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Changes of White Blood Cells in Children Infected with beta Thalassemia (in Babylon)

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Abstract

This study was included the changes that were presented in certain blood characteristics of children suffering from beta thalasemia minor and major, a total number used in this study involved 150 of both healthy children and those affected with beta thalasemia. As far as for thir ages divided into four groups such as (1≤3 years, >3-5 years, >5-10 years and > 10-14 years). The changes that presented in hematological parameter were, total white blood cells recorded a significant increase $p<0.05$ in both types of beta thalasemia when compared with the normal group, as well as differential white blood cells showed a significant increase $p<0.05$ in neutrophel cells and monocytes in both types of beta thalasemia in a comparison with healthy children, but on other hand, other types of white blood cells such as (lymphocytes, basophile cells and eosinophile cells) showed normal values in a comparison with the healthy group.

The changes that are explained may be due to increase bone marrow expansion and its activity. As well as elevation of activity of reticuo- endothelial system that lead to increase phagocytosis.

الخلاصة

تضمنت هذه الدراسة فحص التغيرات الحاصلة في بعض معايير الدم في الاطفال اللذين يعانون من فقر الدم البحري البائي بكلا نوعيه الخفيف والشديد في محافظة بابل. شملت الدراسة فحص 150 طفل من المصابين والاصحاء تراوحت اعمارهم بـين (1-14سنة) وقد قسمت اعمارهم الى اربعة مجاميع (المجموعة الاولى 1-<3 سنوات، المجموعة الثانية>3-5 سنوا، المجموعة الثالثة>5-10 سنة، المجموعة الرابعة>10-14 سنة). تميزت التغيرات في كريات الدم البيضاء. حيث سجلت زيادة معنوية فـي العدد الكلي لخلايا الدم البيضاء بمستوى ($p<0.05$) بالمقارنة مع الاطفال الاصحاء.

اما بالنسبة للعد التفريقي لانواع خلايا الدم البيضاء، حيث لوحظ زيادة بمستوى ($p<0.05$) في عدد الخلايا العدلة والاحادية ولكن لوحظ بان الانواع الاخرى خلايا الدم وهي الخلايا الحامضية والخلايا القاعدية والخلايا اللمفاوية لـم تـسجل اي اخـتلاف معنوي بالمقارنة مع الاطفال الاصحاء.

ان التغيرات الحاصلة في عدد خلايا الدم البيضاء يمكن ان تعزى بصورة رئيسة الى زيادة فعالية نخاع العظـم، وزيـادة فعالية الجهاز البطاني الشبكي بالاضافة الى زيادة البلعمة (phago cytosic) لالتهام كريات الدم الحمراء المشوهة.

Introduction

Beta thalasemia is a genetic disorder of hemoglobin molecules (AL-Awamy, 2000), which is thought to has originated in many parts of the world where malaria was an endemic problem (Thompson, 1984). Thalssemias are consider a haemoglobinpathy, that that are involved insufficient production of structurally abnormal hemoglobin molecules (Bain , 1998).

The beta poly peptide chains of are encoded by two beta genes that have been presented on chromosome number (11) (Dedoussis *et al*, 2000). Generally, all the disorders that effected on beta globin genes represented by point mutations more than other mutations (Cortran *et al.*, 1999). Light point mutations are cause deficiency in biosynthesis of beta globin chains lead to beta thalassemia minor, while sever point mutation cause common defective gene in which no beta globin chain are formed and called beta thalassemia major (Pallister, 1999). On other hand , deficiency of biosynthesis of beta globin chains lead to elevate of accumulation of non companied alpha globin chains and aggregation of these polypeptide chains on the cell membrane of erythrocytes (Hillman and Ault, 1995). Abnormal production of erythrocytes lead to destruction of these cells in reticulo- endothelial system which included (spleen, liver and bone marrow) (Tierney *et al.*, 1994). Sever destruction of abnormal

erythrocytes causes sever anemia, sever hypoxiand and increase activity of reticulo-endothelial system (Bank 1985).

Materialsand Methods

A-Materials

Subjects of study

The subjects that were included in this study were 150 patients and healthy children. Their ages were distributed between one year to fourteenth years, also these ages were divided into four groups first group 1≤3 years, second group>3-5 years, third group>5-10 and fourth group>10-14 years (Gill and Obrien, 1988).

A total number of patients was 100 subject was subdivided into tow groups, 50 children suffering from beta thalassemia minor and 50 children infected with beta thalassemia major, as well as a number of healthy children was 50.

B-Methods

1-Blood collection

Blood was performed in the thalassemia center of Babylon province, tubes to be used for haematological studies contained (EDTA) to prevent collection was always performed between 9 to 11 am by using venipuncture needeles. A tourinqnt was applied directly on the skin around the arm approximately 6 to 8 cm above the site of collection. Needdeles that were used 21 gauge .

