to the General Al-Habobi Hospital / Genetic Hematology; Center in order to receive treatment and care, (60) samples were; obtained from these patients distributed between of either gender. during the research period, their ages ranged ,between (2-20) years, and examinations .were performed in hematology and biochemistry laboratories.

For the period .from December / 2018 to July / 2019, 5 ml of blood was taken for each patient and a hemoglobin,(Hb) test was performed in the blood laboratory, the blood ;serum was obtained by placing ,samples in clean and dry test tubes then placed in a centrifuge At a speed of ;4000 r / min for five minutes and the serum was ,separated from the thrombus by a micro pipette, the serum was placed in labeled Eppendorf ,tubes for the examination of ferritin and liver enzymes (GPT, GOT, ALT).The same amount. of blood was drawn from healthy people (40) samples and they. underwent the same tests for comparison purposes as ,control samples.

2- Hemoglobin measurement:

Hemoglobin was. measured .using the Coulter Horiba blood tester of French origin Emerald. company.

3- Liver enzymes measurement:

3-1- Measurement of Aspartate transaminase (AST) GOT:

This enzyme; was .measured by using a kit of the type. bio Merieux- French, and the, activity of this enzyme was .measured ,by color methods by using the method of .(Gella F.J. *et al*., 1985), according to the following reaction equation:

Aspartate + α Ketoglutarate ↔ Oxaloacetate + glutamate

The formed .oxaloacetate .is measured by color methods through, its interaction with 2,4-. dinitrophenylhydrazne.-.to form the hydrozone derivative. This derivative; is measured through a spectrophotometer .with a wave length of 505nm, the .intensity of .absorption will be proportional. to the activity of this enzyme.

3-2- Measurement of Alanine transaminase (ALT) GPT:

The effectiveness, of this enzyme was measured using a bio Merieux- French kit, and GPT (Glutamic pyruvic transaminase) ,was measured by color methods which described .by (Lustig V. *et al*., 1988) according to the following. reaction:

GPT

Alanine +α-Ketoglutarat Pyruvate + Glutamate

The liberated pyruvate is. measured by the presence of the compound 2- 4- di-nitrophenyl hydrazine .to form the ,hydrazone .derivative, and this compound is measured by a spectrophotometer; with a wave length of 505nm. The. intensity of absorbance, will be proportional. to the activity of this enzyme.

3-3- Measurement of Alkaline, phosphatase (ALP):

This enzyme was; measured by several bio Merieux-. france kit, and the basis of this method depends on the. activity of this enzyme in the basic medium by using the. method described by Tietz , *et al*. [12] according to the

following equation:

Alkaline Phosphatase

Phenylphosphate ----------> Phenol Phosphate

PH 10

The released. phenol is measured by the presence of the 4-aminoantipyren compound, and the potassium, Ferro cyanide, The presence. of sodium arsenate is to stop the. enzyme activity. Then the. solution is measured .over a wavelength of 510 nm. The amount .of absorption is proportional, to the amount of phenol released; and this depends on the activity of the enzyme.

4- Measuring the level of ferritin:

The method for quantifying, protein storage, ziron (Ferritin) is based on the immunoassay. using the Method which described. by Gomez et. al[13].,The ,antibody is used for a specific type of mice and is surrounded by a layer of magnetic atoms, which represent, the solid phase, and another antibody for a type of rat linked to derivatives of. Isoluminol. The work steps were carried out according to the instructions. of the Italian company DiaSorin, and equipped with the equipment. needed to measure the concentration of Ferritin.

5- Determining the incidence of thalassemia among males and females:

Class number) Percentage of thalassemia, incidence)%

$$= \frac{\text{Class number) Percentage of thalassemia, incidence)\%}}{\text{the total number}} * 100$$

6- Divide thalassemia patients into five age groups:

- The first age group. (2-6) years.

- The second age group. (7-11) years.

- The third age group .(12-16) years.

- The fourth age group. (17-21) years.

- The Fifth age group .(22-26) years.

