Anti N Antibody in a Patient of Renal Failure on Hemodialysis

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Abstract Anti N is a rare antibody. It has been reported in renal failure patients on hemodialysis using formaldehyde sterilized equipment. Although anti N is usually clinically insignificant, rarely it can cause hemolytic transfusion reaction. Patients with clinically significant antibodies should receive N antigen negative red cell units. We report a case of anti N antibody active at 37 °C in a patient of chronic renal failure on regular maintenance hemodialysis. The present case is being reported mainly because of its detection during routine blood grouping and compatibility testing by tube method in a patient of chronic renal failure on regular maintenance hemodialysis.

Keywords Anti N antibody · Renal failure · Hemodialysis

Introduction

Alloimmunisation due to previous pregnancy or transfusion can lead to the formation of anti N antibody. Chronic renal failure patients are frequently exposed to allogeneic red blood cell transfusions. Anti N antibodies have been described more frequently in hemodialysis patients [1]. Use of reusable dialysers sterilized with formaldehyde has been reported to lead to formation of anti N like antibody [2]. Antibodies to N antigen are usually rare and clinically insignificant. However, the anti N antibodies in patients with renal failure on maintenance hemodialysis have been associated with reduced survival of red cells [3]. Immediate

renal allograft failure has also been reported in such patients [4, 5]. Anti N antibodies are usually cold agglutinins but antibodies reactive at 37 °C may rarely be encountered.

Case History

A 36 year old male patient presented with breathlessness, distention of abdomen and decreased urine output. Patient was a known case of chronic renal failure on maintenance hemodialysis. The patient had received 2 units of packed red cells 2 months prior to his current visit. Complete blood count of the patient revealed anemia (hemoglobin-5.4 g/dl, hematocrit 18 %, MCV 76 fl, MCH 23 pg, MCHC 30 g/dl), mild leukocytosis (total leucocyte count-11,900/ μ l) and a normal platelet count (3.62×10^5 / μ l). Reticulocyte count was raised (4.8 %). Coagulation profile was normal. Peripheral blood film showed moderate anisocytosis and poikilocytosis, microcytes, mild hypochromia, target cells, elliptical and few tear drop cells. Blood urea was elevated (140 mg/dl) and serum creatinine was also raised (5.2 mg/dl). Serum alkaline phosphatase was deranged (2,620 IU/l). Patient was reactive for hepatitis C. Chest X ray revealed bilateral pleural effusion. Ultrasound of abdomen showed gross free fluid in the peritoneal cavity. Both kidneys showed features of chronic renal failure.

Patient was advised hemodialysis and request for 2 units of packed red cells was received. Blood group of the patient was B Rh(D) positive on cell grouping. However in serum grouping there was reaction with O cells at room temperature which was enhanced at 4 °C and persisted at 37 °C. Auto control and direct antiglobulin test (DAT) were negative. Antibody screening using commercial three cell panel performed with Diamed ID microtyping system

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was negative. Further antibody screening was done by tube method. Screening cell III was positive in immediate spin phase and reaction strength enhanced at 4 °C, weakened at 37 °C and was negative in the antihuman globulin (AHG) phase. The antibody possibilities were c, e, K, k, Fy^b, Jk^b, Le^b, N, s and Lu^a. Antibody identification was performed by tube method using commercial eleven cell panel and findings were consistent with anti N antibody. The reaction strength was stronger in cells carrying double dose of N antigen than in M+N+ cells thereby showing dosage phenomenon. Papainised cells were prepared and the reactivity was again tested with patient serum. The reaction strength diminished with enzyme treatment. Patient was phenotyped as N– and B Rh(D) positive units were phenotyped for N antigen. The patient was safely transfused two B Rh(D) positive N– units compatible by both tube method and gel method. There was improvement in the hemoglobin from 5.4 to 6.7 g/dl. A special immunohematology card was issued to the patient mentioning the nature and type of antibody. Advice for future transfusions was also given.