2-white blood cells count (WBCs).

Blood was diluted with glacial acetic acid solution 1%, and gention 2m1 (Daice,2000).

2-total white blood cells count (WBCS)

Blood was diluted with 0.4 of Turks solution (1m1 of glacial acetic acid, 2m1 of gentian violet, and 100m1 of distilled water). Atwenty microliter of blood was added and mixed in a mechanical mixture, the counting a neubaur chamber was used to count the total WBCS (Dacie and lewis,2001).

3-Determination of differential white blood cells:-

A drop of blood was applicated on the near of sid of slide, and then through other slide (spreader), the blood spread on the slide to prepare a thin blood smear. This smear nemained for 5 minates to dry and then flooded with leishman solution. After 5 minutes, leishman solution washed with normal salin and blood smear left for 5 minutes to dry and then examined under 100 objective with using of oil emmersion (Dacie and Lewis, 2001).

4-Statistical analysis ±SE:-

All values were expressed as means S.

Students T.test was used to examine the differences between different groups.

Results

1-Total white blood cells (WBCs) count.

Results of the total white blood cells in both types of beta thalasscmia and normal control are illusetrated in table(1). Values of (WBCs) for all ages of beta thalassmia minor (9.42± 1.76, 8.81±1.81, 7.91±1.19, 7.73±1.602 cell/mm^3, respectively) were significantly at ($p<0.05$) higher than normal children. Also values of (WBCs) of beta thalassemia major (10.57+1.857, 9.295+2.103,8.685+1.565, 8.325+ 1.892 cell/mm^3, respectively), were significantly at ($p<0.05$) higher than healthy children.

2-Differential white blood cells (WBCs) count:

a-Neutrphils and monocytes count:-

Results of neutrphils and monocytes are shown in table (2-A,B).values of neutrophils for all groups of beta thalassemia minor were (61.8±2.601, 67.1±6.283,

71.0± 0.197, 74.0±0.624%, respectively) significantly at ($p<0.05$) higher than healthy children. As well as ratios of neutrophils in all groups of beta thalassemia major (58.7±0.408, 56.3±0.578, 64.0±0.716,63.0±0.356%, respectively) recorded a significant increase ($p<0.05$) when compared with the normal children. In the same table (2-A,B), results of monocytes in all groups of beta thalassemia minor (11.1+1.757, 9.9+1.972, 10.9+1.577, 10.6+1.21% respectively) were significantly at ($p<0.05$) higher than normal subjects. Also ratios of monocytes in all ages of beta thalassemia major (11.8±4.33, 10.6±1.959, 11.5±2.061, 11.9±1.577%, respectively) were signifantly at ($p<0.05$) higher than healthy children.

b-Basophiels, eosinophils and lymphocytes count:

Ratios of these cells in all groups of beta a thalasemia minor and major are presented in the table (3-A,B). All results of basophils ranged between (1±0.08 and 2±0.003%) in all ages of beta thalassemia minor and major showed no significant difference with normal children. Also values of eosinophils and lymphocytes of all groups of beta thalassemia showed no significant difference when compared with healthy groups.

Table (1):- Change in total white blood cells (WBCs) (cells/mm^3) in beta thalassemia minor and major.

Age Year	WBCs) Beta thalassemia major		(WBCs) Beta thalassemia major	
	Patient groups	Controlgroups	Patientgroups	Controlgroups
1-<3	a 9.42+1.76	b 6.26+0.793	a 10.57+1.857	b 6.26+0.793
>3-5	a 8.81+1.81	b 4.81+0.747	a 9.295+2.103	b 4.81+0.747
>5-10	a 7.91+1.19	b 4.5+0.828	b 8.685+1.565	a 4.5+0.828
>10-14	a 7.73+1.602	b 4.785+0.742	a 8.325+1.892	b 4.785+0.742

-Values were means (±SE)

-Means with the different letters were significantly at ($p<0.05$).

-There was no significant difference between both types of beta thalassemi

Table (2-A):- Change ratio monocyts and neutrophils white blood cells in beta thalassemia minor

Age Year	Neutrophils %		Monocytes %	
	Patient groups	Control groups	Patient groups	Control groups
1-≤3	a 61.8±2.601	b 45.8±0.455	a 11.1±1.757	b 4.8±1.989
>3-5	a 67.1±6.283	b 48.8±0.493	a 9.9±1.972	b 4.0±1.414
>5-10	a 71.0±0.197	b 47.9±1.923	a 10.9±1.577	b 3.8±1.248
>10-14	a 74.0±0.624	b 46.9±0.490	a 10.6±1.21	b 5.9±1.32

-Values were means (±SE)

-Means with the different letters were significantly at ($p<0.05$).

