Abstract:
Immune mediated hemolysis is a rare phenomenon that can occur following renal transplantation. One of the important causes is the passenger lymphocyte syndrome, which occurs following minor ABO blood group incompatibility between donor and recipient. Lymphocytes travelled along with allograft have been reported to cause immune hemolytic anemia in minor ABO incompatible transplants. This case report describes a 35 year old male renal allograft recipient with minor ABO mismatch with mother as a donor who developed Coombs positive hemolytic anemia after renal transplant and recovered spontaneously within 2 months.

Keyword: Passenger lymphocyte syndrome, immune hemolysis, renal transplantation

Introduction:
The passenger lymphocyte syndrome (PLS) is the immune mediated hemolytic anemia occurring after solid organ and hematopoietic transplantation. It is due to concomitant transplantation of donor lymphocytes along with the donor allograft in minor ABO incompatible transplants. The incidence of passenger lymphocyte syndrome is far less in renal transplant recipients than in other solid organ transplants.

Case report:
A 35 year old male underwent ABO compatible living donor renal transplant with his mother as a donor. Donor blood group was O+ve and whereas recipient's was A+ve. His native kidney disease was not known. His preoperative hemoglobin was 8gm/dl. He was on hemodialysis for four months prior to transplant and received parenteral iron and erythropoietin. His intra operative period was uneventful and during the surgery the blood loss was about 250ml. He was started on triple immunosuppresion (cyclosporine, azathioprine and prednisolone.) His immediate post operative course was uneventful and his creatinine was 0.9mg/dl on day 4. On day 6, he developed low grade fever and worsening of serum creatinine to 1.5mg/dl and he was empirically started on methylprednisolone.
pulse in view of suboptimal blood trough level of cyclosporine done on day 5(150ng/ ml). Renal biopsy done on next day showed focal isometric vacuolisation and no tubulitis. In the 2nd week, patient developed low grade fever, jaundice, persistent graft dysfunction and worsening of hemoglobin level from 6.5gm/dl to 4.7gm/dl. Peripheral smear showed hypochromic RBCs with anisopoikilocytosis. Total bilirubin was 1.8mg/dl, Direct bilirubin 0.8mg/dl, AST 48 U/l and ALT 21 U/l. Blood culture and urine culture were sterile. Viral markers for hepatitis like anti HAV , HBsAg , anti HCV and anti HEV were negative. CMV pp65 was negative. At the end of 3rd week, his hemoglobin further declined to 3gm/dl along with mild thrombocytopenia (WBC 4100/cu.mm, platelets 1.2 lakhs/cu.mm). Hence azathioprine was withheld. Transplant renal doppler and USG of transplant kidney were normal. PT/INR and APTT were normal and renal biopsy was repeated to rule out thrombotic microangiopathy. But biopsy revealed only mild focal lymphocytic infiltrate and c4d was negative. 4th week, jaundice further worsened (total bilirubin 3.8mg/dl, indirect bilirubin 2.1mg/dl). LDH was elevated (867 U/l). Coomb’s test was strongly positive. G6PD level was normal. Patient transfused with one unit of packed RBCs. Serum triglycerides was 250mg/dl and serum phosphate was 2.8mg/dl. In view of positive Coomb’s test, causes of immune hemolytic anemia were evaluated and anti A titre was done which was significant (Anti A titre was 1:16). ANA and dsDNA were negative. Patient was serially monitored anti A titer which progressively declined. During 7th week of post transplant, anti A titre became negative and hemoglobin rose to 8.5gm/dl and creatinine returned to baseline of 1.1mg/dl.

