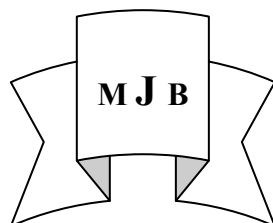


Hematological Changes in Children Suffering From Beta Thalassemia In Babylon Governorate

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Abstract

This study was designated to investigate the changes that occurs in certain blood characteristics in beta thalassemia minor and major .The total number used was 300,150 healthy children and 150 suffering from beta thalassemia. Their ages (1 – 14 years old) were divided into four age groups (1 ≤ 3 years ; >3 – 5 years ; >5 - 10 years and >10 – 14 years).Hematological changes ,covered red blood cells(RBCs) count and hemoglobin concentration (Hb) in both types of beta thalassemia were significant ($P<0.01$) decrease than control subject. The values of packed cell volume (PCV)in two types of beta thalassemia showed significant ($P<0.05$) decrease than control subject. RBCs indices (MCV and MCH) showed a significant ($P<0.05$) decrease when compared to control subject, while MCHC showed no significant difference. Osmotic fragility of RBCs showed a significant decrease ($P<0.01$) in two types of beta thalassemia when compared to control subject.Concerning hemoglobin electrophoresis in both types of beta thalassemia. The values of HbA showed significant decrease ($P<0.01$)than control subject. As well as a significant difference ($P<0.05$) in HbA values recorded between beta thalassemia minor and major. Values of HbA2 and HbF showed significant ($P<0.05$) higher concentration than control subject.It has also been found that there is significant difference ($P<0.05$) in HbF values between the two types of beta thalassemia. Changes may be attributed to the decrease or absence of biosynthesis of beta globin chains which lead to severe hemolytic anemia and severe hypoxia.

الخلاصة

صممت هذه الدراسة لمعرفة تأثير مرض فقر الدم البحرى البائى وبكلا نوعيه الصغير والكبير على بعض المتغيرات الدمويه. شملت هذه الدراسة فحص ٣٠٠ طفل من المصابين وغير المصابين (الاصحاء) تراوحت اعمارهم بين (1 الى ١٤ سنة) وقد قسمت اعمارهم الى اربع مجاميع (المجموعة الاولى ١- ٣ سنوات، المجموعة الثانية ٣- ٥ سنوات ، المجموعة الثالثة < ٥ - ١٠ سنوات ، المجموعة الرابعة < ١٠ - ١٤ سنة) . تميزت التغيرات الدموية بانخفاض العدد الكلى لكريات الدم الحمراء (RBCs) وتركيز خضاب الدم (Hb) وبصورة معنوية ($P<0.05$) مقارنة بالاطفال الاصحاء ، اما قيم حجم الخلايا المضغوطة (PCV) فقد اظهرت انخفاضاً معنوياً ($P<0.01$) في هذا المرض . سجلت تراكيز الهيموكلوبين الخلوي (MCV و MCH) انخفاضاً معنوياً ($P<0.05$) اما الدليل (MCHC) فانه لم يسجل انخفاضاً معنوياً مقارنة بالاطفال الاصحاء ولوحظ بان الهشاشة الازموزية لكريات الدم الحمراء (RBCs Fragility) قد انخفضت بصورة معنوية ($P<0.01$) . سجلت انواع خضاب الدم (HbA,HbA2,HbF) انخفاضاً معنوياً ($P<0.05$) في قيم ال (HbA) في الاطفال المصابين بهذا المرض ولوحظ ايضا هناك فرق معنوي في قيم ال (HbA) بين كلا النوعين من هذا المرض . اما بالنسبة للنوعين (HbA2 , HbF) لوحظ هناك ارتفاع معنوي ($P<0.05$) بين كلا النوعين من هذا المرض . ان المتغيرات الحاصلة في نتائج هذه الدراسة يمكن ارجاعها بصورة رئيسية الى انخفاض او فقدان التصنيع الحيوي لسلاسل متعدد الببتيد من نوع بيتا والتي تؤدي الى تكسير كريات الدم الحمراء ونقص الاوكسجين وترسيب الحديد في اعضاء الجسم .

