ANTI-NUCLEAR ANTIBODY POSITIVITY IN MULTI-TRANSFUSED THALASSEMIA MAJOR

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ABSTRACT

The frequency of anti-nuclear antibodies (ANA) was evaluated in multi-transfused patients of thalassemia major. Twelve out of 83 patients (14.5%) had positive ANA at titres of 1 : 80 or above. The results were compared with age and sex matched healthy controls who showed positive results in only 1 of 52 cases (1.9%; $p<0.05$). Antibody against double stranded DNA was absent. ANA positivity was found to correlate with higher age ($p<0.01$), more amount of blood transfused ($p<0.01$), splenectomy status ($p<0.01$), higher levels of serum ferritin ($p<0.01$) and presence of hepatitis B surface antigen ($p<0.01$) and antihepatitis C antibody ($p<0.01$).

Key words: *Antinuclear antibody, Thalassemia major.*

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Multi-transfused patients with or without iron overload and cases who are splenectomised are known to develop immunological abnormalities(1,2). Patients with recurrent infections show increased evidence of various autoantibodies(3). In thalassemia major (TM), multiple transfusions, iron overload, splenectomy and various infections can lead to immunological abnormalities as well as production of autoantibodies(4,5).

We studied 83 patients of multi-transfused thalassemia major for the incidence of anti-nuclear antibody (ANA). Those with positive ANA, were further investigated to detect presence of antibody against double stranded DNA (anti-ds-DNA).

Material and Methods

Patients of thalassemia major receiving regular transfusions were eligible to participate in the study. The clinical and transfusion records were reviewed. Review of patients' data included age at diagnosis and at first transfusion, amount of blood transfused, date of last transfusion, time and amount of iron chelation received, splenectomy status, any other medication, history of past or present illness including infections. Any significant complication was recorded in detail. Patients were carefully reviewed for the presence of any concomitant disease known to be associated with the presence of ANA positivity. Patients with symptoms of arthralgia, arthritis or other systemic features of rheumatic fever, or collagen vascular diseases including rheumatoid arthritis were excluded. Those on treatment with oral iron chelator (L_1, *i.e.*, 1-2-dimethyl-3hydroxypyride-4-one) were not included as the drug is known to produce arthralgia or arthritis(6-8) and variable of ANA positivity(8,9).

Sera from 52 age and sex matched healthy normal subjects were used as controls. Blood was collected in fasting state in morning, centrifuged immediately and serum was separated. ANA was measured using an indirect immunofluorescent antibody technique (Fluoro-Kit™ test systems, Incstar Corp., Stillwater, Minnesota). Sera were tested in serial doubling dilutions starting at 1 : 20. All positive samples were further tested for presence of anti-ds-DNA. Iron overload was studied by serum ferritin assay using ELISA technique. Hepatitis B surface antigen (HBsAg) and antibody against hepatitis C virus (anti-HCV) were also tested by ELISA technique. Incidence of ANA positivity in patients versus controls was compared and significance was found by assessing p value. Similarly, attempt was made to assess the significance of various factors responsible for ANA positivity by calculating p values. These factors included age, amount of blood transfused, splenectomy status, HBsAg and anti-HCV positivity and serum ferritin level. A p value less than 0.05 was said to be significant.

Results

Eighty-three patients of transfusion dependent thalassemia were investigated. The patients included 49 males and 34 females (ratio 1.4 : 1). All had homozygous beta-thalassemia major except three patients who had HbE/beta thalassemia. The mean age was 9.2 ± 7.6 years (range 2.5 to 23.5). The control population included 30 males and 22 females (ratio 1.4 : 1) with a mean age of 7.3 ± 5.3 (range 3-27.5) years. Twenty one (25.3%) patients were splenectomised. The amount of blood received varied from 12 to 293 (mean 109 ± 32.4) units. Twelve patients (14.5%) gave history of overt attack of jaundice (one or more). Hepatitis markers showed presence of HBsAg in 9 (10.8%) and presence of Anti-HCV in 11 (13.3%) cases.

Positive ANA test with titre of 1 : 80 or more was found in 12 patients (14.5%). These results were significant when compared to the control group where ANA positivity (1 : 80 or above) was seen in only one case (1.9%; $p<0.05$). Six patients (7.2%) had ANA titres of 1 : 160 or greater while none had similar titre in the control group ($p<0.05$). All patients with ANA positivity were negative for anti-ds-DNA antibody.

Analysis of various factors between the two groups of patients with ANA positivity and negativity showed that a higher age, larger amount of blood transfused, splenectomy, presence of hepatitis markers (hepatitis B and C) and higher levels of serum ferritin were more commonly seen in the ANA positive group and the differences were significant (*Table I*).