7- Statistical analysis:

The results were analyzed by using the, SPSS 17 statistical program to determine the mean , standard deviation, and other variables. The differences in the case of the probability $p < 0.05$ were, considered statistically significant.

Results

1 -Thalassemia relationship with gender:

The current study, indicated that there, was a high percentage of thalassemia in males, which was 61.70%, while in females it decreased to 38.30%.

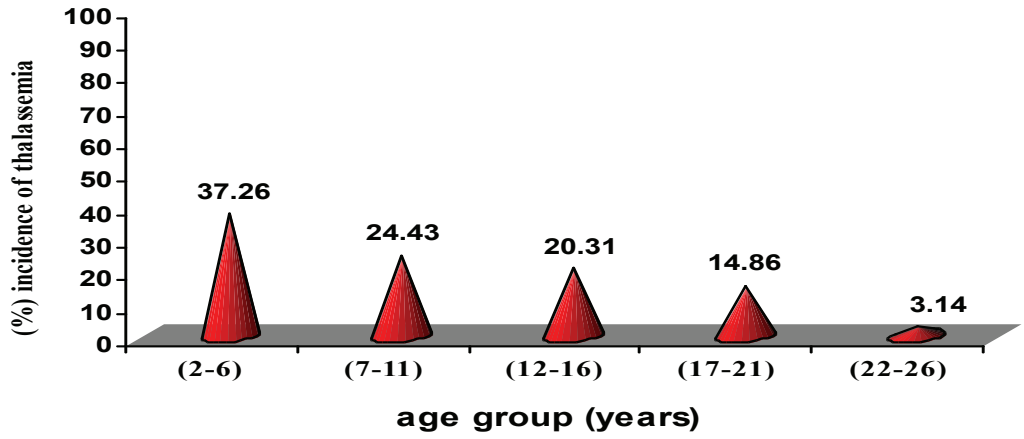

Figure 1: Thalassemia incidence, depending on gender.

2 -Thalassemia relationship with age:

The current ,study showed that the incidence ,of thalassemia reached its peak in the first age group (2-6) years, so the percentage was (37.26%), then the infection rate decreases, as the age progresses until the ,ratio gradually decreases to the lowest level in the, fifth age group (22-26 years), so it was (3.14%).

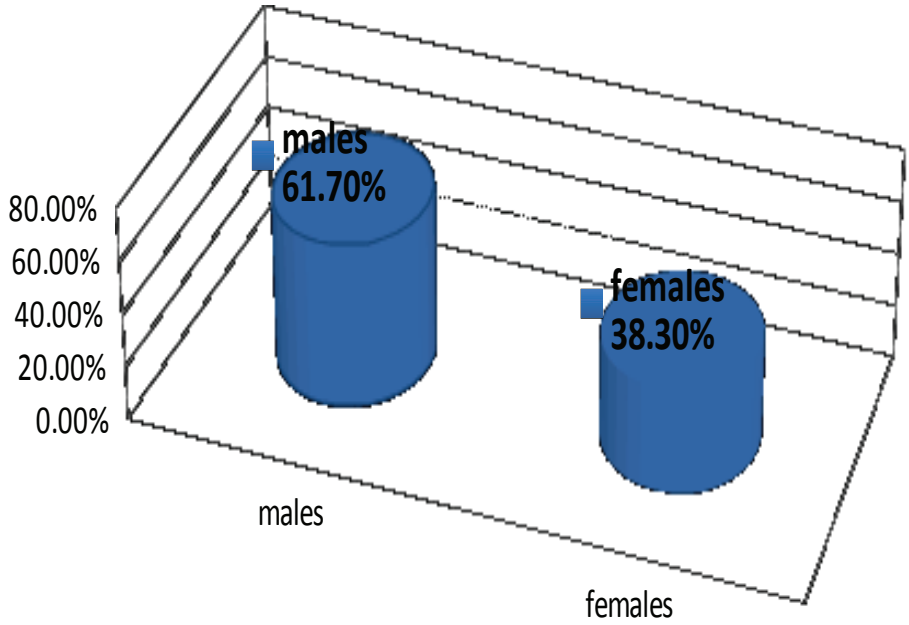

Figure 2: Distribution of age groups in thalassemia patients.

