

A STUDY OF THE INTRACELLULAR KILLING OF THE PERIPHERAL BLOOD NEUTROPHILS IN β - THALASSEMIA MAJOR PATIENTS

MOHAMMAD JAAFARI*, MINA IZADYAR**, MOHAMMAD
RAKSHAN***, ABUL HASSAN FARHOUDI**, TAHEREH ZAN-
DIEH****, AND GHOLAM-REZA BABAI*

From the Tarbiat Modarres University, **Children's Medical Center, Tehran University of Medical Sciences,
Dept. of Pathology, Shaheed Beheshti University of Medical Sciences, and *Blood Transfusion Organization,
Tehran, Islamic Republic of Iran.*

ABSTRACT

52 β - thalassemia major patients were studied. Their ages ranged from two to 20 years. The group consisted of 22 girls and 30 boys. Candida intracellular killing and NBT (nitroblue tetrazolium) tests were performed on the peripheral blood neutrophils of these patients.

Average results of the intracellular killing test was within normal range for the patients, but showed an appreciable decrease in comparison with the results of the normal control tests (50 normal persons of the same age group were tested as normal controls. This decrease was more pronounced in those patients who had undergone splenectomy.

A converse relationship was observed between the results of the patients' intracellular killing and their serum ferritin level and age.

Average results of the patients' NBT tests showed an increase in comparison with those of the control group. The average results for the patients who had their spleens removed due to hypersplenism was the same as in those who had normal spleens and had no relation to the serum ferritin level but did show a moderate relationship with the age of the patients.

A suggestion that can be put forth is the likelihood that intracellular killing of peripheral blood neutrophils in the β - thalassemia patients is conducive to the determination of those with greater susceptibility to infection.

MJIRI, Vol. 6, No. 2, 135-138, 1992

INTRODUCTION

Beta- thalassemia is prevalent in Iran. Its homozygous forms are usually accompanied by severe anemia within the first year and require blood transfusion for survival. It is known that the homozygous form is accompanied by recurrent infections, but the probable mechanism for this is not known. Perhaps it is due to the increased iron load in these patients. There also exists

the probability of a defect in the alternate pathway of complement.²

The red blood cells of these patients contain abnormal hemoglobins and are therefore destroyed before their normal life-span by the spleen. Hyperactivity of the erythropoietic organs and increased iron absorption via the digestive system as well as immature hemolysis of red blood cells impose an iron overload on these patients.

With the passage of time these patients develop hypersplenism in which case splenectomy increases the interval between blood transfusions. Studies have been made to determine the cause of these patients' propensity for infection. The immunoglobulins' opsonic activities and phagocytosis that were studied by Khalifa and his colleagues in Egypt in 1983,³ were considered. As Khalifa and his group have contended, the phagocytic and opsonic activities are abnormal in these patients.

In the present study, we have performed the intracellular killing and the NBT test in order to evaluate the efficiency of phagocytosis (phagocytosis is effective when the ingested organism can be destroyed within the macrophage or neutrophil) in the peripheral blood neutrophils of patients with beta thalassemia.

PATIENTS AND METHODS

Fifty-two patients with beta thalassemia major were included in the survey. The patients were all Iranians aged two to 20 years. The group was made up of 22 girls and 30 boys. 24 patients had splenectomy. The normal group consisted of 50 persons (within the same age range). The same patient group plus eight other patients (a total of 60) were studied for the NBT test. These were aged two to 21 years. 25 of them were female and 35 were male. 28 patients had their spleens removed, the remaining 32 had not. In this connection 30 persons were subjected to normal control test. All patients were receiving blood transfusions (once every 3-5 weeks repeatedly). All patients were taking desferrioxamine, and none showed any acute or overt infection.

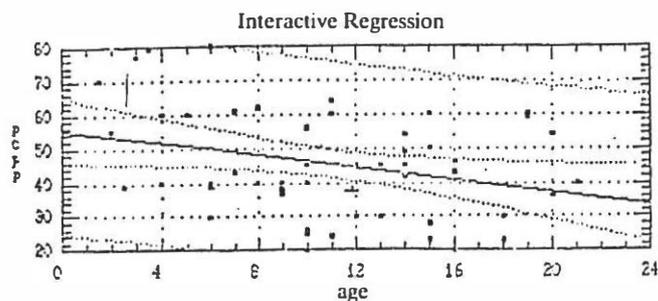
Separation and preparation of peripheral blood neutrophils was carried out with the help of a 66% dextran solution.⁴ The performed tests included the intracellular killing^{5,6} and the NBT test.⁴

RESULTS

Results of the intracellular killing are shown in Table I.

