

## **Physiological study for some blood parameters in children with major B-Thalassemia in Al- Najaf governorate / Iraq.**

**دراسة التغيرات في بعض معايير الدم الفسلجية لدى الأطفال المصابين بمرض بيتا-تلاسيميا العظمى في محافظة النجف الأشرف / العراق .**

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### **Abstract.**

$\beta$ -Thalassemia is more an inherited anemia publicity that common in the world and especially in Mediterranean region, and the most important cause of mortality in patients with  $\beta$ -thalassemia . The present study carried out on ( 75 ) children of males and females thier age from (1-12 ) years with B- thalassemia in Al- Najaf ,there were (60%)child of them infected with B- thalassemia major , who consult the thalassemia center for teaching Al- Zahra Hospital for birth and children in Najaf governorate, while ( 15 ) healthy child used as a control group. The results presnt study showed that a significant decrease (  $p < 0.05$  ) in number of red blood cells and the concentration of heamoglobin and packed cell volume ( PCV ) and the values of the mean corpuscular volume ( MCV ) and the number of blood platelets in the blood of infected children with  $\beta$ - thalassemia major compared with healthy children, while the results showed a significant increase (  $P < 0.05$  ) in the total number of white blood cells in children infected with the disease compared with the control group, as the current research distncted that the ratio of males infected with  $\beta$ - thalassemia higher than the ratio of females ( % 68.33 , % 31.67 respectively ) , also children had blood group ( A ) were the most susceptible to the infection with  $\beta$ -thalassemia ( 38.33 % ) compared with the other blood groups , as the infected children who carry the factor rhesus positive (  $Rh^+$  ) were ( % 65 ) higher than the patients with rhesus factor negative (  $Rh^-$  ) (35 %).

Key words:-  $\beta$ -Thalassemia major, MCV, PCV, heamoglobin, blood parameters.

### **الخلاصة**

مرض التلاسيميا - نوع بيتا من أكثر أمراض فقر الدم الوراثية الشائعة على مستوى العالم بشكل عام وفي منطقة البحر الابيض المتوسط بشكل خاص , و يعد سبب مهم في ارتفاع معدل الوفيات بين الاشخاص المصابين به , هدفت الدراسة الحالية دراسة التغيرات في بعض معايير الدم الفسلجية لدى الأطفال المصابين بالبيتا - تلاسيميا العظمى في محافظة النجف , شملت الدراسة متابعة (75) طفلا من الذكور والإناث تراوحت اعمارهم (1-12) سنة , كان بينهم (60) طفلا مصابا بمرض بيتا - التلاسيميا الكبرى والذين يراجعون مركز التلاسيميا التابع لمستشفى الزهراء التعليمي للولادة والاطفال في محافظة النجف , بينما كان (15) طفلا أصحاء مثلوا مجموعة السيطرة .

لقد أظهرت نتائج الدراسة الحالية وجود انخفاض معنوي ( $p < 0.05$ ) في اعداد كريات الدم الحمر وتركيز الهيموغلوبين (Hb) وحجم الخلايا المضغوطة (p.c.v) و قيم معدل حجم الكرية (MCV) وأعداد الصفائح الدموية في الاطفال المصابين بالمرض مقارنة مع مجموعة السيطرة , بينما بينت النتائج ارتفاعا معنوياً ( $p < 0.05$ ) في العدد الكلي لخلايا الدم البيض في الاطفال المرضى بالمقارنة مع الاطفال الاصحاء . كما تبين من خلال البحث الحالي ان نسبة الذكور المصابين بمرض بيتا-تلاسيميا العظمى (68.33%) كانت أعلى من نسبة الإناث المصابات (31.67%) , في حين كانت نسبة الاطفال المصابين بالمرض من فصيلة الدم A والتي بلغت (38.33%) هم الاكثر عرضة للإصابة بالمرض مقارنة مع المصابين من فصائل الدم الاخرى , كما كانت نسبة الاطفال المرضى الحاملين لعامل الريسوس الموجب  $Rh^+$  والتي بلغت (65%) اكثر من نسبة الاطفال المصابين الاخرين ذات العامل الريسوس السالب  $Rh^-$  (35%) .

## **Intoduction-**

Thalassemia is the most important diseases of hemolytic anemia, and it is a Greek word originally that means Mediterranean anemia ,this disease was known and resounded in this region significantly so-called anemia Mediterranean(1, 2, ,3)and was diagnosed and known their kinds by scientists Lee and Cooley in 1925,so it was called as Cooley's anemia in the United States (4, 5) .