3 - Thalassemia effect on concentration of hemoglobin and Serum ferritin :

The results. of the current study showed a significant, decrease (P <0.05) in the hemoglobin Hb concentration in Thalassemia patients group (2) with a concentration of (7.76 ± 0.58 g / dL) compared, to the control group (1) it was (13.40 ± 0.92) g / dL.

The results recorded, a significant increase (P <0.05) in the concentration of ferritin in thalassemia, patients, group (2), it reached (3841.79 ± 942 µg / L) compared to the control .group (1) as it was (150.69 ± 44.72 /g / L) as shown In Table (1).

Table (1): The effect of thalassemia on hemoglobin concentration (Hb g / dL) and serum ferritin (µg / L).

Number of samples	Serum ferritin (µg/L)	Hb (g/dL)	Studied Parameters groups
40	150.69±44.72 b	13.40±0.92 a	(1) Control group
60	3841.79±942 a	7.76±0.58 b	(2) Thalassemia group
-	237.89	0.27	LSD

- The values represent the mean ± the standard deviation.

- The letters a, b indicate a significant difference (P <0.05) between the studied groups depending on the value of LSD.

4 - Effect of Thalassemia on liver enzymes levels :

The results. of the current study showed a significant increase (P <0.05) with GPT enzyme concentration in. Thalassemia patients (Group 2), it was (29.74 ± 5.40 U / ml) compared, to the control group (Group 1) which reached (17.04 ± 2.07 U / ml).

The results also recorded, a significant increase (P <0.05) with a concentration of GOT enzyme in group (2) reached ,(30.02 ± 7.23 U/ml) compared to control group (1) was (13.87 ± 0.70 U / ml).

The results indicated, a significant increase (P <0.05) with the, concentration of ALP enzyme in, group (2), it was (166.23 ± 8.45 U/ml) compared. to the control group (1) that, recorded (81.06 ± 26.79 U / ml).

Table (2): The effect of thalassemia on liver enzyme levels.

LSD	(2) Thalassemia group	(1) Control group	Groups Studied Parameters
1.45	29.74±5.40 a	17.04 ±2.07b	GPT (U/ml)
1.84	30.02±7.23 a	13.87±0.70 b	GOT (U/ml)
7.01	166.23±8.45 a	81.06±26.79 b	ALP (U/ml)

- The values represent the mean ± the standard deviation.

- The letters a, b indicate a significant difference (P <0.05) between the studied groups depending on the value of LSD.

Discussion

1 -Thalassemia relationship with gender:

It is clear. from Figure No. 1 that the males are more infected than females. The percentage, of thalassemia in males was 61.70%, while in females it decreased to 38.30%. There is no clear explanation. for this, although it is consistent with previous studies [4],, but the reason may be attributed to the possibility of a genetic association, with gender, as it may reflect the reality of the areas where. the disease is spreading where, increased Attention to males compared to females, in addition to the reluctance; of some families to visit hospitals when; the disease is present in females.

2 -Thalassemia relationship with age:

The current study ,showed in Figure (2) that the incidence of thalassemia reached its peak in the first age group (2-6) years, ;so the ratio was (37.26%), and the incidence, rate gradually decreased with age ,until it reached the lowest level in the fifth, age group (22-26) year , it was (3.14%), and the. reason for this may be an increase, in deaths of children with thalassemia. as the age progresses, either due to the cost of treatment., which is offset by the poor physical condition; of the patients, the reason may be the ,delayed of disease diagnosis to advanced. stages , when the treatment .may be not benefit with it, or the cause may be attributed; to the patient's poor health condition and suffering from severe complications of the disease .that may eventually lead to death [3] .