Introduction

The thalassemia syndrome was first recognized by Vondaksh1889, beta thalassemia major was first recognized by Cooley and Lee 1925, while beta thalassemia minor was determined by Wintrobe 1940 [1]. The thalassemia is the term derived from Greek word “thalassa” which means the sea because of high incidence of such cases in the area surrounding the Mediterranean sea [2]. The thalassemia syndromes are a heterogeneous of mendelian disorders, all characterized by decreasing or absent biosynthesis of either alpha or beta globin chains of adult hemoglobin [3]. Thalassemias are classified into two types, alpha thalassemia which is characterized by decrease or absence of biosynthesis of alpha globin chains and beta thalassemia which is characterized by decrease or absence of biosynthesis of beta globin chains [4]. Beta thalassemia is the commonest type of thalassemias which can be classified into two types, beta thalassemia minor and major [5]. The beta thalassemia caused by several point mutations rather than other mutations [6, 7]. Hence, there is a defective production of beta chain mRNA because of abnormal transcription or premature termination of mRNA [2]. This decrease or absence of beta chains that caused several hematological changes lead to a decrease in survival of red blood cells [8, 9]. Beta thalassemia is considered the common type of thalassemias which occurs at sixth month after the birth [10]. This type of thalassemias occurs due to genetic defect in one or both beta globin genes which lead to impairment of messenger RNA transcription resulted in the decrease or the absence of beta globin chains. The disorder occurs in beta globin genes due to point mutations rather than other mutations such as deletion or insertion mutations

[6]. Beta thalassemia minor is characterized by decrease of beta globin chains biosynthesis leads to the moderate increase HbF and HbA₂, While beta thalassemia major characterized by absence or severe decrease of beta globin chains biosynthesis resulted in high increase of HbF with a moderate increase of HbA₂ [11, 12, 13]. The decrease or absence of biosynthesis of beta globin chains resulted in accumulation of alpha globin chains within the developing red cells [8, 4]. The alpha chains production increased in some amounts and causing insoluble aggregations in red cells [14]. These chains are precipitated on the cell membranes causing abnormal red cells to the destruction of red cells in bone marrow (ineffective erythropoiesis) and also the destruction occurs during the passage in the spleen [15]. Gamma and delta chains are increased to compensate to some extent for the lack of beta chains lead to increase HbA₂ and HbF [16]. The destruction of red cells leads to chronic anemia, increased erythropoietin, hypoxia, hemosiderosis and bone marrow hyperplasia [10]. Several studies recorded a decrease count of RBCs in beta thalassemia [17, 18]. Other studies showed an increase count of RBCs [19, 20]. Several studies which showed a decrease of PCV in beta thalassemia [21, 22, 23]. Several studies recorded the decrease of MCV, MCH and MCHC in beta thalassemia [16, 11, 24, 25, 26]. The osmotic fragility of the red blood cells reflects their ability to take up water without lysis and it is determined by their volume to surface ratio [27]. The ability of the normal RBCs to withstand hypotonicity results from its biconcave shape which allows the RBCs to increase its volume by about 70% before the surface membrane is stretched. Several studies showed the

decrease of osmotic fragility in beta thalassemia [28, 22, 29 ,7].The adult Hb is the common type of hemoglobin during adult life , with minor component of HbA2 and HbF.The beta thalassemia is characterized by decreasing HbA and increase HbA2 with HbF[3].The production of delta and gamma globin chains lead to elevated HbA2 and HbF [30, 8].Several studies showed the decrease of HbA and increase of the HbA2 with HbF in beta thalassemia [31, 32 , 23].To fulfill these objectives, the present study has dealt with a relatively large sample of children of different ages in Babylon Province to determine: Hematological changes in both types of beta thalassemia.

