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Cryoglobulinemia in Thalassemia Major A Preliminary Report*

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Serum immunoglobulins which reversibly precipitate when exposed to cold are commonly called cryoglobulins or more appropriately cryoimmunoglobulins. They have been commonly reported as an incidental finding in a variety of diseases and received little attention until recently. Normal serum generally does not contain cryoglobulins. They are usually detected in cases with increased levels of IgM and IgG. Cryoglobulins belonging to the IgG or IgM or both classes have been found in a variety of disease states such as multiple myeloma, primary macroglobulinemia and related malignancies, the so-called connective tissue diseases (S.L.E.. periarteritis nodosa, rheumatoid arthritis, Sjögren's syndrome) cirrhosis, chronic infections (kalaazar, subacute bacterial endocarditis, syphilis, etc.)[1,7] A high incidence of cryoglobulinemia in acute glomenulonephritis is reported[8]. They may be detected in angina pectoris and acute myocardial infarction without the symptoms mentioned above.[9,10] Some authors believe that cryoglobulins are autoantibodies against IgG which are effective in the cold. Recently in some patients with thrombotic thrombocytopenic purpura, after exposure to cold, development of IgG and IgM autoantibody complexes have been reported.

Arthralgia, Raynaud's phenomenon, purpura, weakness, lymphadenopathy, hepatomegaly, splenomegaly, signs of peripheral vas-

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cular stasis and nephritis are the commonly recognized symptoms and findings in patients with cryoglobulinemia.

During a study of immunoglobulins in sickle-cell anemia and thalassemia we incidentally observed that the serum of these anemic children developed precipitates during a conservation period of 24 hours in a refrigerator at 4 °C. With the heating of the serum at 37 °C these cryoprecipitates completely disappeared. Since we could not find any report of cryoglobulinemia in thalassemia patients, we believe this is the first study on this subject.

Material and Methods

We studied serum samples of 23 children with beta thalassemia major. Their ages varied between 3 and 16 years. The diagnosis in those children was made by hemoglobin electrophoresis, fetal hemoglobin, sickling test, red-cell fragility, serum iron, and iron binding capacity, bone marrow aspiration, reticulocyte count in addition to routine hematological tests, using conventional laboratory methods.[11] Those tests were also done on parents. For cryoglobulin studies, blood was drawn and kept at room temperature at 24 °C for an hour. After clotting took place, the serum was separated and was kept in the refrigerator at 4 °C for a period of 48-72 hours. An aliquot was heated to 37 °C and disappearence of the cryoprecipitate was observed. Degree of the cryoprecipitation was graded from + to +++ according to Charmot and co-workers which assessed precipitation, flocculation and gel formation respectively[12]. Since we used only serum in this study, the observed precipitates were cryoglobulin, but not cryofibrinogen which is seen in the plasma. From the cryo-precipiate immunoglobulin levels were determined by the method of Fahey and Mc Kelvey, using Hyland plates[13].

Results

Cryoglobulins were observed to be positive in 6 out of 23 children with thalessemia major (26 %).

Table I shows the results of serum electrophoresis and immunoglobulin levels in 6 thalassemic children with cryoglobulinemia.

High levels of beta and gamma fractions in serum electrophoresis is noticed. Immunoglobulins are also high in these patients.

Table II shows the immunoglobulin levels in an 8 year-old thalassemic girl M.G. who had splenectomy two years before this study.

She had +++ cryoprecipitate in her serum and after separation of this, immunoglobulins were determined in both, in this fraction and in the serum. She had 130 blood transfusions. As is seen, the IgG and IgM levels were much higher in the cryoportion than that of the serum portion suggesting that cryoglobulinemia in this patient might be a mixed (IgG+IgM) type.

