A WARM ANTIBODY MEDIATED ACUTE HEMOLYTIC ANEMIA WITH RETICULOCYTOPENIA IN A FOUR – MONTH – OLD GIRL REQUIRING IMMUNOSUPPRESSIVE THERAPY*

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SUMMARY: Olcay L, Düzova A, Gümrük F. (Hematology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). A warm antibody mediated acute hemolytic anemia with reticulocytopenia in a four-month-old girl requiring immunosuppressive therapy. Turk J Pediatr 1999; 41: 239-244.

We present a four-month-old girl with severe hemolytic anemia and reticulocytopenia. This case is the youngest with hemolytic anemia encountered in our hospital. Findings of autoimmune hemolytic anemia were preceded by diphtheria-pertussis-tetanus (DPT) and oral polio vaccines which were given one month before. At admission, she had heart failure, her hemoglobin (Hb) was 27 gm/L, hematocrit (Hct) 8.5 percent, reticulocyte count 0.2 percent, and gamma and non-gamma Coombs tests were positive. Plasma Hb was 23 percent (N < 3%) and haptoglobin 0 mg/dl. Bone marrow aspiration smear revealed erythroid hyperplasia. No infection, immunodeficiency or malignancy could be established. She received multiple transfusions and did not respond to methyl prednisolone therapy of seven days' duration, but was successfully treated with a combination of immunosuppressive therapy (cyclophosphamide, 6-mercaptopurine, intravenous immunoglobulin and prednisolone, which was added later). This case is interesting in that the disease was preceded by DPT vaccination, was associated with reticulocytopenia and was resistant to steroids. *Key words: autoimmune hemolytic anemia, childhood, reticulocytopenia, warm antibody.*

Autoimmune hemolytic anemia (AIHA) of childhood is generally mediated by warm antibodies and is almost always extravascular; however, when hemolysis is severe intravascular hemolysis may coexist[1]. It generally pursues a chronic course, especially in children under two years of age, although this is not a rule[2]. There have been few patients having reticulocytopenia despite severe hemolytic anemia who responded to steroids[3-5]. We report herein a four-month-old girl with AIHA mediated by warm antibodies which pursued an acute course. She also had reticulocytopenia and signs of intravascular hemolysis. She did not respond to steroid therapy and was treated with immunosuppressive drugs.

Case Report

A four-month-old girl was referred to our hospital with jaundice and anemia. She had begun vomiting three days before and had no history of respiratory tract infection, or symptoms like diarrhea, skin lesions or fever. Two days prior to

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admission jaundice appeared. She had not been given any medication. She we breast-fed. She had been vaccinated regularly: BCG at the 2nd month diphtheria, pertussis, tetanus (DPT) and oral polio at the 2nd and 3rd months. Diphtheria-pertussis-tetanus and oral polio vaccines were the last administered one month prior to admission. She was the second child of healthy non-consanguineous parents. On admission, physical examination revealed a head circumference of 39.5 cm (50th percentile), weight of 5,300 g (25th-50th percentile), and length of 62 cm (75th-90th percentile). Skin and conjunctiva were icteric, there was no skin lesion and no lymphadenopathy. Heart rate was 162/min and the liver and spleen extended 4 and 2 cm below the right and left costal margins, respectively.

Hemoglobin (Hb) was 27 g/L (2.7 g/dl), hematocrit (Hct) 8.5 percent, mean corpuscular volume 76.9 fl, white blood cell count 16.4 x 10^9/L (16,400/mm^3) and platelet count 380 x 10^9/L (380,000/mm^3). Differential count revealed 44 percent neutrophils, 3 percent monocytes, 53 percent lymphocytes, and anisocytosis and poikilocytosis.

Reticulocyte count was 0.2 percent, total bilirubin 3.9 mg/dl, direct bilirubin 1.1 mg/dl, serum glutamic-oxaloacetic transaminase 66 IU/L, glutamic-pyruvic transaminase 16 IU/L, gamma Coombs test (+), non-gamma Coombs test (+), cold agglutinin (-), plasma Hb 23 percent (normal < 3%), and haptoglobine 0 mg/dl. The bone marrow aspiration smear revealed erythroid hyperplasia. Erythrocyte glucose-6-phosphate dehydrogenase, urinary and blood amino acid examination, and immunoglobulin G, A, M levels were normal. Epstein-Barr virus (EBV) IgM was (-), EBV IgG (+), cytomegalovirus (CMV) IgM (-), CMV IgG (+), Parvovirus (PV) B19 IgM (-), PV B19 IgG (+), hepatitis B surface antigen (-), and hepatitis B surface antibody (-).