Materials and Methods

Materials

The subject of the study

This study was carried out over a nine month period , from October 1999 to june 2000 .The subjects of the study were 300 patients and healthy children.Their ages ranged from 1 year to 14 years.These ages were divided into four groups ; first group $1 \leq 3$ years (toddler child) ; second group $>3 - 5$ years (preschool child) ; third group $>5 - 10$ years (older child) and fourth group $>10 - 14$ years (adolescent)⁽³³⁾.All subject belong to the middle class of the society.The study was carried out before doing blood transfusion for all patients .The total number of patients were 150 ; 80 children suffering from beta thalassemia minor and 70 children suffering from beta thalassemia major.All children were attending the Packed cell volume

$$\text{MCV} = \frac{\text{Packed cell volume}}{\text{Red cell count per / liter}} \times 10^{15} \text{ femtoliters}$$

b-The MCH was calculated as follows

$$\text{Hemoglobin in gm \%} \quad \text{MCH} = \frac{\text{Hemoglobin in gm \%}}{\text{RBCs count per / liter}} \times 10^{13} \text{ (pg)}$$

c- The MCHC was calculated as follows

thalassemia center for the blood transfusion and other drug requirements, while the control children (150) to the care centers in Babylon Governorate.

Methods

Blood collection

The collection of blood was performed in the thalassemia center in Babylon.Before the collection, the patients were asked to rest on a chair for an hour.Collection was always performed between 9 to 11 a.m.by using venipuncture needles [34].

Hematological studies

1):-Red blood cells count (RBCs count)

Blood was diluted with formal citrate solution (1% formalin in 38 gm / L trisodium citrate).20 microliter of blood was added into four ml of diluting fluid, after mixing by a mechanical mixture ; the neubaur hemocytometer chamber was filled before being examined under the microscope to count RBCs [35].

2):-Estimation of hemoglobin (Hb)

The Hb was estimated using the cyanmethaemoglobin method.The method was based on Drabkin's cyanide-ferricyanide solution[36].

3):-Determination of packed cell volume (PCV)

Microhematocrit method was used , heparanized capillary tubes were used , tubes were permitted to fill to approximately three quarters of its length.Then the unmarked end is closed with modeling clay and put in the microhematocrit centrifuge[35].

4):-Measurement of RBCs indices :

a-The MCV was calculated as follows

$$\text{MCHC} = \frac{\text{Hemoglobin gm \%}}{\text{PCV}} \text{ gm / dl (Dacie and Lewis [37]).}$$

5- Osmotic fragility of red blood cells

Equal volume of blood (50 μ) were added to a series of 10 ml tubes containing buffered hypotonic sodium chloride solution (starting 0.9 – 0.15%).A further tube containing distilled water was also used as test tube (100 % hemolysis) , and left for 30 minutes at room temperature .A further mixing was done before being centrifuged for 10 minutes to allow the deposition of RBCs in the bottom of the tubes, degree of hemolysis was recorded photo metrically at 540 nm and expressed as percentage of lyses at each sodium chloride osmotically equivalent to 100 gm [38 , 39].

6-Hb electrophoresis

Cellulose acetate electrophoresis method was used to determine hemoglobin quantitation (HbA ; HbA2 and HbF) to diagnosis the thalassemia minor and major.Hb will migrate from cathode to the anod in the following order.First Hb constant spring , then HbA2 ,C and E migrate in the same band , next HbS and Lepore, again in the same band next HbF followed by HbA then Hb Bart's and last HbH [39].

Statistical analysis

The results were expressed as mean $\pm$ SE.The data were analyzed by using T- test (MS – works version 1.5) and taking ($P < 0.05$) as the lowest limit of significance [40].

Result

1-Red blood cells(RBCs)count

Result of RBCs in both types of beta thalassemia and control are presented in table (1- A , B) values of RBCs count (million / mm^3) is presented for all groups in beta thalassemia minor (3.451 ± 0.082 ; 3.435 ± 0.103 ; 3.614 ± 0.050 ; $3.521 \pm$

$0.084 / \text{mm}^3$, respectively).These values were significantly ($P < 0.01$) lower than control. Also RBCs values were recorded in all groups in beta thalassemia major (3.328 ± 0.071 ; 3.171 ± 0.065 ; 3.185 ± 0.074 ; $3.178 \pm 0.100 / \text{mm}^3$, respectively) were significantly ($P < 0.01$) lower than control. There was no significant difference between beta thalassemia minor and major.