TABLE I

SERUM PROTEINS AND IMMUNOGLOBULINS IN PATIENTS WITH THALASSEMIA MAJOR AND CRYOGLOBULINEMIA

	Serum Proteins (gm/100 ml)						Immunoglobulins (mg/ml)			
Pt.	TP.	Albumin	α_1	α_2	β	γ	IgG globulins	IgM	IgA	Cryoglob.
1. M.G. (8 y)	9.1	4.48	0.48	0.53	0.83	2.78	17	1.2	6.4	+++
2. Ş.A. (5 y)	7.8	4.87	0.58	0.59	0.29	1.45	20	2.5	3.9	+
3. Ş.A. (7 y)	9.0	5.31	0.34	0.88	1.13	1.32	14	0.9	2.0	+
4. Ş.H. (16 y)	9.6	5.07	0.48	0.67	0.76	2.59	14	2.5	2.9	+
5. N.K. (16 y)	8.3	4.23	0.30	0.73	1.29	1.71	12	0.6	3.9	++
S. SAL (3 y)	6.9	2.07	0.46	0.72	0.85	2.76	14	0.22	1.7	+

TABLE II

IMMUNOGLOBULIN LEVELS IN THE SERUM AND CRYOPRECIPITATE OF M.G.

	IgG (mg/ml)	IgM (mg/ml)	IgA (mg/ml)	Cryoglob.
Cryo-portion	40	3.5	1.1	+++
Serum-portion	28	0.9	1.95	+

We also found β_1C in cryoportion in 2 patients. The first patient was the same one who had mixed cryoglobulinemia (Table III).

TABLE III

β_1C LEVELS IN TWO PATIENTS

	Cryoportion	Serum portion
M. G. (Hb 5 gm/100 ml, WBC – 1700)	90 %	135 %
Ş. H. (Hb 4 gm/100 ml, WBC – 2100)	50 %	190 %

Discussion

In this preliminary study we found a 26 % incidence of cryoglobulinemia in patients thalassemia major. One of the common findings in our thalassemics with cryoglobulinemia was splenectomy, which was done at least a year before this study, in three patients (Nos. 1,3 and 4).

They exhibited a moderate to marked degree of hepatomegaly with disturbed liver function tests. Their hemoglobin levels were below 8 gr. per 100 ml. All had hypergammaglobulinemia on paper electrophoresis of the serum and had high levels, of IgG and IgM.

All but one (the last patient in this series) received multiple blood transfusions of which the effects on cryoglobulinemia is not known. Our first patient had acute glomenolonephritis and recovered. The second and third patients had hepatitis. The latter had Au antigen positive in her serum. Cryoglobulinemia lasted between 1 and 4 months in our patients.

Hepatitis and nephritis are both known to cause cryoglobulinemia.[8,14] Grupe[8] observed cryoglobulinemia in 8 out of 28 children with acute post streptococcal nephritis (31%). The cryoglobulins were of the mixed type of IgG, β1C and persisted from 2 weeks to 3 months. In renal biopsy, fluorescent studies showed granular deposition of IgG and β1C along capillary loops. In view of these findings, he suggested that cryoglobulins might be of immunopathologic importance in certain cases of acute nephritis.

Feizi and Gitlin also described a patient (21 year-old man) with chronic hepatitis who showed mixed (IgG + IgM) type cryoglobulinemia after development of glomenulonephritis following exposure to cold.[14] Granular deposits containing IgG + IgM were found in his renal glomeruli with only trace amounts of β1C. In the absence of evidence of recent streptococcal infection and on account of the similarity of the glomerular immunoglobulin deposits and the cryoglobulin, it seemed likely that this man might have had immune-complex disese of the kidney due to deposition of cryoglobulin.

The high incidence of pericarditis of unexplained etiology in splenectomized thalassemic children is recently brought into attention by Wasi and co-workers who found high ASO titers in majority.[15] The frequency of arthralgia, arthritis, mitral valve lesions compatible with rheumatic disease and of proliferative glomerulonephritis, are unusually high among thalassemic patients and ASO titers were frequently raised in a large number of these patients with cardiac and

renal involvement, especially after splenectomy. Therefore, it is suggested that thalassemia is associated with increased susceptibility to streptococcic infection which is enhanced by splenectomy.

In our thalassemic patients, in view of the fact mentioned above, one might expect high incidence of streptococcic infections and nephritis associated with cryoglobulinemia. Further studies are in progress in our laboratory for the explanation of cryoglobulinemia in these patients.

Summary

Six children with thalassemia and cryoglobulinemia are presented and the related literature is reviewed briefly.

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