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Research Article

**ANALYSIS OF THE INTRACELLULAR KILLING OF THE
PERIPHERAL BLOOD NEUTROPHILS IN PATIENTS OF B
THALASEMIA MAJOR**Dr Arwa Shahzad¹, Dr Huma Tariq², Dr Mohammad Hamza Kiyani³¹ Avicenna Medical College, Lahore² Sahiwal Medical College, Sahiwal³ Rawalpindi Medical College, Rawalpindi**Article Received:** January 2020 **Accepted:** February 2020 **Published:** March 2020**Abstract:**

Aim: This study aimed to study the peripheral blood neutrophils intracellular killing in patients of b- thalassemia major.

Place and Duration: In the Department of Pathology, Mayo Hospital Lahore for one year duration from March 2019 to March 5, 2020.

Methods: 60 patients with B thalassemia were examined. Their age ranged from two to 20 years. The group consisted of 24 girls and 36 boys. The Candida intracellular killing and NBT (nitroblue tetrazolium) were performed in peripheral blood neutrophils of these patients.

The average intracellular killing test results were within the normal range for patients, but showed a significant decrease compared with the results of normal control tests (60 normal individuals in the same age group were considered normal control. The reduction in the number of patients is significant who had splenectomy. An inverse relationship was observed between the results of intracellular death and serum ferritin level and age.

Results: Average results of the patients' NBT tests showed an increase in comparison with those of the control group. The average results of patients whose spleens were removed due to hypersplenism are the same as those of ordinary spleens and are not associated with serum ferritin levels, but have a moderate relationship with the age of patients.

Conclusion: One suggestion that can be made is the possibility of intracellular death of peripheral blood neutrophils in patients with B thalassemia to help identify the most susceptible to infection.

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INTRODUCTION:

Beta-thalassemia is common in Pakistan. Homozygous forms are often accompanied by severe anemia in the first year and a blood transfusion is necessary for survival¹⁻². It is known that recurrent infections accompany homozygous forms. However, the possible mechanism for this is unknown. Perhaps this is due to the increased iron load in these patients³⁻⁴. There is also a possibility of a defect in the alternative genetic path⁵. These patients' red blood cells contain abnormal hemoglobin, which is why they destroy them before normal life. Hyperactivity of the erythropoietic organs and increased absorption of iron by the digestive system, as well as immature hemolysis of red blood cells causes iron overload in these patients⁶.

Over time, these patients develop hypersplenism, in which case splenectomy prolongs the interval between blood transfusions. Studies have been conducted to determine the cause of infection susceptibility in these patients. Opsonic immunoglobulin and phagocytosis activities studied by Khalif *et al.* In 2014, in Egypt according to the caliph and his group, phagocytic and opsonic activities are abnormal in these patients⁷⁻⁸.

RESULTS:

The results of intracellular death are shown in Table I.

Title of the test	Patients		Total patients	Normal control
	N S	S		
Intracellular killing	48.75 (n 29)	41.95 (n 26)	"45.61 ± 15.31 (n 60)	"73.1 ± 11.98 (n 60)

The results show a significant decrease (in contrast to the normal control group). The reduction was greater in patients with splenectomy. An inverse relationship was observed between patient age and study results.

In addition, an inverse relationship was observed between serum ferritin and the result of an intracellular destruction test.

The results of the NBT test showed a significant increase in patients in contrast to the normal control group (Table II), but no significant difference was observed between the splenectomized (S) group and the group of patients that had not yet been performed. The spleen has been removed (NS).

Patients were also examined in the age group 10 or less for ten years and no significant difference was observed.

Test Title	Patients		Total Patients	Normal control group
	N S	S		
NBT	87.30	+ 88.57	• 87.90 ± 6.02	• 83.29 ± 3.40
%	n 31	n= 29	n= 60	n 35

There was no difference between the S (without spleen) and NS (spleen) groups. In other words, there is no correlation.

In this study, we conducted an intracellular death test and NBT to assess the effectiveness of phagocytosis (phagocytosis) when an organism swallowed in peripheral blood neutrophils of patients with beta thalassemia can be destroyed within macrophages or neutrophils.

PATIENTS AND METHODS:

60 patients with major beta thalassemia were included in the study. All patients aged from 2 to 20 years. The group consisted of 24 girls and 36 boys. Splenectomy was performed in 28 patients. The normal group consisted of 60 people (in the same age range). The same group of patients and eight other patients (60 in total) were tested for NBT. They were people from 2 to 21 years old. 25 are women and 35 are men. The spleen of 28 patients was removed, the remaining 32 had not removed. In this context, 30 people received a normal control test. All patients received blood transfusions (every 3-5 weeks in a row). All patients used desferrioxamine and none of them had an acute or open infection.

Separation and preparation of neutrophils in peripheral blood was carried out with a 66% dextran solution. "The tests performed included NBT test and intracellular killing.

Occurred between NBT test results and the presence or absence of spleen. Comparison of the results of studies with ferritin in patients' serum did not show any relationship, but the increase in age of patients increased the results of these studies.

DISCUSSION:

Intracellular Killing: This study reveals a significant reduction in the intracellular killing of BTM candidates, in contrast to the normal control group, with peripheral blood neutrophils. This decrease was inversely proportional to the age and amount of serum ferritin in patients and could not be corrected by spleen removal⁹⁻¹⁰. Reports indicate the presence of disorders in the humoral and cellular immune system as well as in patients with thalassemia in PMN and mononuclear lytic system¹¹.

Patients with BTM experience iron overload due to frequent blood transfusions and ineffective hematopoiesis. It plays a catalytic role in the production of active oxygen (bactericidal) radicals, such as iron, OH. These oxygen metabolites have a toxic effect on PMN cells and reduce their activity¹².

A different interpretation of immune suppression and frequent blood transfusions may be suggested in these patients. This means that patients with BTM motivated by chronic alloantigens have a tolerance mechanism that can be explained by a decrease in the number of T4 + cells and an increase in the number of T8 + cells.

There are also suggestions for system-related disorders that are independent of myeloperoxidase in the functioning of the immune system and the destruction of microorganisms¹³.

NBT test result: The result of this test shows both phagocytosis and oxidative metabolism of the PMN cell. By producing exciting PMN leukocytes and phagocytosis of particles and superoxide anions in cells, the color of NBT decreases and turns into dark blue crystals (formazan). Iron plays a catalytic role in the production of peroxide anions (O₂⁻). Therefore, given the increase in iron load in these patients, this process can be suggested to increase NBT results¹⁴⁻¹⁵. In addition to repeated blood transfusions and PMN shock in these patients with allo-antigens, continuous stimulation of this cell may also be a possible test and other recommendation (regarding the increase in NBT results).

CONCLUSION:

Beta thalassemia is one of the cases in which the NBT test becomes false positive (without phagocytosis as a stimulant). When a patient receives a blood transfusion or undergoes splenectomy, the results of the NBT test show an increase. It can be suggested that this condition is associated with non-specific phagocytic activity (in vivo) and may improve the view that blood transfusion or spleen removal may increase the phagocytic activity of a vivo patient. In addition, it can be said that in patients with splenectomy, the removal of active neutrophils (NBT-positive) from the bloodstream is delayed.

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