2-Hemoglobin (Hb) concentration

Values of Hb concentration of both types of beta thalassemia and control are shown in table (1-A , B). The results of Hb concentration obtained in all groups of beta thalassemia minor (8.78 ± 0.551 ; 9.12 ± 0.722 ; 9.75 ± 0.477 ; 8.75 ± 0.375 mg / dl, respectively)and significantly($P < 0.05$) lower than control.The values of Hb concentration in all groups of beta thalassemia major (7.72 ± 0.390 ; 6.79 ± 0.528 ; 6.21 ± 0.481 ; 6.34 ± 0.520 mg / dl, respectively). These values were significantly ($P < 0.01$) lower than control. There was no significant difference between beta thalassemia minor and major.

3-Packed cell volume (PCV)

The values of PCV in both types of beta thalassemia and control are depicted in table (1- A , B). The results of PCV in all groups of beta thalassemia minor (0.27 ± 0.015 ; 0.28 ± 0.022 ; 0.30 ± 0.014 ; 0.27 ± 0.011 % respectively)were significant ($P < 0.05$) lower than control. The values of PCV in all groups of beta thalassemia major (0.22 ± 0.024 ; 0.21 ± 0.016 ; 0.19 ± 0.012 ; 0.19 ± 0.051 % , respectively).These values were significantly ($P < 0.05$) lower than control. No significant difference in PCV values

was recorded in two types of beta thalassemia.

4-Red blood cells (RBCs) indices

a)-Mean cell volume (MCV)

The values of MCV obtained from all groups of beta thalassemia minor (75.71 ± 2.569 ; 74.1 ± 5.656 ; 76.21 ± 1.032 ; 74.22 ± 5.499 fl , respectively). These values were significantly ($P < 0.05$) lower than control. Also the values of MCV in all groups of beta thalassemia major (66.44 ± 5.93 ; 64.28 ± 4.507 ; 62.11 ± 4.445 ; 62.42 ± 2.918 fl , respectively) were significantly ($P < 0.05$) lower than control. There was no significant difference ($p > 0.05$) in the values of MCV between beta thalassemia minor and major.

b)-Mean cell hemoglobin (MCH)

The results of MCH obtained in all groups of beta thalassemia minor (25.06 ± 1.292 ; 24.61 ± 1.253 ; 25.86 ± 0.896 ; 25.41 ± 0.535 pg , respectively) were significantly ($P < 0.05$) lower than control. Also the values of MCH in all groups of beta thalassemia major (21.73 ± 0.934 ; 21.17 ± 1.285 ; 20.17 ± 1.601 ; 20.24 ± 0.992 pg , respectively). These values were significantly ($P < 0.05$) lower than control. However, there was no significant difference ($p > 0.05$) recorded between both types of beta thalassemia.

c)-Mean cell hemoglobin concentration (MCHC)

The results of MCHC were obtained in all ages of beta thalassemia minor (32.08 ± 1.281 ; 31.66 ± 0.278 ; 32.12 ± 0.101 ; 31.74 ± 0.136 gm / dl, respectively) recorded no significant difference between each of the groups and control. As well as the values of MCHC in all groups of beta thalassemia major (31.18 ± 0.0429 , ; 31.43 ± 2.300 ; 31.42 ± 0.369 ; 31.45 ± 0.490 gm / dl, respectively) were no significant difference when compared with control. However, there was no

significant difference between the two types of beta thalassemia.