Thalassemia is a genetic disorder in biosynthesis in the globin chains of normal hemoglobin because genetic mutations in the genes that responsible for the globin chains production (Alpha and Beta) (6 , 7 ), the alpha- globin chains were encoded by four genes are located on chromosome 16 while beta -globin chians located on chromosome 11( 8,9), mutation happend in one of the globin chains (beta or alpha) or both,therefore, thalassemia are classified depending on the type of affected globin chain to the Alpha and Beta thalassemia also each type is divided by the number of genetic mutationsins globin chains to secondary varieties, beta-thalassemia is more frequent and widespread to few of controlling genes( 10,11,12).

The types of thalassemia (alpha and beta) which undergo all genes to amutations are the species most dangerous to a pantient's life ( 13,14),alpha- thalassemia major causes hydrops fetalis of the embryo and his death before he was born, either  $\beta$ - thalassemia major causes severe anemia, life-threatening and the patients need to blood transfusion periodicity each(3-4)weeks according to age and lack degree of hemoglobin ( 15,16). as a result of the inability of the body to form normal red blood cells, but the red blood cells are abnormal and small-sized and full of inculsion bodies that make up the result of the accumulation of alpha and beta chains inside the corpuscular making hemoglobin insoluble and this slushy abnormal condition which the hemoglobin is dissolved in corpuscular( 17,18)or a result to continuation production of alpha chains and the lack or absence of the production of beta chains leading to accumulation the alpha chains in corpuscular causing broken the plasma membrane and damage the corpuscular in bone morraw( 19,20 ). and when abnormal corpuscular exit to blood cycle would removed by the spleen, causing severe anemia (21,13) .

The constant blood transfer causes a condition called iron accumulation resulting from the deposition of iron in the body of the patients gradually leading to derangement of the functions of the cells and thus her death(22,23) and affects many organs of the body, such heart, liver, lungs, kidney and the endocrine glands causing failure( 24,25), beta-thalassemia major may detect during the first months six from the child's age, and the disease leads to a number of complications if not treated as yellowing of the skin (jaundice) and enlargement of the liver and spleen and growth retardation in addition to malformations in the bones of the face and diabetic and disease of the heart muscle(19,26). Beta-thalassemia major are diagnosed by examining the haemoglobin etectrophoresis movement ,where a high amount of fetal hemoglobin due to loss of beta chains leads to increased production of gamma chains which combine with alpha chian to form the fetal hemoglobin which is very familiarity to oxygen and prevents the liberation to the tissue(27,28,29) the aim of this study to detection some physiological blood parameter ,sex ,blood group and factor rhesus related with B-thalasemia.

## **Materials and Methods. -**

### **-Patients**

The present study included examne (60) child of( male and female) patients with B-thalassemia major and who are reviewing periodically the center of the thalassemia of the teaching Alzahra hospital for birth and children in the Najaf government and then the results that obtained compared with the control group that included (15) healthy child.

**The collection of information.-**

Information was collected by preparing special menus recorded the necessary information and special of the research samples have included the age / sex / blood group/rheuses factor.

**-The collection of blood samples.**

Blood samples were collected between the hours of nine and ten in the morning using medical syringes and then the samples placed in a tubes container the material anti- coagulation (EDTA), to do blood tests included in this study .

**Study the physiology blood parameters. -**

**Count the number of red blood cells.-**

Used absorbent with Bead red ,the blood was pulled to the mark (0.5) and then pull the Hyem's solution to the mark (101), Shake the mixture lightly, then put a drop of on a slide count and left the slide to stabilize the cells and then count cells under (40 X) and then extracted the number of red blood cells by applying the following equation(30):-

Red blood cell count/mm<sup>3</sup> =  $N \times 80/400 \times 200$

N: number of red blood cells calculated .

**Estimate the concentration of hemoglobin. -**

Use solution's Draken who put (5 ml) in the tube, then Added (0.02) ml of blood sample to the tube and shook the tube well, and left for (10) minutes and then placed in a hemoglobinmeter with wavelength (450)nm (31).

**-Measurement the packed cells volume(PCV).**

Put amount of blood in the capillary tube to three quarters and then fill one end with the mud artificial,then tube placed in a microcentrifuge and the exact role of the fast (10000) cycle / minute for ( 5) minutes and then read a percentage of the packed cells volume(PCV) by using special standard ruler (32).