Discussion

Anti N is a rare antibody which was first described in patients on hemodialysis by Howell and Perkins in 1972. Antibody appearance was not related to blood transfusion [1]. Patients on chronic hemodialysis who are treated with reusable dialyzers sterilized with formaldehyde are known to form antibodies with N-like specificity irrespective of whether the patient is N+ or N– [6–9]. Various mechanisms have been proposed (1) antigens on foreign surface of the extracorporeal circuit lead to anti N antibody formation, (2) exposure to foreign surface or trauma leads to modification of N antigen. (3) The formaldehyde used to sterilize the dialysis membranes alters the N antigen which is further recognized as foreign leading to antibody formation [10]. The last mechanism is more likely. The antibody titre decreases when dialysis treatment and exposure to formaldehyde are stopped. Harrison et al. found no antibodies in sera of patients using dialysers sterilized with ethylene oxide [2]. In the present case, formalin was used to sterilize the dialysers and patient was on hemodialysis for 2 years.

Sandler et al. suggested that anti N antibody may be of two types; anti N-like and anti formaldehyde [6]. Sharon showed that initially formaldehyde antibodies are produced 6 months after hemodialysis, later anti N-like antibodies appear thus framing the hypothesis of cross reactive antibodies. He suggested that N-like antibodies are predominantly of IgM type and formaldehyde antibodies are IgG type [9]. Lynen et al. found sequential production of

formaldehyde related antibodies in hemodialysis patients and showed that formaldehyde dependent red blood cell immunization occurred in several stages. The authors found a considerable amount of warm anti N antibodies (IgG) in hemodialysis patients. The antibody production was dependent on the time of hemodialysis treatment [11].

Various studies have demonstrated the frequency of anti N from 3 to 27 % [1, 3, 6, 12]. Hemodialysis associated anti N antibodies can cause problems in blood grouping and cross matching. In our patient there was a reaction with O cells in serum grouping and cross match was incompatible by the tube method in immediate spin phase. Anti N is usually clinically insignificant but rarely has been reported to cause acute and delayed hemolytic transfusion reaction. A few cases of warm autoimmune hemolytic anemia have also been described due to auto anti N [13]. Clinically significant anti N antibodies in renal failure patients can cause unexplained anemia. Patients with N antibodies active at 37 °C should be provided antigen negative compatible red cells [14]. Our patient had anemia with hemoglobin of 5.4 g/dl before transfusion which improved to 6.7 g/dl after transfusion of B Rh(D) positive N– compatible packed red cells. Although the antibody reactivity was maximum at 4 °C, it persisted at 37 °C also hence it could be possible that a clinically significant anti N antibody was developing in our patient.

In a study on 96 patients on chronic hemodialysis with formaldehyde sterilized equipment, 19 % had a positive direct antiglobulin test significantly associated with anti-Form (anti formaldehyde) in the patient's serum. The eluate from DAT positive cells was non reactive with standard reagent cells but reacted with formaldehyde treated cells. In our patient the DAT was negative [15]. In their study on 87 chronic renal failure patients on hemodialysis, Fonseca et al. found anti N like and anti Form antibodies in 5.7 and 60.9 % patients respectively. They used indirect antiglobulin test (IAT), DAT and direct polybrene test (DPT) and found higher sensitivity of DPT over DAT. However the specificity of DAT was more. They concluded that formaldehyde specific antibodies could increase the risk of hemolysis, reduce the survival of red cells and increase the need for blood transfusions in these patients [16].

Anti N antibodies have been reported to cause failure of renal allografts in patients who undergo kidney transplantation [4, 5]. This could be due to microvascular agglutination of red cells in the chilled kidney as anti N antibodies are mostly cold agglutinins. Patients on dialysis should be investigated for the occurrence of these antibodies especially if they are candidates for renal transplant or if there is unexplained progression of anemia in such patients. The present case highlights the fact that anti N antibodies in patients on hemodialysis may be missed routinely because

of their typical serological characteristics. We recommend inclusion of O cells in serum grouping and compatibility testing by tube method in addition to gel method whenever room temperature reacting agglutinins are detected. In addition, routine antibody screening and identification by tube method in patients of chronic renal failure is suggested so that such antibodies are detected early. Patients with anti N active at 37 °C should receive N antigen negative red cell units. Although it is not feasible to stop the use of formaldehyde sterilized dialysers particularly in developing countries due to cost constraints, advice about the use of disposable dialysers or alternative sterilization methods such as ethylene oxide should be given in patients with antibodies to formaldehyde.

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