5:-Red blood cells (RBCs) osmotic fragility

The results of RBCs osmotic fragility in both types of beta thalassemia are presented in figure (1). These results showed the osmotic fragility of RBCs , as percentage of lysed RBCs at different sodium chloride concentration. The initial lysis of RBCs recorded was at 0.40% NaCl concentration in two types of beta thalassemia, while the initial lysis of control was at 50% of NaCl concentration.

a)-0.40 %NaCl

The percentage of lysis obtained in beta thalassemia minor (9.1 ± 1.526 %) showed no significant difference when is compared to the control . Also the percentage of lysis in beta thalassemia major (8.9 ± 2.456 %) recorded no significant difference with control.

b)-0.35 % NaCl

The values of percentage of lysis were obtained in beta thalassemia minor (25.8 ± 2.455 %) recorded significantly ($P < 0.01$) lower than control. Also the values of % lysis in beta thalassemia major (25.6 ± 2.114 %) were significantly ($P < 0.01$) lower than control. However, there was no significant difference ($p > 0.05$) in both types of beta thalassemia.

c)-0.30 % NaCl

The percentage of lysis in beta thalassemia minor (51.3 ± 4.338 %) were significantly ($P < 0.01$) lower than control. As well as the values of % of lysis in beta thalassemia major (51.1 ± 8.858 %). There was no significant difference ($p > 0.05$) between both types of beta thalassemia.

d)-0.25 % NaCl

The percentage of lysis in beta thalassemia minor (85.9 ± 0.577 %) recorded significantly ($P < 0.01$) lower than control. The values of lysis in beta

thalassemia major (85.8 ± 0.504 %) were significantly ($P < 0.01$) lower than control. However, there was no significant difference ($p > 0.05$) between

two types of beta thalassemia.

e)-0.15 % NaCl

The percentage of lysis in beta thalassemia minor ($96.9 \pm 0.60\%$) were significantly ($P < 0.01$) lower than control. The values of % of lysis in beta thalassemia major (96.4 ± 52.5 %). These values were significantly ($P < 0.01$) lower than control. There was no significant difference ($p > 0.05$) between both types of beta thalassemia.

f)-0.1 % NaCl

The percentage of lysis of all subjects studies included two types of beta thalassemia and the control was complete (100 %).

6-Hb electrophoresis

a)Adult hemoglobin (HbA)

The results of HbA in all groups of beta thalassemia minor (90 ± 2.088 ; 93 ± 1.316 ; 92 ± 0.600 ; 94 ± 0.930 % , respectively) were significantly ($P < 0.05$) lower than control. Also the values of HbA in all groups of beta thalassemia major (50 ± 5.773 ; 53 ± 8.027 ; 48 ± 8.571 ; 55 ± 4.45 % , respectively) these values were significantly ($P < 0.05$) lower than

control. As well as there was a significant difference ($P < 0.05$) in the values of HbA between beta thalassemia minor and major.

b)Adult hemoglobin 2 (HbA2)

The values of HbA2 of all groups of beta thalassemia minor (6 ± 1.832 ; 6.6 ± 1.400 ; 5.42 ± 0.719 ; 6.14 ± 0.769 % , respectively) were significantly ($P < 0.05$) higher than control. Also the results of HbA2 in all groups of beta thalassemia major (6.6 ± 0.926 ; 6.5 ± 1.231 ; 6.5 ± 0.846 ; 6 ± 1.366 % , respectively) these values were significantly ($P < 0.05$) higher than control. However , there was no significant difference ($p > 0.05$) in the values of HbA2 between beta thalassemia minor and major.

c)Fetal hemoglobin (HbF)

The values of HbF of all groups of beta thalassemia minor (5.2 ± 0.489 ; 6 ± 0.707 ; 5.8 ± 0.969 ; 5 ± 0.969 % , respectively) were significantly ($P < 0.05$) higher than control. The results of HbF in all ages of beta thalassemia major (49.21 ± 4.96 ; 42.83 ± 9.329 ; 49.85 ± 8.359 ; 45.41 ± 7.528 % , respectively) these values were significantly ($P < 0.05$) higher than control. However, there was significant difference ($P < 0.05$) in the values of HbF between beta thalassemia minor and major.

