ORIGINAL ARTICLE

COMPARISON OF NEUTROPHIL FUNCTION IN PATIENTS WITH THALASSEMIA MAJOR AND HEALTHY CONTROLS

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Background– There are many reports of defective neutrophil function in patients with thalassemia major that lead them to increased susceptibility to acquire infections.

Methods– In this study, neutrophil function (i.e. chemotaxis, *Candida albicans* killing, phagocytosis, opsonization and nitro blue tetrazolium [NBT] reduction) were evaluated in 30 patients with thalassemia major (16 splenectomized and 14 nonsplenectomized) and 30 controls.

Results– There were no significant differences in phagocytosis, NBT reduction, and opsonization in neutrophil function between patients and controls. Chemotactic migration and *C. albicans* killing in the thalassemic patients were found to be defective. There was also a significant difference in chemotactic migration between splenectomized and nonsplenectomized patients.

Conclusion– Patients with thalassemia major showed a greater degree of susceptibility to infections than controls.

Keywords ● *Candida albicans* killing ● nitro blue tetrazolium (NBT) ● opsonization ● phagocytosis ● thalassemia major

Introduction

The immunologic defects observed in patients with thalassemia major make them susceptible to different kinds of infections, both before and after splenectomy.[1] The reasons for immunodeficiency are:

1. The immunosuppressive effects of increased ferritin concentrations due to multiple blood transfusions[1,2] and the immunosuppressive effects of blood transfusion.[3]

2. Cytomegalovirus (CMV) infection is responsible for increased susceptibility to infection because of the immunosuppressive properties of CMV. This is particularly true in splenectomized patients.

3. Abnormalities in humoral immunity such as defects in alternative complement pathways[4] and abnormal immunoglobulin levels[5,6], abnormalities in cell mediated immunity (CMI) such as decreased natural killer (NK) cell activity, defective neutrophil function,[6] decreased T-helper/T-suppressor ratio[3] and T-cell subset abnormalities.[1] Defective neutrophil function in patients with thalassemia has been suggested in different reports.

In this study we assessed different aspects of neutrophil function by measuring nitro blue tetrazolium (NBT) reduction, chemotaxis, opsonization, phagocytosis and *C. albicans* killing in patients with thalassemia major. We also determined if serum ferritin concentrations, splenectomy, transfusion number, sex and age affected these functions.

Patients and Methods

In a 12-month period, 30 consecutive patients (36.7 % females; 63.3 % males) with thalassemia

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Table 1. Comparison of opsonization, phagocytosis and nitro blue tetrazolium (NBT) reduction in patients with thalassemia major and controls.

Groups	% Opsonization (mean ± SD)	*p* Value	% Phagocytosis	*p* Value	% NBT (mean ± SD)	*p* Value
Patients (*n* = 30)	73.23 ± 7.9	0.36	261.9 ± 52.1	0.07	98.62 ± 1.31	0.8
Controls (*n* = 30)	75.10 ± 8		285.9 ± 48.9		98.50 ± 1.4	

major, of whom 16 were splenectomized and 14 were nonsplenectomized, and 30 healthy controls (43.3% females, 56.7 % males) were included in the study. The mean age of the patients ± SD was 13.5 ± 5 years (range, 2–24 years). All of the patients with thalassemia major had required blood transfusions since childhood and received blood transfusions at 1-month intervals. All of the patients had been treated with deferoxamine (Desferal) regularly.

Separation of neutrophils

Five mL of blood was drawn into plastic tubes containing 10 IU heparin/mL (Sigma, St. Louis, MO, USA). Mononuclear cells and platelets were separated from polymorphonuclear cells (PMNs) and red blood cells by centrifugation. Erythrocytes were sedimented for 45 minutes at 4ºC in 6% dextran saline (Pharmacia, Sweden) and lysed with 0.2% NaCl. PMNs were washed twice and counted with Neobar slide. Phagocytosis with *C. albicans*[6–8], *C. albicans* killing and nitro blue tetrazolium (NBT, Sigma) slide testing were used to evaluate neutrophil killing activity. An opsonization test[8,9] and chemotaxis (with Boyden chamber technique) and formyl-leucin-methionin-phenylalanine (FLMP, Sigma) as a chemotactic factor were used to evaluate other neutrophil functions. Chemotaxis was assessed by placing a chemotactic factor in the lower half of a two-part chamber.[10] Immunoradiometric assay was used to measure ferritin concentrations (IRMA kit, Specteria, Finland).

Statistical evaluation

Data were expressed as mean ± SD. Student *t*-

test and analysis of variance were used for comparison. The relationship between neutrophil function, sex, age, splenectomy, ferritin concentration and the frequency of transfusions was determined using Pearson correlation.

Results

The total neutrophil count for patients and controls showed no significant difference ($p > 0.05$). No significant difference was noted between the two groups in terms of NBT reduction, opsonization and phagocytosis of *C. albicans* ($p > 0.5$) (Table 1).

The results of chemotaxis and *C. albicans* killing tests did show a significant difference between thalasssemic patients and controls. For chemotatic migration, the measurement was 83.2 μm versus 98.6 μm in thalassemic patients and controls respectively; for *C. albicans* killing it was 21.6% versus 24.5% (Table 2).