**Count the total number of white blood cells.-**

The blood was hauled by the special pipette after the white blood cells which has the white bead to the sign (0.5) .Then, with the same pipette diluted solution was hauled to the sign (11)and shook the pipette slightly and put a drop of the diluted blood on the count slide after putting a cover on it ,the slide was left in order to make the cells be stable ,then it was examined under small force (10x).In the four big squares (the 64 small squares ) ,the white blood cells were counted in mm<sup>3</sup> according to the following equation (31):-

Total count of white blood cells in mm<sup>3</sup> =  $N \times 4/10 \times 20$

N: number of counted white blood cells.

**Count number of the blood platelets.-**

Draw blood by a pipette with bead red to mark( 0.5), then pull dilution solution to the mark (101)and the pipette shaking lightly and put a drop on the silde of counting,then left to settle cells then the platelets were counted in mm<sup>3</sup> according to the following equation (33):-

The number of blood platelets =  $N \times 200 \times 5$

N: number of blood platelets calculated.

**Measure Mean Corpuscular Volume(MCV). -**

The values of mean corpuscular volume(MCV)were extracted according to Godkar(34).

### **Statistical analysis. -**

The results were analysed by using F-test with a significant level ( $p < 0.05$ ) for showing the results significant (35).

### **Results**

Effect  $\beta$ -thalassemia major on number of the red blood cells, concentration of haemoglobin, packed cell volume (PCV) and mean corpuscular volume (MCV). The results showed a significant decrease ( $p < 0.05$ ) in the number of the red blood cells and concentration of haemoglobin and packed cell volume and mean corpuscular volume in patient children with  $\beta$ -thalassemia major ( $31.4 \pm 0.39$ ,  $6.14 \pm 1.59$ ,  $24.87 \pm 4.51$ ,  $68.14 \pm 21.25$ ) respectively compared with the control group ( $4.46 \pm 0.51$ ,  $13.24 \pm 0.59$ ,  $37.68 \pm 0.91$ ,  $82.54 \pm 1.65$ ) respectively (Table 1).

Effect  $\beta$ -thalassemia major on the total number of white blood cells and number of the blood platelets. Table (2) showed that a significant increase ( $p < 0.05$ ) on the total number of white blood cells in blood of patient children with  $\beta$ -thalassemia ( $3.144 \pm 0.39$ ) and showed a significant decrease ( $p < 0.05$ ) in the number of the blood platelets in patient children with  $\beta$ -thalassemia major ( $3.144 \pm 0.51$ ) compared with the control group ( $9.550 \pm 0.80$ ,  $4.467 \pm 0.51$  respectively).

The correlation between the sex, blood group and Rhesus factor (Rh) and  $\beta$ -thalassemia major. The results of present study revealed that the ratio of patient males with  $\beta$ -thalassemia major (%68.33) was higher than the ratio of patient females (%31.67) (figure 1), while the figure (2) illuminates that the patient children with  $\beta$ -thalassemia major from blood group A (%38.33) more infection with the disease, then patient children with blood group O (%23.33), then patient children with blood group AB (%20) and last patients with blood group B (%18.34). as the infected children who carry the factor rhesus positive (Rh+) were (%65) higher than the patients with rhesus factor negative (Rh-) (35 %) (figure 3). As the interference between the blood group and Rhesus factor (Rh) and infection repetition with  $\beta$ -thalassemia major might be arranged by the following figure (A+>O+>B+>A-, AB+>O-, B-).

### **Discussion**

Effect  $\beta$ -thalassemia major on number of the red blood cells, concentration of haemoglobin, packed cell volume (PCV) and mean corpuscular volume (MCV). The results showed a significant decrease in the number of red blood cells in children with  $\beta$ -thalassemia major when compare with the control group (Table 1). The cause returns to the genetic mutations that occur in the genes responsible for the synthesis of protein chains in hemoglobin which lead to disorder in the biosynthesis of the chains globin and thus loss the imbalance in the manufacture of hemoglobin (36,12) and this affects in the number and shape and size of red blood cells during the formation stages in the bone marrow, where red blood cells are small size and therefore red blood cells do not hold the same volume occupied by normal red blood cells (37,1), as lack the correlation of globin chains by wall membrane red cell lead to collect globin chains inside the red cell and formation the inclusion bodies that are prone to phagocytosis by macrophages that exist numerously in bone marrow and remove the red blood cells during development stages (38,22) and the red blood cells that enter into the circulatory system may be broken yours truly or broken inside the spleen by macrophages that riddance the blood from the malformed and aged (13,37).