-There was no significant difference between both types of beta thalassemia

Table (2-B):- change in ratio of monocyte and neutrophel white blood cells in beta thalassemia major.

Age Year	Neutrophils%		Moncys%	
	Patient group	Control group	Patient group	Control group
1-<3	a 58.7±0.408	b 45.8±0.455	a 11.8±4.33	b 4.8±1.989
>3-5	a 56.3±0.578	b 48.8+0.493	a 10.6+1.959	b 4.0±1.414
>5-10	a 64.0±0.716	b 48.8±0.493	a 11.5±2.061	b 3.3±1.248
>10-14	a 63.0±0.356	b 46.9±0.490	a 11.9±1.577	b 5.9±1.32

-Values were means (±SE)

-Means with the different letters were significantly different at ($p<0.05$).

Table (3-A):- changes in the ratio of lymphocys, eosinophils and basophils white blood cells in beta thalassemia minor.

Age Year	Lymphocytes%		Eosinophils%		Basophils%	
	Patient group	Control group	Patient group	Control group	Patient group[	Control group
1≤3	a 35±0.451	a 32±0.321	a 3±0.071	a 4±0.925	a 2±0.003	a 1±0.031
>3-5	a 33±0.021	a 31±0.421	a 4±0.058	a 4±0.831	a 1±0.08	a 1±0.004
>5-10	a 32+0.909	a 34±0.905	a 5±0.061	a 3±0.21	a 1±0.001	a 1±0.003
>10-14	a 25±1.231	a 24±1.575	a 4±0.781	a 3±0.313	a 1±0.021	a 1±0.007

-Values were means (+SE)

-Means with the same letters were non significantly different.

Table (3-B):- changes in the ratio of lymphocys, eosinophils and basophils white blood cells in beta thalassemia major.

Age Year	Lymphocyte%		Eosinophel%		Basophile%	
	Patient Subject	Control group	Patient group	Control group	Patient group	Control group
1≥3	a 38±1.284	a 32±0.321	a 3±0.952	a 4±0.925	a 4±0.003	a 1±+0.003
>3-5	a 30±1.782	a 31±0.421	a 4±0.052	a 4±0.831	a 1±0.008	a 1±0.002
>5-10	a 34±1.560	a 34±0.905	a 5±0.061	a 3±+0.21	a 1±0.001	a 1±0.005
-14 >10	a 22±1.784	a 24±1.575	a 4±4+0.78	a 3±0.313	a 1±0.021	a 1±+0.007

-Values were means (+SE)

-Means with the same letters were non significantly different.

Discussion

Leukocytes count changes that have been described in table (1) showed elevation in the total white blood cells count in both types of beta thalassemia, these results agree with other studies (Krupp and Chatton 1981; Penington *et al.,* 1984; William et at.,1990; William *et al.,* 1997). From pathophysiologic stand point, the central features of beta thalassemia are: deficient hemoglobin synthesis resulting from deficiet production of beta globin chain and precipitation of free polypeptide chains in the red corpuscles to form inclusion bodies lead to red cell membrane damage and shortened red cell survival (Wintrobe *et al.,* 1976; Thompson 1984; Firkin *et al.,* 1989). When the magnitude of the erythropoietic defect is sufficiently great, the consequences are anemia attempted compensation by increasing activity of reticuloendothelial system (liver, spleen and bone marrow) because of the need for removing the products of corpuscular break down. On the other hand , sever hypoxia that resulted causes increased production of erythropoietin in blood circulation which lead to extra medullary blood formation with myelocytes and macrophages caused in liberation additional new blood cells into blood circulation (Frikin *et al.,* 1989 ; Mazza, 1995; Rodak, 1995). Concerning differential white blood cells that have been illustrated in table (2-A,B) and table (3-A,B). These values recorded a significant increase in the ratios of neutrophels and monocytes.

As explained previously that, increase of uncompained alpha chains production and accumulation or these uncompained chains on the cell membranes of erythrocytes resulted in production abnormal erythrocytes such as anisocytosis , poikilocytosis , target cells and other abnormal shapes of red cells (Bilto, 1998), these cells removed from blood circulation by reticuloendothlial system through netrophils and monocytes via phagocytosis proceses. Also increasing of extra medullary erythropoiesis leads to liberation additional new blood cell in to blood circulation which may leads to elevate number of these cells.

On the other hand, results of basophels, eosinophels and lymphocytes recorded normal values and pointed out a non significant different with control group. (Krihnadas, 1986; Frikin *et al.,* 1989; Walter and Talboti, 1996).

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