Discussion

This study showed ANA positivity in titre of 1 : 80 or above in 14.5% of multi-transfused patients. The results have also correlated the ANA positivity to various factors including age, number of transfusions, splenectomy, higher levels of serum ferritin and infections with Hepatitis B or C.

The exact mechanism underlying the higher incidence of ANA positivity in patients of thalassemia multi-transfused could be multifactorial. Immunological abnormalities have been described in multi-transfused patients(1). Iron overload(2,4,5,10), chronic and recurrent infections(3) and splenectomy(2) are also important factors.

Patients of sickle cell disease are known to have higher incidence of ANA

TABLE I – *Comparison of Certain Characteristics between ANA Positive and ANA Negative Multi Transfused Thalassemics*

Parameter	ANA positive (≥1 : 80)	ANA negative (≤1 : 40)	p value
Number of cases total	12	71	
(ANA ≥ 80)	12	71	<0.05
(ANA ≥160)	6	Nil	<0.05
Age in years (Mean ± SD)	15.2 ± 7.9	8.7 ± 4.7	<0.01
Blood transfusion (units)	172.2 ±33.8	9.6 ±28.5	<0.01
Splenectomy	9 (75%)	1 (16.9%)	<0.01
HBsAg positive	6 (50%)	3 (4.2%)	<0.01
Anti HCV positive	7 (58.3%)	4 (5.6%)	<0.01
Serum ferritin (ng/ml)	5120 ± 2330	2960 ± 1510	<0.01

positivity(11). Altered immunological function secondary to multiple transfusions, infections and functionally asplenic state are the various proposed mechanisms(11). Same may apply to patients of thalassemia major.

ANA positivity in thalassemia with or without oral chelator L_1 (1-2-dimethyl-3-hydroxypyrid-4-one) has been described(8,9). The subject is important as arthralgia and arthritis have been noted in patients receiving L_1(6-8). Further studies are required to evaluate the immunological alterations and ANA status of cases of thalassemia major and its clinical significance.

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REFERENCES

1. Fisher E, Lenhard V, Seifert P, Kluge A, Johannsen R. Blood transfusion-induced suppression of cellular immunity in man. Hum Immunol 1980, 3: 187-192.
2. Kapadia A, de Souza M, Markensol AL, Miller DR, Good RA, Gupta S. Lymphoid cell sets and serum immunoglobulins in patients with thalassemia intermedia: Relationship to serum iron and splenectomy. Br J Hematol 1980, 45: 405-416.
3. Morton JI, Siegel BV. Stimulation of the reticuloendothelial system and autoimmunity. Adv Exp Med Biol 1979, 121: 3107-3115.
4. Grady RW, Akbar AN, Giardina PJ, Hilgartner MW, de Souza M. Disproportionate lymphoid cell subsets in thalassemia major: The relative contribution of transfusion and splenectomy. Br J Hematol 1985, 59: 713-720.
5. Akbar AN, Fitzgerald-Bocarsly PA, de Souza M, Giardina PJ, Hilgartner MW, Grady RW. Decreased natural killer activity in thalassemia major: A possible consequence of iron overload. J Immunol 1986, 136: 1635-1646.

6. Agarwal MB, Vishwanathan C, Ramanathan J, *et al*. Oral iron chelation with L_1. Lancet 1990, 335: 601.

7. Agarwal MB, Gupte SS, Vishwanathan C, *et al*. Phase II trial of 1-2-dimethyl-3-hydroxypyrid-4-one (L_1)–the oral iron chelator in 52 patients of transfusion dependent thalassemia. Blood 1990, 76 (Suppl i): 52a.

8. Bartlett AN, Hoffbrand AV, Kontoghiorghes GJ. Long term trial with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L_1). II Clinical observations. Br J Hematol 1990, 76, 301-304.

9. Agarwal MB, Gupte SS, Vishwanathan C, Vasandani D, *et al*. Effective oral iron chelation in patients of thalassemia. Proceedings of the International Symposium cum workshop on "Anemia in Children". Bombay, February 1991, p 13.1-13.13.

10. de Souza M. Immune cell functions in iron overload. Clin Exp Immunol 1989, 75: 1-9.

11. Ofosu MD, Saunders DA, Dunston GM, Castro O, Alarif L. Association of HLA and autoantibody in transfused sickle cell patients. Am J Hematol 1986, 22: 27-33.

NOTES AND NEWS

SUPPLEMENT ON DEVELOPMENTAL ASSESSMENT FOLLOW UP AND INTERVENTIONS IN HIGH RISK NEONATES

In the supplement published by this office on "Developmental Assessment Follow Up and Interventions in High Risk Neonates" the names of Dr. Anand Pandit and Dr. Sheila Bhave were inadvertently listed on the first page as editors, instead of Dr. Sudha Chaudhari and Dr. Sujata Kulkarni. The error is regretted.

Dr. R.K. Puri
Editor