Table1-A Changes in Red blood cells (RBCs) count, hemoglobin concentration (Hb) and packed cell volume (PCV) in beta thalassemia minor.

Age (year)	RBC _s count million/mm ³		Hb concentration mg/dl		PCV%	
	Patient subject	Control subject	Patient subject	Control subject	Patient subject	Control subject
1 - ≤ 3	a* 3.451± 0.082	b* 5.412± 0.178	a 8.78± 0.551	b 15.66± 0.408	A 0.27± 0.015	b 0.47± 0.012
> 3 – 5	a* 3.435± 0.103	b* 4.831± 0.225	a 9.12± 0.722	b 15.13± 0.349	A 0.28± 0.022	b 0.40± 0.012
> 5 – 10	a* 3.614± 0.050	b* 4.891± 0.055	a 9.75± 0.447	b 13.84± 0.297	A 0.30± 0.014	b 0.40± 0.001
> 10 – 14	a* 3.521± 0.084	b* 4.771± 0.033	a 8.75± 0.375	b 13.18± 0.365	A 0.27± 0.011	b 0.40± 0.009

- Values are means (± SE).

- Means with the different letters are significantly different at P < 0.05 .

- Means with the different letters containing asterisk are significantly different at P < 0.01 .

Table (1-B): Changes in Red blood cells (RBCs) count, hemoglobin concentration (Hb) and packed cell volume (PCV) in beta thalassemia major.

Age (year)	RBC _s count million/mm ³		Hb concentration mg/dl		PCV%	
	Patient subject	Control subject	Patient subject	Control subject	Patient subject	Control subject
1 - ≤ 3	A* 3.328 ±0.071	B* 5.412±0.178	A* 7.72± 0.390	B* 15.66±0.408	A 0.22± 0.024	B 0.47±0.012
> 3 – 5	A* 3.171± 0.065	B* 4.831±0.225	A* 6.79± 0.528	B* 15.13±0.349	A 0.21± 0.016	B 0.40±0.012
> 5 – 10	A* 3.158± 0.074	B* 4.891±0.055	A* 6.21± 0.481	B* 13.84±0.297	A 0.19± 0.012	B 0.40±0.001
> 10 – 14	A* 3.178± 0.100	B* 4.771± 0.033	A* 6.34± 0.520	B* 13.18± 0.365	A 0.19± 0.015	B 0.40±0.009

- Values are means (± SE).

- Means with the different letters are significantly different at P < 0.05 .

- Means with the different letters containing asterisk are significantly different at P < 0.01 .

-There is no significant difference between both types of beta thalassemia.

Table 2-A Changes in Red blood cells (RBCs) indices(mean cell volume-MCV; mean cell hemoglobin-MCH and mean cell hemoglobin concentration-MCHC) in beta thalassemia minor.

Age (year)	MCV fl		MCH pg		MCHC gm/dl	
	Patient subject	Control subject	Patient subject	Control subject	Patient subject	Control subject
1 - ≤ 3	a 75.71± 2.569	b 85.57± 1.306	a 25.06± 1.292	b 31.58± 0.411	A 32.08± 1.281	a 32.56± 0.128
> 3 – 5	a 74.11± 5.656	b 83.8± 1.157	a 24.61± 1.253	b 31.12± 0.624	A 31.66± 0.278	a 32.64± 0.183
> 5 – 10	a 76.21± 1.032	b 84.3± 1.316	a 25.86± 0.896	b 31.51± 0.431	A 32.12± 0.101	a 32.56± 0.132
> 10 – 14	a 74.22± 5.499	b 82.5± 0.562	a 25.41± 0.535	b 30.25± 0.217	A 31.74± 0.136	a 32.68± 0.149

- Values are means (± SE).

- Means with the different letters are significantly different at P < 0.05 .

Table 2-B Changes in Red blood cells (RBCs) indices(mean cell volume-MCV; mean cell hemoglobin-MCH and mean cell hemoglobin concentration-MCHC) in beta thalassemia major.