The result of this study are consistent with the results of several studies (5,3,39). Either decrease in hemoglobin concentration was due to a genetic defect in synthesis globin chains leading to the lack or low production globin chains ( $\alpha$ ,  $\beta$ ) in hemoglobin (40) and this leads to the production of a small number of red blood cells that are small in size and hypochromic (22,20) as well as broken a large number of red blood cells due to a significant decrease in hemoglobin concentration (13). and this result agreed with the results of many studies (3,39). Either respect by the packed cells volume (PCV) due to the PCV depends mainly on the number, size and shape of red blood cells and the extent of blood dilution and viscosity (30).

But with regard to the decline in the values of the mean corpuscular volume (MCV) was due to the relationship between MCV and the values of PCV and number of red blood cells , which were low, so down the values of MCV about the normal range(MCV<75fl), which indicate that patients with  $\beta$ - thalassemia major undergo from microcytic anemia (34,20).

Effect  $\beta$ - thalassemia major on the total number of white blood cells and number of the blood platelets.

Table(2)showed that a significant increase ( $p<0.05$ ) on the total number of white blood cells in blood of patient children with  $\beta$ -thalassemia major and showed a significant decrease ( $p<0.05$ ) in the number of the blood platelets in patient children with  $\beta$ -thalassemia major compared with the control group.

The increase of the total number of white blood cells in the blood of children with  $\beta$ -thalassemia major may be due to break high percentage of red blood cells inside and outside bone marrow which stimulates secretion erythropoietin hormone from the kidney which stimulates the bone marrow to increase formation of different blood cells included white blood cells (41,26).or due to rise some of cells of liver and spleen to formation centers to generation the blood cells (42),in addition the infection with thalassemia causes increasing of monocytes which broken the malformed red cells (15,43). These results agreed with results of some studies(21 ,39). And the cause of decline in the number of platelets in the blood of infected children went to the enlargement of the spleen, which is destruction of blood cells, including blood platelets(44). The result of the present study are consistent with studies of researchers (3) and (39). The correlation between the sex ,blood group and Rhesus factor(Rh) and  $\beta$ -thalassemia major.But with regard to the relationship of sex and thalassemia (Figure 1) shows that the percentage of infected males with  $\beta$ -thalassemia major higher proportion of females infected with the disease and in fact there is no relationship between sex and thalassemia because the genes controlling in the production globin chains in hemoglobin located on chromosomes somatic (11, 16) and is not located on the sex chromosomes(X,Y) (17,12).

Either the relationship between blood groups and factor Rhesus and repeat infection with  $\beta$ -thalassemia Figure (3,2) is observed that the blood group (A) and children with factor Rhesus positive(Rh+) were more compared with the other blood groups may be due to the small sample or because of varying proportions of these groups in community.

Table(1):- Effect  $\beta$ - thalassemia major on number of the red blood cells , concentration of haemoglobin,packed cell volume(PCV) and mean corpuscular volume(MCV).

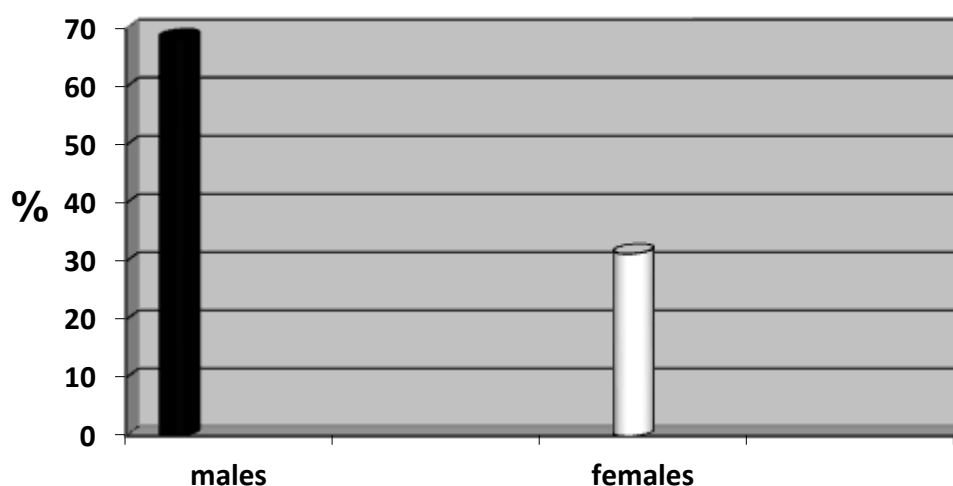
Mean Corpuscular Volume fl (MCV)	packed cells volume (%) (PCV)	concentration of hemoglobin. mg/dL	number of red blood cells. Million/mm <sup>3</sup>	samples
Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	
82.54 $\pm$ 6.1	37.68 $\pm$ 0.91	1 3.24 $\pm$ 0.59	4.46 $\pm$ 0.51	Control group
68.14* $\pm$ 2.25	24.87 * $\pm$ 4 .5	6.14* $\pm$ 1.59	3.14* $\pm$ 0.39	Patients
P<05.0	P<05.0	P< 05.0	P< 05.0	Probable level