3 - Thalassemia effect on concentration of hemoglobin and Serum ferritin :

It was noted through .the results of the current study in Table (1) that there was a significant decrease (P <0.05) in the hemoglobin, concentration in thalassemia patients, as its. concentration (7.76 ± 0.58 g / dL) compared to the control group was (13.40 ± 0.92 g

/ dL), and the reason. for this may be attributed to the genetic mutation occurring. in both genes of beta-globin, resulting in a severe decrease in the ratio of, beta- globin, thus a decrease in hemoglobin, as a result of the breakage, of abnormal erythrocytes before the end of their shelf life 120 days [11], The Erythropoiesis ,process is ineffective and manifests itself through the apoptosis of .erythrocytes as a result of obstructing the manufacture of .heme units, as hemoglobin is mainly composed of two parts, heme and globin .protein, so the resulting red blood cells, are in small size , small numbers and low hemoglobin, and that high fracture ,due to the decrease Severe hemoglobin concentration, and the results of the; current study are consistent with what it reached [14], and this ,condition occurs when two of the damaged ,gene carriers of beta thalassemia .decide marriage and childbearing, as a result of ;which the patient will need to periodically, blood transfer every 3-4 weeks to ,maintain a high rate of Hb to allow the body to grow. in a healthy way [4].

Because thalassemia. is the most common genetic defect, in humans, around the world [15], genetic blood .disorders are characterized by a low level of hemoglobin (Hb) in red blood cells (RBC) and a very low production of ,red blood cells [6], ,and although this disease ,is characterized by a number of ,signs and symptoms, severe anemia is the only crucial thing [10].

The results, recorded a significant increase ($P < 0.05$) in the concentration of ferritin in ,thalassemia patients, as it reached (3841.79 ± 942 µg / L) compared to the control ,group, as it was 150.69 ± 44.72 µg / L), and the .reason for this may be periodic blood .transfusions Every 3-4 weeks, as multiple blood transfusions lead to increased. iron loading, which increases its; sedimentation in the body, which in turn. leads to an increase in ferritin .levels (the protein-storing iron) in the blood ,serum of patients. Thus, iron levels in the blood serum increase as they increase .and repeat blood transfusions [5]. Iron is stored primarily in the liver at 70%, and the remainder is .stored in the spleen and bone .marrow in the form of ferritin in addition to the presence of additional amounts of it in the .blood [3].

When conducting. blood transfusions to thalassemia .patients, the percentage of iron will increase., which leads to the inability of the ferritin to absorb it. Ultimately, free iron, is deposited in the blood and tissues [16]. This iron ,burden is usually reflected by increasing serum .ferritin levels and controlling it on Long-.term correlation with survival, estimating the concentration of ,ferritin in the blood is an indicator for measuring total iron in the body [15].

4 - Effect of Thalassemia on liver enzymes levels :

Table 2 shows a significant, increase ($P < 0.05$) in GPT enzyme concentration in Thalassemia patients, as it .was 29.74 ± 5.40 U / ml) compared to the control group that reached (U / ml 17.04 ± 2.07). The .results also recorded a significant increase ($P < 0.05$) in a concentration of GOT ,enzyme in Thalassemia patients, as it reached (30.02 ± 7.23 U / ml) compared .to the control group that was (U / ml) 13.87 ± 0.70. The results ,indicated a significant increase ($P < 0.05$) in the ALP enzyme .concentration of thalassemia patients it was ((166.23 ± 8.45 U / ml) compared to the control group that, recorded (81.06 ± 26.79 U / ml), may be the reason. for the high concentrations of liver enzymes due to very high levels of. ferritin that collects primarily in The liver shows its .negative effects on the liver, enzymes clearly [4,17].

A slight positive .relationship has been observed between serum ferritin with hepatic enzymes and hemoglobin in thalassemia patients., as high levels of hepatic enzymes are. related to some extent to the concentration of, ferritin in the serum, Or because of the need to build peptide .chains through the effectiveness of ,this enzyme In the formation of amino acids required for construction [16].