Age (year)	MCV fl		MCH pg		MCHC gm/dl	
	Patient subject	Control subject	Patient subject	Control subject	Patient subject	Control subject
1 - ≤ 3	A 66.44± 5.931	B 85.57± 1.306	A 21.73± 0.934	B 31.58± 0.411	A 31.18± 0.429	A 32.56± 0.128
> 3 – 5	A 64.28± 4.507	B 83.8± 1.157	A 21.17± 1.285	B 31.12± 0.624	A 31.43± 2.300	A 32.64± 0.183
> 5 – 10	A 62.11± 4.445	B 84.3± 1.316	A 20.17± 1.601	B 31.51± 0.431	A 31.42± 0.369	A 32.56± 0.132
> 10 – 14	A 62.42± 2.918	B 82.5± 0.562	A 20.24± 0.992	B 30.25± 0.217	A 31.45± 0.490	A 32.68± 0.149

- Values are means (± SE).

- Means with the different letters are significantly different at P < 0.05 .

- Means with the same letters are not significantly different.

-There is no significant difference between both types of beta thalassemia.

Table 3-A Changes in values of adult hemoglobin A (HbA),ad*ult hemoglobin A2(HbA2) and fetal hemoglobin (HbF) in beta thalassemia minor.

Age (year)	HbA* %		HbA2 %		HbF* %	
	Patient subject	Control subject	Patient subject	Control subject	Patient subject	Control subject
1 - ≤ 3	a 90± 2.088	b 98± 0.577	a 6± 1.822	b 1.66± 0.333	a 5.2± 0.489	b 0.875± 0.125
> 3 – 5	a 93± 1.316	b 97± 0.577	a 6.6± 1.400	b 2± 0.577	a 6± 0.707	B 0.625± 0.125
> 5 – 10	a 92± 0.600	b 98± 0.666	a 5.42± 0.719	b 1.66± 0.333	a 5.8± 0.969	B 0.75± 0.144
> 10 – 14	a 94± 0.930	b 97± 0.333	a 6.14± 0.769	b 2.0± 1.0	a 5± 0.969	B 0.75± 0.144

- Values are means (± SE).

- Means with the different letters are significantly different at P < 0.05 .

Table (3-B) Changes in values of adult hemoglobin A (HbA), adult hemoglobin A2 (HbA2) and fetal hemoglobin (HbF) in beta thalassemia major.

Age (year)	HbA* %		HbA2 %		HbF* %	
	Patient subject	Control subject	Patient subject	Control subject	Patient subject	Control subject
1 - ≤ 3	A 50± 5.773	B 98± 0.577	A 6.6± 0.927	B 1.66± 0.333	A 49.21± 4.964	B 0.875± 0.125
> 3 – 5	A 53± 8.027	B 97± 0.577	A 6.5± 1.231	B 2± 0.577	A 42.83± 9.239	B 0.625± 0.125
> 5 – 10	A 48± 8.571	B 98± 0.666	A 6.5± 0.846	B 1.66± 0.333	A 49.85± 8.359	B 0.75± 0.144
> 10 – 14	A 55± 4.54	B 97± 0.333	A 6± 1.366	B 2.0± 1.0	A 45.41± 7.528	B 0.75± 0.144

- Values are means (± SE).

- Means with the different letters are significantly different at P < 0.05 .

- (HbA^{*}, HbF^{*}): There is significant difference ($P < 0.05$) in the results of HbA and HbF between both of beta thalassemia.

Discussion

As far as changes are concerned, table (1 -A, B) shows hematological decrease of RBCs count in the beta thalassemia minor and major. These results agree with other studies [1, 41]. This reduction may have been resulted from RBCs destruction in the reticuloendothelial system by macrophages due to abnormal red blood cells such as poikilocytosis, anisocytosis and target cells because of accumulation of alpha chains on RBC membrane [16, 2, 42]. At the first time, these results contradict to what is known about the erythroid hyperplasia of the bone marrow, extra medullar hematopoiesis associated with the increase erythropoietin level [43, 44].