:- represents a significant difference at the control group. \*

Table(2):- Effect  $\beta$ - thalassemia major on on the total number of white blood cells and number of the blood platelets.

total number of white blood cells/ mm <sup>3</sup>	number of blood platelets / mm <sup>3</sup>	samples
Mean $\pm$ SD	Mean $\pm$ SD	
9.550 $\pm$ 0.80	4.467 $\pm$ 0.51	Control group
14.839* $\pm$ 1.02	3.144* $\pm$ 0.39	Patients
P< 05.0	P< 05.0	Probable level

:- represents a significant difference at the control group.\*



Figuer(1):Relationship between  $\beta$ - thalassemia major and the Sex .

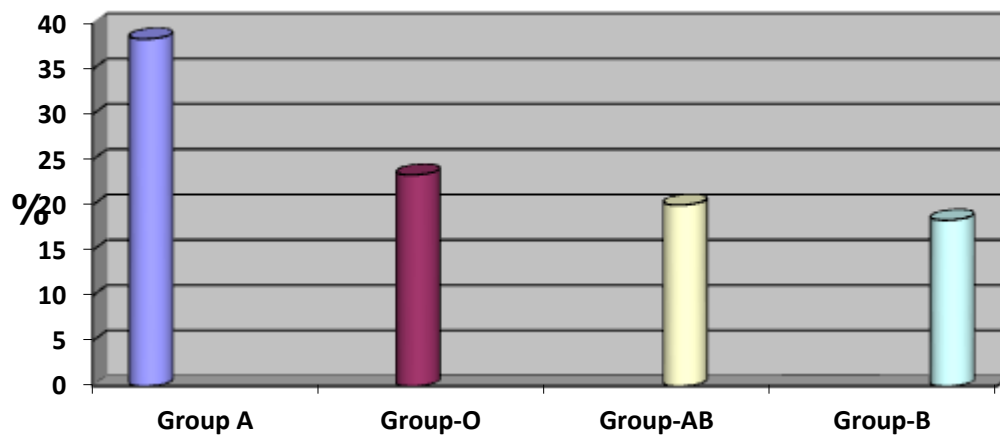


Figure:(2)Relationship between  $\beta$ - thalassemia major and the Blood groups .

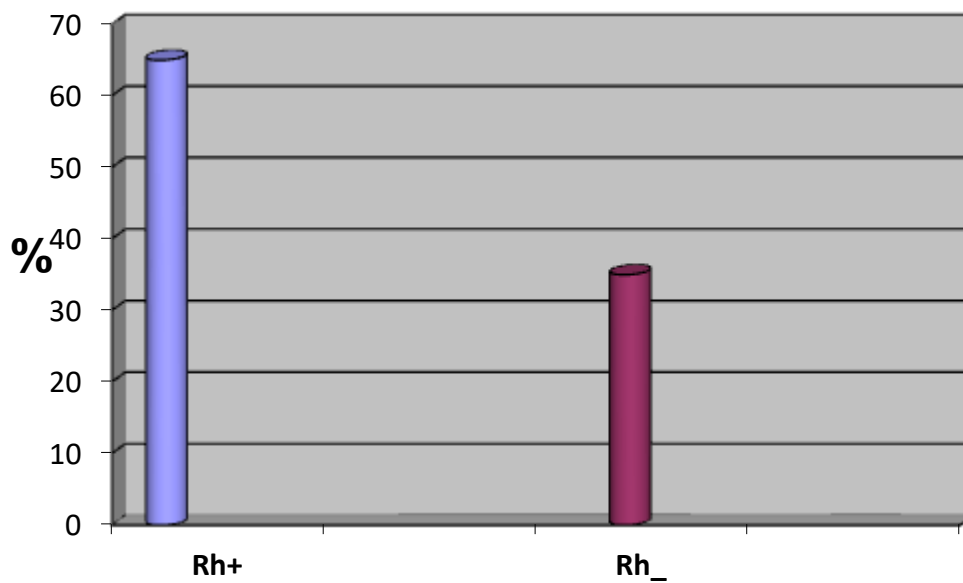


Figure:(3)Relationship between  $\beta$ - thalassemia major and the Rhuses factor(Rh).

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