The GOT enzyme is more. effective than the GPT enzyme. The first enzyme is more common in the tissue of the heart, liver, and ,skeletal muscles as well as the kidneys, while the liver .contains large amounts of the GPT enzyme, and other tissues .such as the kidneys, heart, and skeletal muscles ,contain abundant amounts of this enzyme, possibly due to the amounts, of iron present in patients' serum, which is deposited in these organs, which results, in the breakdown of the fat of some cells of ,these organs [3].

As for the ALP enzyme, the research, results showed a significant increase in the activity of this enzyme compared to the enzyme, activity in the healthy serum.

The reason for this may be due to; the fact that most of The activity of this enzyme comes from bone tissue and ,since thalassemia patients suffer from the dissolution of this tissue, this leads to the leakage, of this enzyme into the blood circulation ,and then an increase in the effectiveness of this enzyme [15].

Iron is one of the essential minerals that. the body needs to complete the formation of ,erythrocytes, oxidative .metabolism, and cellular immune response, and plays, an essential role in many body ;functions. Iron is the site of oxygen binding in Heme-containing proteins such as, hemoglobin and myoclobin,[18]. Iron is absorbed ,by the gut and transported to the blood by a special ,protein called Transferrin, as the receptors for this carrier protein are found on; bone marrow cells and some ,other cells of the body, and the ability of this ,protein to bind to iron increases. in the event of high iron ,consumption by the body [15].

Iron deposition in the liver, spleen and bones resulting from the blood transfusion ,process may cause distortions in the shape of the body and its deposition in the organs that we have mentioned;, which leads to their enlargement [5]. Therefore., this requires working to displace iron to reduce ,complications, by giving the patient medications to remove iron from the body and other drugs. such as folic acid, Vitamin C, including, desiferal injections, is injected under ,the skin for five days a week, in addition to another ,treatment, Exjade pills, given ,daily once, according to the child's weight, given, his dose of (15 mg per kilo - 40 mg per kilo) depending on the patient's weight and ,high iron content. In His Blood [19,20].

Conclusions and recommendations:

Conclusion

We conclude from this study:

1. The higher. incidence of thalassemia in males than in females

2. The incidence of infection decreased ,with age, and it peaked in the age group (2-6) years and decreased to the lowest. levels in the age group (22-26) years.

3. There is a decrease in hemoglobin .concentration and high serum ferritin concentration in people with. thalassemia, compared with healthy people.

4. There was an increase in the; levels of hepatic enzymes (GPT, GOT, ALP) in patients ,compared to healthy levels.

5. Deposition, of iron in patients' bodies adversely affects liver function.

6. There is a positive .correlation between serum ferritin and liver enzymes in thalassemia patients.

Recommendations

1. Intensifying awareness; campaigns through various media such as newspapers, magazines, television and lectures and publishing, brochures on thalassemia as it is a genetic, disease that may be limited by proper, planning and avoiding ,marriage of relatives as much as possible in families with a history of the disease.

2. Specialized, centers shall be determined to work on finding a formula for recording the numbers. of the injured, their addresses and the sick, history of the family, from grandparents to parents. And the establishment of centers specialized in bone marrow transplantation as part of the. treatment requirements for thalassemia.

3. Emphasis on conducting a pre-marriage screening, test for thalassemia, and adding it to the list of examinations, conducted for this purpose may help with medical advice ,before marriage.

4. Emphasis on prenatal, diagnosis, especially in families where the disease appears frequently.

5. Urging families with a history of .thalassemia to determine childbearing in order to reduce the frequency of infection, in the same family.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