Methyl prednisolone treatment was started with a dose of 30 mg/kg/day and was continued for seven days. However, blood transfusions had to be performed four times within the first week because of severe anemia. Plasmapheresis was attempted because of the intravascular component of the hemolysis, but was technically unsuccessful; therefore, intravenous immunoglobulin (400 mg/kg/day), cyclophosphamide (5 mg/kg/week), and 6-mercaptopurine (1.5 mg/kg/day) were started on the eighth day, when Hb was 38 g/L (3.8 g/dl). Cyclophosphamide was stopped at the third week when an Hb level of 125 g/L (125 g/dl) was maintained. Prednisolone (2 mg/kg/day) was then added to 6-mercaptopurine. Prednisolone was given for one month and was stopped after tapering the dose over 15 days. 6-mercaptopurine was continued for three months and was then stopped. The child has maintained an Hb level of 120 g/L (12 g/dl). Hemoglobin and Hct levels; reticulocyte counts; and gamma, non-gamma, and indirect Coombs tests during the follow-up are shown in Table I.

Table I: Hemoglobin and Hematocrit Levels, Reticulocyte Counts, and Direct and Indirect Coombs Tests During Follow-up

	Hb (gm/L)	Htc (%)	Ret (5)	Gamma Coombs Test	Non-Gamma Coombs Test	Indirect Coombs Test	Drugs*
1st week	27	8.5	0.2	+	+	+	HDMP
2nd week	38	12	0.2	+	+	+	Iv IgG Cyc 6MP
3rd week (discharge)	125	37	0.2	+	+	+	6MP continued Pred
5th week	99	32	0.4	+	+	+	6MP continued Pred continued
7th week	125	37	0.8	–	–	–	6MP continued Pred stopped
9th week	125	37	0.4	–	–	–	6MP continued
14th week	134	39	0.4	–	–	–	6MP stopped
16th week	126	36	0.2	–	–	–	
19th week	120.8	36	0.2	–	–	–	

High dose methylprednisolone (HDMP).. : 30 mg/kg/day x 7 days po.
Intravenous immunoglobulin G (Iv IgG) . : 400 mg/kg/day x 5 days iv.
Cyclophosphamide (Cyc)..... : 5 mg/kg/week iv.
6-mercaptopurine (6MP) ..... : 1,5 mg/day, po.
Pednisolone (Pred)..... : 2 mg/kg/day, po.
* Folic acid was given between 1st-4st weeks.

Discussion

In Childhood and adolescence the incidence of AIHA has been reported as 1/267,000 to 1/1,780,000. In the literature, patients' ages ranged from eight weeks to 10 years[4]. This patient is the youngest patient with hemolytic anemia encountered in our hospital.

Autoimmune hemolytic anemia can be associated with infections (respiratory infections, EBV, CMV, mycoplasma, human immunodeficiency virus); immunodeficiency syndromes (X-linked agammaglobulinemia, dysgammaglobulinemia, IgA deficiency, Wiskott-Aldrich syndrome, common variable hypogammaglobulinemia); malignancies (non-Hodgkin's lymphoma, Hodgkin's disese, acute lymphocytic leukemia, thymoma, ovarian cysts and tumors); and disorders associated with autoantibody production (systemic lupus erythematosus, neonatal lupus syndrome, rheumatoid arthritis, chronic active hepatitis, ulcerative colitis, thyroid disorders)[3, 1]. However, the detailed past history and laboratory examinations of our patient revealed none of these etiologic agents except the vaccines of DPT and oral polio which were done one month prior to illness. Among Zupanska et al.'s[6] cases of children with AIHA, in two out of 13 acute AIHA and one out of 12 subacute AIHA, immunization (against typhoid fever together with poliomyelitis, DPT and typhoid fever, respectively) preceded the

development of hemolysis. In all three patients AIHA was mediated by warm antibodies as it was in our patient. Symptoms of AIHA started one or two weeks after typhoid vaccine in the patient with subacute AIHA versus the four weeks in our case.

The onset of the illness may be acute with abdominal pain and vomiting (as in our patient) with or without fever, general weakness, anorexia, diarrhea, pallor, hemoglobinuria[6,7].