We evaluated the relationship between age, sex, splenectomy status, ferritin concentrations and the frequency of blood transfusions with neutrophil functions. In all patients, ferritin concentrations were increased. The frequency of blood transfusion showed no relationship with neutrophil function parameters. A significant association between splenectomy and chemotaxis was found in neutrophil chemotactic migration; (79.8 ± 8.75 in splenectomized patients and 88.07 ± 11.03 in nonsplenectomized patients). Therefore, neutrophil chemotactic migration in nonsplenectomized patients is greater than in splenectomized patients and reflects the important role of spleen.

Table 2. Comparison of chemotactic migration and *C. albicans* killing in patients with thalassemia major and controls.

Groups	Chemotaxis (μm) (mean ± SD)	*p* Value	% *C. albicans* killing (mean ± SD)	*p* Value
Patients (*n* = 30)	83.23 ± 11.1	0.0001	261.6 ± 2.9	0.0001
Controls (*n* = 30)	98.63 ± 1.7		24.53 ± 3.5	

Discussion

Neutrophils, the body's primary defense against bacterial invasion, engulf and destroy bacterial and foreign particles by a process known as phagocytosis. In patients with bacterial infections, neutrophil function tests may show that neutrophils are unable to kill target bacteria or migrate to the infection site (chemotaxis).[10] Chemotaxis in patients with thalassemia major was found to be defective when compared with healthy controls. In 1993, Matzner et al noted that the chemotactic defect was encountered in all patients except one who suffered from pyogenic infections.[11] In 1996, Palacios et al found an impairment in directed chemotaxis, and it was further depressed after addition of thalassemic serum.[12] Kutukculer et al in 1996 showed that chemotactic and random migrations in a group of patients were defective, and may partially account for the increased susceptibility to infection, occasionally observed among them. [1] Kutukculer et al also suggested that defects in chemotaxis are due to an inhibitory factor in the sera of thalassemic patients, and iron-dextran complexes induce defects in chemotaxis. In 1990, Speer et al examined neutrophil function parameters including adherence, random migration, chemotaxis, killing of *Escherichia coli* and production of superoxide radicals and found all the parameters to be normal.[13] In addition, the same study found lymphocyte proliferation in response to different lectins (phytohemagglutinin, concanavalin A, and pokeweed mitogen) did not differ between patients and controls. However, the numbers of circulating T-lymphocytes, helper T-cells and B-lymphocytes were increased among some of the patients. This phenomenon probably reflects a nonspecific stimulation of the antibody-producing cells by repeated blood transfusions.[13] Another study showed a decrease in CD4+ / CD8+ ratios in the beta-thalassemia major group, and no difference in terms of absolute T-lymphocyte numbers and activated T-cell numbers was observed. These results do not correlate with the tendency toward infection.[14]

In this study, there was a significant difference in neutrophilic chemotaxis between splenectomized and nonsplenectomized patients. Wysocki et al in 1990 reported a defective chemo-tactic migration in splenectomized patients.[15] Foster et al found that the mean distance migration by normal neutrophils in the presence of serum from post-

splenectomized patients was significantly less than this measure when normal serum was used ($p < 0.005$).[16] Defective chemotactic migration may be due to splenectomy following trauma. The trauma induces an increase in corticosteroids, adrenaline and noradrenaline secretion. This increased hormonal release has a role in determining mono-cytic and neutrophilic migration prevention.[17] The defect found in these patients might be caused by iron overload.[12] Lianou and Bassaris showed that splenectomy transiently stopped the process of being able to generate chemotactic factors in the serum of thalassemic patients.[18]

Our study showed that, in peripheral blood of thalassemic patients, neutrophils have a normal ability to reduce NBT and to ingest and opsonize *C. albicans*. There is a report that reflects *C. albicans* phagocytosis in most of the thalassemia cases.[19] Sen et al in 1989 reported that the capacity to ingest *C. albicans* is preserved while the killing activity against *C. albicans* and the generation of toxic oxygen metabolites during the respiratory burst are diminished, and are inversely related to age and the serum ferritin concentration.[20] There was no significant relationship between age and ferritin concentration and phagocytosis but data confirmed that *C. albicans* killing between patients and controls was significantly different.

It has been shown that normal PMNs act against infections by different lytic mechanisms that depend on microorganism species.[6] Lahler and Cline documented that *C. albicans* killing is mediated through the myeloperoxidase-dependent system.[21] NBT testing relies on neutrophil generation of bactericidal enzymes (like NADPH-oxidase) and toxins during killing. This action increases cellular oxygen consumption and glucose metabolism that reduces colorless NBT to blue formazan.[10] Superoxide is immediately oxidized by superoxide dismutase to hydrogen peroxide (H_2O_2).[22] Myeloperoxidase is another enzyme that produces the strong oxidant hypochloric acid from hydrogen peroxide and chloride.[23] Myeloperoxidase is an enzyme necessary for normal intracellular killing of certain organisms. It catalyzes the oxidation of microorganisms by intracellular H_2O_2 in the presence of halids.[24]

With regard to normal NBT and impaired *C. albicans* killing, our data suggest a probable myeloperoxidase deficiency in patients. It would appear that neutrophils have normal oxygen consumption and superoxide anion production, however, myeloperoxidase-dependent intracellular

killing is impaired in comparison with controls.

In conclusion, our data show that there is an impairment in neutrophil migration and killing activity. Increased susceptibility to infection in patients with thalassemia major could be explained by a decrease in neutrophil function.

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