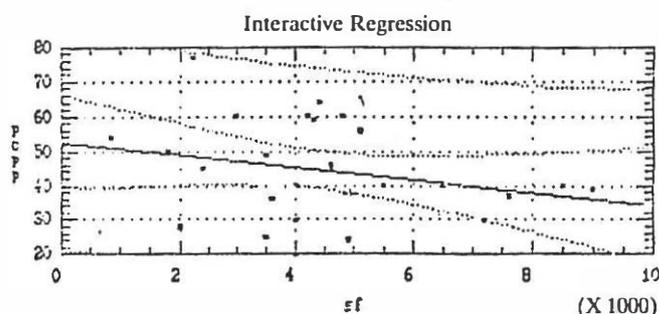
Table I. The results of the intracellular killing *Candida* tests for the BTM patients. NS= non-splenectomized, S= splenectomized, + = mean, * = mean \pm SD

Title of the test	Patients		Total patients	Normal control
	NS	S		
Intracellular killing	+ 48.75 (n= 28)	+ 41.95 (n= 24)	*45.61 \pm 15.31 (n= 52)	*73.1 \pm 11.98 (n= 50)



BO: 55.622 SE: 4.7245 T: 11.53
 B1: -0.50956 SE: 0.15615 T: -2.6234
 CORR: -0.3073 MSE: 216.51 DF: 50
 POINTS DELETED:

Fig.1. Relationship between the age of the patients and the result of the intracellular killing (PePP) test. The relationship between them is inverse (correlation = -0.3073).



BO: 52.633 SE: 6.7375 T: 7.812
 B1: -1.8475E-3 SE: 1.15895E-3 T: -1.5326
 CORR: -0.26357 MSE: 192.94 DF: 24
 POINTS DELETED:

Fig.2. Comparison between serum ferritin (SF) and the intracellular killing (PePP) test results in BTM patients. An inverse relation exists between these two quantities (correlation = -0.26357).

The results show a marked decrease (in contrast with the normal control group). The decrease was higher in patients who had their spleens removed. A converse relationship was observed between the ages of the patients and the test results (Fig. 1).

Furthermore, an inverse relationship was noted between the amount of serum ferritin and the result of the intracellular killing test (Fig. 2).

The result of the NBT test in patients showed a meaningful increase in contrast with the normal control group (Table II), but no appreciable difference was

Table II. The results of the NBT tests on beta thalassemia major patients (BTM) [all of the patients including the splenectomized (S) and non-splenectomized (N.S.) groups and the normal control group were considered] (n: number, +: mean, *: mean \pm SD)

Test Title	Patients		Total Patients	Normal control group
	NS	S		
NBT %	+ 87.28 n= 32	+ 88.57 n= 28	* 87.88 \pm 6.04 n= 60	* 83.33 \pm 3.39 n= 30

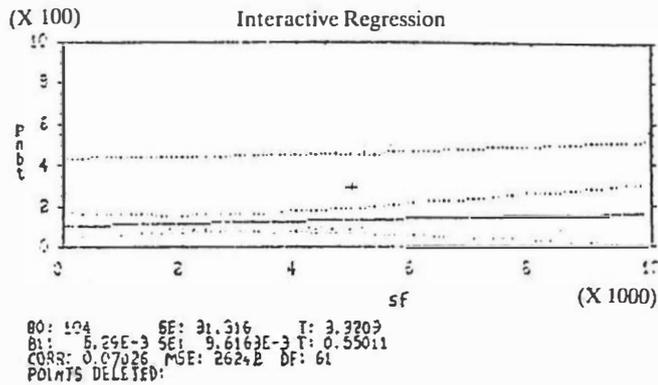


Fig.3. As seen in the above diagram no relationship is indicated between the serum ferritin level (SF, ng/ml) in the BTM patients and the results of the NBT test (Pnbt) (correlation= 0.07).



Fig.4. Relationship between the results of the NBT test performed on patients (pnbt) and their ages (calculated by year).

noted between the splenectomied group (S) and the group of patients who had not had their spleens removed (NS).

The patients were also studied in two age groups of ten years and below, and over ten years, and no significant difference was noted.

The S (without spleen) and NS (with spleen) groups showed no difference. In other words, no correlation existed between the results of the NBT test and the presence or absence of the spleen.

The comparison between the test results and the patients' serum ferritin showed no relationship (Fig. 3), but the increase in the patients' age boosted the results of these tests (Fig. 4).