Constant physical findings such as pallor, tachycardia, jaundice, moderate splenomegaly and mild hepatomegaly, and mixed or unconjugated hyperbilirubinemia may be encountered[4], as in our patient. Leukopenia and thrombocytopenia, which were not features in our case, may accompany anemia in some patients.

Positive gamma and non-gamma Coombs tests which demonstrated the presence of IgG and C3, respectively, and the absence of cold agglutinins, confirmed that the hemolysis in our case was mediated by a warm antibody of IgG with C3. (In childhood, AIHA due to warm antibodies is more common than AIHA due to cold antibodies and generally pursues a chronic course[3,5]). However, in our patient the disease pursued an acute course. Moreover, it has been reported that AIHA in patients less than two years of age (and over 12 years of age) generally presents as chronic forms[2]. However, there are enough exceptions to these generalizations to preclude applying them in individual cases[8]. Signs of hemolysis in our patient disappeared within two weeks and the serologic tests were negative within a further six weeks. The disease has not relapsed for two years. Zupanska et al.'s[6] patients with AIHA mediated by warm antibodies and preceded by vaccination also did not have a chronic course. In warm antibody mediated AIHA, hemolysis is almost always extravascular; however, in severe cases intravascular hemolysis may occur[1], as in our patient.

This case is the first case of severe AIHA with reticulocytopenia we have encountered. Liesveld et al.[9] showed that median reticulocyte percentage of 109 cases with AIHA at diagnosis was 9%; 20% of the cases had an initial reticulocyte count < 4%, the lowest being 0.4%.These reticulocytopenic patients were nearly evenly distributed between warm and cold antibody mediated cases. Fifty-four percent of reticulocytopenic cases had a bone marrow examination. Three fourths of these marrows showed erythroid hyperplasia; erythroid hypoplasia was seen in only one case. The bone marrow of our patient also revealed erythroid hyperplasia.

Our patient differs from Liesveld et al.'s[9] reticulocytopenic patients, 76 percent of whom had increases in their reticulocyte index, the increase being more prominent among patients who received steroids. Among Liesveld et al.'s[9] reticulocytopenic patients, ony four out of 33 did not show any increase in reticulocyte counts as with our patient. Unlike Conley et al.'s[10] patients, who

displayed brisk reticulocytosis within a few days to a week after diagnosis and initiation of therapy, our patient's reticulocyte count never changed with therapy, in spite of recovery.

It has been suggested that reticulocytopenia in adults indicates poor prognosis, while in childhood reticulocytopenia this is not necessarily the case[5].

Delay in proliferation of red cell precursors[10] or preferential destruction of them[11], by a complement-dependent serum IgG inhibitor directed against erythroid colony and burst-forming units[12], are suggested as possible mechanisms for reticulocytopenia in AIHA.

Treatment of the underlying disorder brings the hemolytic anemia under control. Corticosteroids are the first agents to be used[5,7]. Patients with warm antibody induced AIHA generally respond to steroid therapy in dosages up to 2-10 mg/kg day of prednisolone. An increase in Hb can be accomplished within one to four days, and for reticulocytopenic patients within eight days[9].

Our experience shows that high dose methyl prednisolone is quite effective in patients with hemolytic anemias who are resistant to low dose prednisolone[13]. Thus, we immediately started high dose methyl prednisolone as the first choice of treatment rather than a low dose steroid to avoid a possible delay due to resistance to low dose prednisolone, in view of the severity of anemia in our case.

Transfusions should be given if AIHA is of life-threatening severity. As the response to high dose methyl prednisolone was inadequate, intravenous immunoglobulin, 6-mercaptopurine and cyclophosphamide were started. Plasmapheresis, exchange transfusion, other alkylating agents (chlorambucil), vitamins B_{12} and B_6, Vinca alkaloids, splenectomy, cyclosporin-A, monoclonal antibodies and danazol are other therapeutic strategies, although the roles of the last three have yet to be evaluated[3,1]. Improvement in AIHA with immunosuppressive drugs was reported in 60 percent of children[14].

The case was interesting as there was no history or finding of preceding infection only DPT and oral polio vaccines administered one month prior to illness, which is a rare cause of AIHA; reticulocytopenia occurred despite severe hemolysis; and there was no increase in reticulocyte count during steroid therapy. Moreover, forms of AIHA which are resistant to steroids and require additional immunosuppressive drugs are generally chronic forms and not acute types, as in our patient.

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