Discussion: In 1971, Beck et al first described the appearance of antibodies against A and B blood group antigens in recipients of kidney allograft from ABO minor mismatched donors. Antibodies to red cell antigens outside the ABO system commonly anti D and rarely isolated cases of anti c, anti e, anti kell, anti JKa or anti FYa have been reported. Stevens coined the term "passenger lymphocyte syndrome" in 1981. Passively transferred viable donor B cells in transplanted graft act as a source of isohe-magglutinins. Viable donor lymphocytes which reside in the allograft when stimulated by the recipient or by transfused red cell antigens, produce antibodies. These antibodies have been demonstrated in patients who underwent graft nephrectomy, which suggest that donor lymphocytes migrate from the graft to other lymphoid organs of the host. Schilt opined that immunologically active cells are transplanted with the allograft itself and they are not removed by perfusion during harvesting. Ramsey reported that the frequency of passenger lymphocyte derived antibodies and hemolysis was highest at 70% (for both) in heart-lung transplant recipients. In renal transplant recipients antibodies were present in 17% and 9% of the recipients had hemolysis. The passenger lymphocyte syndrome is most likely to occur when the donor group is O and the recipient group is A, due to the fact that IgG anti A and anti B are more common in group O than in A or B. Anti D mediated hemolysis tends to last longer than hemolysis induced by ABO antibodies and can persist up to 6 months. In our case the recipient blood group is A +ve and donor blood group is O+ve. This is the most common combination shows
passenger lymphocyte syndrome in minor ABO mismatch renal transplants. Donor derived ABO antibody mostly develops 7-14 days after transplantation, the time course being independent of type of organ transplanted. The appearance of donor derived antibody in the serum and the development of positive direct Coomb’s test are generally concurrent. The serum antibody is predominantly IgG, but it may also be IgM. These antibodies are short lived, persisting for 2 to 13 weeks with a median of 5 weeks in kidney transplant recipients. Factors affecting hemolytic severity are amount of lymphoid tissue in the transplanted organ, number of B-lymphocytes/plasma cells in the graft, pre-transplant titer of donor’s antibody, rapidity of antibody titer rise in the recipient and immunosuppressive induction regimen. In our patient, hemolysis started 1 week after transplantation and spontaneously improved after 6 weeks. Antibodies to recipient’s RBCs may be acquired passively from ABO unmatched blood components, intravenous immunoglobulin and anti lymphocyte globulins. Other positive DATs are often due to nonspecifically bound antibody, but they can occasionally represent a delayed alloimmune response. Such immunohematological alterations may reflect hemolysis associated with the multifactorial etiology of anemia after solid organ transplant. Our patient did not receive ATG or IVIG or mismatched blood transfusion. Other causes of hemolytic anemia should be considered in a patient with hemolysis after transplantation. This patient was taking trimethoprim-sulfamethoxazole which was stopped when hemolysis was noted. Sulfonamides can cause hemolytic anemia in the setting of G6PD deficiency. Cyclosporine can cause microangiopathic hemolytic anemia. Our patient did not have a microangiopathic process or G6PD deficiency. Severe hypophosphatemia can cause nonimmune hemolysis. Our patients serum phosphorus was 2.5mg/dl at the time of hemolysis. Uncommonly hemolysis can be severe enough to cause acute renal failure requiring hemodialysis and rarely death. Our patient had mild graft dysfunction which was probably attributed to hemolysis which recovered spontaneously.

Passenger lymphocyte syndrome is considered as a minor form of graft versus host disease. It is confirmed by detection of either anti A or anti B titre or anti D titre. Treatment of PLS is supportive. If any blood transfusion required, it should be donor compatible group. For example A +ve recipient with donor group O, donor should receive O group RBCs. If repeated transfusion is required, donor group blood should be given until antibody is not detected. If hemolysis is severe, red cell exchange with compatible red blood cells is advocated. If more than 2 units of RBCs need to be transfused within the first 24 hrs after the onset of hemolysis, RBC exchange should be considered or plasma exchange to reduce antibody titer. Cryoprecipitate can be given without regard to ABO type because this product contains only small volume of plasma.

In the presence of massive hemolysis, plasmapheresis and exchange transfusion with group O RBCs and intravenous immunoglobulin have been found to be effective. Passenger lymphocyte syndrome is documented more with use of cyclosporine(CsA). It is because cyclosporine usage resulted in permissive effect attributable to predominant inhibition of T lymphocyte regulatory function. Secondary immune response of competent primed B lymphocytes are resistant to Cyclosporine (AB and H blood group antigens are T cell
The inhibition of T lymphocyte function by cyclosporine may prevent the recipients immune system from recognising and damaging donor derived passenger B lymphocytes. Compared with cyclosporin, tacrolimus has a greater potency to inhibit B cell proliferation. The acute or chronic symptoms of hemolysis disappeared, at 2 and 5 weeks, respectively, after conversion from CsA to tacrolimus\textsuperscript{15}. Use of immunosuppressive agents with anti B lymphocyte activity would be expected to prevent alloimmune hemolysis. Mycophenolate mofetil (MMF) and sirolimus possess anti B cell activity may be useful in this situation.\textsuperscript{16} Some case report shows that after withdrawal of azathioprine alloimmune hemolytic anemia recovered.\textsuperscript{17} A response to high dose corticosteroids has been reported.\textsuperscript{18} Whether immunosuppression hastens the resolution (as a result of lymphocytotoxicity) or worsens the hemolysis (by reducing immune surveillance of abnormal population) is unknown.\textsuperscript{19} Reduction in alloimmune hemolytic anemia is noted in patients receiving prophylactic allograft irradiation.\textsuperscript{20} Rituximab and splenectomy were also tried in some case reports\textsuperscript{21}. Prognosis of passenger lymphocyte syndrome is self-limiting and remits spontaneously within 2 months. It is due to gradual depletion of passenger lymphocytes which are deprived of signals from corresponding T helper cells and follicular dendritic cells.\textsuperscript{22}

**Conclusion:**

PLS is an important rare complication observed with every organ type. Clinicians must be vigilant in the ABO minor mismatched transplant setting in order to recognise hemolysis and implement appropriate therapy. As of now, there is no specific treatment is available for PLS. PLS can be treated with transfusion, plasmapheresis, red cell exchange, IVIG and anti CD20 monoclonal antibody.

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