DISCUSSION

1- Intracellular killing: This study reveals a considerable decrease in the candida intracellular killing by the peripheral blood neutrophils of the BTM patients in contrast with that of the normal control group. Such a

decrease was in inverse relation to the age and the amount of serum ferritin of the patients, and it could not be corrected by removal of the spleen. Reports indicate the presence of disorders in the humoral and cellular immune system and in the PMN and mononuclear lytic system of patients with thalassemia.⁷

BTM patients develop iron overload as a result of frequent blood transfusions and ineffective hematopoiesis. Iron has a catalyzer role in the production of active oxygen radicals (microbicidal) such as OH. These oxygen metabolites have toxic effects on the PMN cells, and decrease their activity.^{8,9}

Another interpretation can be suggested with regard to the suppression of the immune system and frequent blood transfusions in these patients. That is to say, a mechanism of tolerance exists in BTM patients motivated by chronic alloantigens which can be explained by the reduction of T4⁺ cells and the increase in T8⁺ cells.¹⁰

There exists also a suggestion concerning disorders in the performance of the immune system which is dependent on myeloperoxidase and the system that is independent of myeloperoxidase for the killing of microorganisms.¹¹

2- Result of the NBT test: The result of this test also shows both the phagocytosis and the oxidative metabolism of the PMN cell. By exciting the PMN leukocytes and the phagocytosis of particles and by production of superoxide anions within the cells, the NBT color is reduced and turns into dark-blue crystals (formazan).

Iron has a catalyzer role in the production of superoxide anions (O₂⁻).⁷ Thus in view of the increase in the iron load of these patients, it can be suggested that the process boosts the results of the NBT. Repeated blood transfusions and the clash of these patients' PMN with the alloantigens as well as the sustained excitement of this cell too, can be a probable proof and another suggestion (concerning the increase in the NBT results).

Thalassemia beta is one of the cases in which the NBT test becomes falsely positive (without phagocytosis as a stimulus).¹² This is so while the patient has received blood transfusion or has had splenectomy as a consequence of which their NBT test results show an increase. It can be suggested that this condition is related to non-specific phagocytic (*in vivo*) activity and it may develop the view that blood transfusion or removal of the spleen may increase *in vivo* phagocytic activities.¹³ Also it can be suggested that in splenectomied patients the clearance of the activated neutrophils (NBT- positive) from blood circulation becomes retarded.¹³

REFERENCES

- 1- Eraklis HJ, Keny SV, Diamond LK, Gross RE: Hazard of overwhelming infection after splenectomy in children. *New Eng J Med* 276: 1225-9, 1976.
- 2- Germenis A, et al: C₃ polymorphism in β -thalassemia. *Acta Haematol* 71: 45-50, 1984.
- 3- Khalifa AS, Abdel Fallah S, Maged Z, Sabri F, Mohamed HA: Immunoglobulin levels, opsonic activity and phagocytic power in Egyptian thalassemic children. *ACTA Haematol* 69: 136-9, 1983.
- 4- Thompson RA: *Techniques in Clinical Immunology*. London, Blackwell Scientific Publications, second edition, 1981.
- 5- Ghavami-Nejad A, Zandieh T: Inhibition of «¹⁴C» uptake by *Candida albicans*: A new method for investigation of intracellular killing by phagocytes. *Med J Islam Repub Iran* 2(3): 237-42, 1988.
- 6- Yamamura M, Boler J, Valdimarsson H: Phagocytosis measured as inhibition of uridine uptake by *Candida albicans*. *J Immunol Methods* 14: 19-24, 1977.
- 7- Ballart IJ, Ester Vez ME, Sen L, Diez RA, Guintoli J, Demiani SA, Penarver J: Progressive dysfunction of monocytes associated with iron overload and age in patients with thalassemia major. *Blood* 67 (1): 105-9, 1986.
- 8- Sweder Van Asbeck, B, et al: Effect of iron (III) in the presence of various ligands on the phagocytic and metabolic activity of human polymorphonuclear leukocytes. *J Immunol* 132 (2): 851-6, 1984.
- 9- Gutteridge GMC, Paterson SK, Segal AW, Halliwell S: Inhibition of lipid peroxidation by the iron binding protein lactoferrin. *Biochem J* 199: 259, 1981.
- 10- Pardalos G, Kanakoudi-Tsakalidis F, Malakazafiriu M, Tsantali H, Thanasiou-Metaxa M, Kallinikos G, Papaevangelou G: Iron-related disturbances of cell-mediated immunity in multitransfused children with thalassemia major. *Clin Exp Immunol* 68, 138-145, 1987.
- 11- Guintoli JMT, et al: Defective function of the peripheral blood neutrophils in thalassemia major. *AMJ Pediatr Hematol Oncology* 2: 215-6, 1984.
- 12- Liakakos D, Viachos P: NBT test in thalassemia (letter). *J Pediatr* 82: 352, 1973.
- 13- Pier Angelo Tovo PA, et al: NBT test in thalassemia. *J Pediatr* 4: 666, 1977.