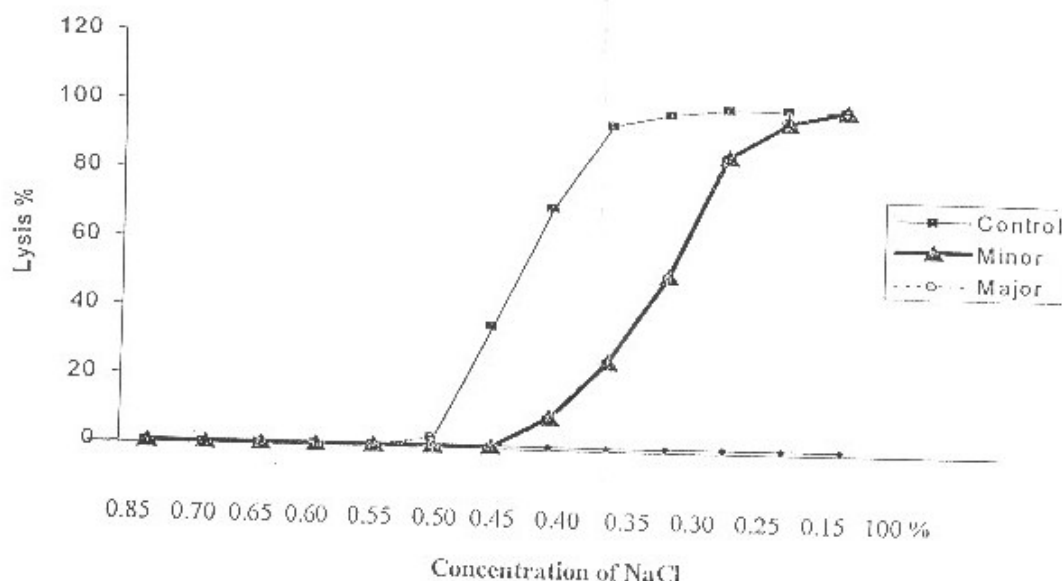
The significant decline in Hb concentration as showed in table (1 -A, B) in both types of beta thalassemia as compared to the control subject is in agreement with other studies [45, 46, 47]. This decrease in Hb concentration is due to impairment of heme biosynthesis and decrease or absence of beta globin chains synthesis that associated with decrease RBCs count [14, 16].

As shown in table (1 -A, B) the decrease of PCV in two types of beta thalassemia as a compared to the control subject is in agreement with the results of other studies [20, 18, 48]. This decrease in PCV as a result from the decrease of RBCs count, microcytosis and the decrease of Hb concentration [4, 9, 50]. The decrease in RBCs indices in both types of beta thalassemia and the control subject table (2 -A, B). These results are in agreement with

other studies [19, 24, 51, 52, 53]. Beside the component of these indices included RBCs count, Hb concentration and PCV, the decrease in these values occur in beta thalassemia minor and major lead to decrease in these indices [9, 10].

The decrease of osmotic fragility of RBCs in both types of beta thalassemia in a comparison with the control subject (fig.1). These results are in agreement with other studies [45, 29]. The decrease of osmotic fragility of RBCs is due to the presence of target cells and poikilocytosis are relatively resistance to the osmotic fragility [39]. As well as the decrease of MCV and MCH with the presence of normoblasts and reticulocytes lead to decrease osmotic fragility [54, 37, 55].

The results of Hb electrophoresis in both types of beta thalassemia in comparison with the control table (3 -A, B). The increase in HbA₂ and HbF associated with the decrease of HbA in comparison with the control subject in agreement with the other studies [56, 57]. This increase in HbA₂ and HbF has been attributed to the increase of production of delta and gamma globin chains to compensate the decrease or absence of beta globin chains (HbA) [58, 21, 2, 59]. The significant increase in HbF associated with significant decrease in HbA values in beta thalassemia minor and major in agreement with other studies [60, 50]. These changes in both HbF and HbA values may be due to the severe decrease in the beta chains biosynthesis [28, 61, 5, 62].



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