References

1. Baig SM, Azhar A, Hassan H, Baig JM, Aslam M, Ud Din MA, et al. Prenatal diagnosis of β-thalassemia in Southern Punjab, Pakistan. Prenatal diagnosis. 2006;26(10):903–905.
2. Azarkeivan A, Hashemieh M, Akhlaghpoor S, Shirkavand A, Yaseri M, Sheibani K. Relation between serum ferritin and liver and heart MRI T2* in beta thalassaemia major patients. East Mediterr Health J. 2013;19(8):727–32.
3. Wahidiyat PA, Iskandar SD, Rahmartani LD, Sekarsari D. Liver iron overload and hepatic function in children with thalassemia major. Paediatrica Indonesiana. 2018;58(5):233–7.
4. Al-Moshary M, Imtiaz N, Al-Mussaed E, Khan A, Ahmad S, Albqami S. Clinical and Biochemical Assessment of Liver Function Test and Its Correlation with Serum Ferritin Levels in Transfusion-dependent Thalassemia Patients. Cureus. 2020;12(4).
5. Sobhani S, Rahmani F, Rahmani M, Askari M, Kompani F. Serum ferritin levels and irregular use of iron chelators predict liver iron load in patients with major beta thalassemia: a cross-sectional study. Croatian medical journal. 2019;60(5):405.
6. Cappellini M, Cohen A, Eleftheriou A, Piga A, Porter J, Taher A. Guidelines for the Clinical Management of Thalassaemia [Internet]. Thalassaemia International Federation TIF; 2008.; 2014.
7. Koonrungsesomboon N, Chattipakorn SC, Fucharoen S, Chattipakorn N. Early detection of cardiac involvement in thalassemia: From bench to bedside perspective. World journal of cardiology. 2013;5(8):270.
8. Vichinsky E, Butensky E, Fung E, Hudes M, Theil E, Ferrell L, et al. Comparison of organ dysfunction in transfused patients with SCD or β thalassemia. American journal of hematology. 2005;80(1):70–74.
9. Kontoghiorghes GJ. Iron mobilization from transferrin and non-transferrin-bound-iron by deferiprone. Implications in the treatment of thalassemia, anemia of chronic disease, cancer and other conditions. Hemoglobin. 2006;30(2):183–200.
10. Galanello R, Origa R. Beta-thalassemia. Orphanet journal of rare diseases. 2010;5(1):11.
11. BORGNA-PIGNATTI C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, et al. Survival and complications in thalassemia. Annals of the New York Academy of Sciences. 2005;1054(1):40–47.
12. Tietz NW, Burtis CA, Duncan P, Ervin K, Petitclerc CJ, Rinker AD, et al. A reference method for measurement of alkaline phosphatase activity in human serum. Clinical chemistry. 1983;29(5):751–761.
13. Gella FJ, Olivella T, Pastor MC, Arenas J, Moreno R, Durban R, et al. A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clinica chimica acta. 1985;153(3):241–247.
14. Agarwal MB. Advances in management of thalassemia. The Indian Journal of Pediatrics. 2009;76(2):177–184.
15. Sengsuk C, Tangvarasittichai O, Chantanaskulwong P, Pimanprom A, Wantaneeyawong S, Choowet A, et al. Association of iron overload with oxidative stress, hepatic damage and dyslipidemia in transfusion-dependent β-thalassemia/HbE patients. Indian Journal of Clinical Biochemistry. 2014;29(3):298–305.
16. Al Hawsawi ZM, Sairafy MH, Tarawah AM, Zolaly MA, Al Hegaily ARS. Experience with Combination Therapy of Deferiprone and Desferrioxamine in β-Thalassemia Major Patients with Iron Overload at Maternity and Children Hospital, Al Madinah Al Munawarah, Saudi Arabia. Journal of Taibah University Medical Sciences. 2010;5(1):27–35.
17. Ameli M, Besharati S, Nemati K, Zamani F. Relationship between elevated liver enzyme with iron overload and viral hepatitis in thalassemia major patients in Northern Iran. Saudi medical journal. 2008;29(11):1611–1615.
18. Mariani R, Trombini P, Pozzi M, Piperno A. Iron metabolism in thalassemia and sickle cell disease. Mediterranean journal of hematology and infectious diseases. 2009;1(1).
19. Makis A, Hatzimichael E, Papassotiriou I, Voskaridou E. 2017 Clinical trials update in new treatments of β-thalassemia. American journal of hematology. 2016;91(11):1135–1145.
20. Ellis, J N. Update on iron chelators in thalassemia. Hematology. 2010 Dec 4;2010(1):451–5.