RESEARCH BRIEF

Immunoglobulin Levels and CD4 / CD8 Counts in β – Thalassemia Major

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Objective: This cross-sectional study determined the CD4, CD8 counts and serum immunoglobulins in transfusion dependent β - thalassemic patients, and correlated them with anti-HIV, anti-HCV and HBsAg status, number of transfusions, iron overload and splenectomy. **Methods:** Patients with acute or chronic diseases (except HIV, Hepatitis B and C), on immunosuppressive drugs or vaccinated within one month prior to study were excluded. CD4, CD8 counts and serum Immunoglobulins were documented. **Results:** Increasing transfusions led to higher IgA and IgM as well as a decline in CD4 and CD8 levels. Higher ferritin correlated with high IgM. CD4, CD8 and IgA were significantly higher in splenectomized subjects. HCV correlated significantly with lower IgA values. **Conclusion:** Higher transfusion requirement, iron overload, splenectomy and HCV infection correlated with alterations in different immunological parameters.

Keywords: *Immunity, Iron, Transfusion.*

Advances in transfusion regimens and iron chelation therapy have improved the quality of life as well as life-expectancy of transfusion-dependent thalassemics. Nevertheless, this has led to disclosure of several newer complications such as immunological alterations causing increased infections. Several studies have found infections to be the second commonest cause of death after heart failure in thalassemia [1-3]. This has been attributed to various immunological abnormalities. Factors that might lead to these abnormalities include iron-overload, splenectomy, Desferioxamine, exposure to allogenic antigens in blood, immunosuppressive viruses, and liver damage following hepatitis. The disease itself does not seem responsible as non-transfusion dependent thalassemics do not exhibit similar immunologic changes [4]. The current study was therefore designed to study the CD4 and CD8 counts, and immunoglobulin levels in multi-transfused patients with β–thalassemia.

METHODS

This was a cross-sectional, observational study conducted at a tertiary care hospital between July 2010 and June 2011. All patients with transfusion-dependent β-thalassemia were included. Those with any acute/chronic illness or infection (excluding HIV, Hepatitis B and C) and who had received any vaccinations or immunosuppressive medications including steroids, hydroxyurea etc. during one month prior to enrolment were excluded.

History and examination including number of transfusions, chelation, signs of iron overload, and splenectomy-status was noted. Apart from routine investigations, anti-HIV antibody, HBsAg, anti-HCV antibody and serum ferritin were performed. Mean pre-transfusion hemoglobin, and yearly packed red blood cell requirement were calculated. CD4, CD8 levels and Serum IgG, IgM, IgA levels were analyzed using standard methods at National Institute of Immunohaematology (NIIH), ICMR, Parel, Mumbai. Institutional Review Board approval was obtained.

The CD4, CD8 and immunoglobulin levels were compared with age-matched normal values [5,6]. Statistical analysis was done using Chi-square test, Fisher exact test, Student's t-test, correlation co-efficient and linear regression models.

RESULTS

Data from total of 100 patients (60 males) were analyzed. The mean (SD) age of the subjects was 10.9 (5) years. Nineteen, 56 and 25 patients had low, normal and high CD4 values, respectively. CD8 counts were normal in 69, low in 11, and high (abnormal) in 20 subjects. IgG levels were normal in 48 patients and high in 51 patients. Only 1 subject had low values. Ninety-three subjects had normal IgA levels while 4 and 3 subjects had low and high values, respectively. Ninety-five subjects had normal IgM levels. The remaining five had a high value. The mean (SD) number of transfusions received was 155.8 (81.44). There was a significant positive correlation between number of

transfusions and IgA (r = 0.2985) and IgM (r = 0.2637). Serum ferritin was available in 69 patients. The mean (SD) serum ferritin level was 4296.83 (2576.35) ng/mL. We noted increasing IgG (P>0.05) and IgM levels with increasing serum ferritin levels. The mean (SD) packed RBC requirement in the subjects was 171.9 (33.38) cc/kg/year. Results depicted a significant decline in CD4 (r = - 0.379) and CD8 (r = - 0.3659) counts with higher RBC requirement.

Two subjects each were anti-HIV and HBsAg-positive, whereas 34 patients were anti-HCV positive. A significant decrease in IgA levels was noted in those with anti-HCV antibodies (P = 0.048). Ten children were splenectomized. As shown in ***Table* I**, the CD4 (P< 0.0001), CD8 (P < 0.0001) and IgA (P = 0.01) levels were significantly higher in splenectomized subjects.

DISCUSSION

We found that higher number of transfusions increased the IgA and IgM significantly, whereas iron overload increased the IgM significantly. Low CD4 and high CD8 counts (abnormal) were observed in those requiring higher amount of packed red cells. CD4, CD8 and IgA were significantly higher in spenectomized patients. Low IgA levels were noted in those with anti-HCV antibodies.

The present study had some limitations. We evaluated only selected immunological parameters. Neutrophil functions, other lymphocyte subsets, IgD and IgE were not done. Moreover, serum ferritin was done only in 69 subjects.

Interestingly, low CD4 counts were observed only in 19 subjects, with high CD8 values in 20 subjects. This was contrary to published studies [7,8]. Twenty five of our subjects had unexpectedly high CD4 values. This has been described in a study by Nualart, *et al*. [9] to be due to functionally defective cells. Similarly, low CD8 count was another surprising finding. A description of L CD8 phenotype, wherein intrinsically these persons have a low CD8 count, has been described [10], and was likely the case here.

TABLE I CD4, CD8, IgG, IgA AND IgM VALUES IN SPLENECTOMIZED *VS* NON-SPLENECTOMIZED CHILDREN

	Splenectomized	*Non-splenectomized*
*CD4 (cells/μL)	3200 (2133.08)	1233.2 (865.70)
*CD8 (cells/μL)	2452.9 (1813.37)	789.3 (514.0)
IgG (g/L)	16.8 (5.37)	16.1 (5.31)
*IgA (g/L)	2.4 (1.75)	1.5 (0.88)
IgM (g/L)	1.2 (0.71)	1.4 (0.72)

*All values in mean (SD); *P<0.005.*

Evaluation of serum immunoglobulins revealed results differing from most previous studies, which have shown elevated levels of IgG, IgM and IgA [11]. Similar trend has been observed in other recent studies also [12]. This could be due to better chelation as well as leuco-depleted red cell transfusions currently in use for thalassemic patients. With increasing number of transfusions, there was an increase in the levels of immunoglobulins,. It has been postulated that donor alloantigens lead to development of alloantibodies to the patients' RBCs, leading to conformational changes on the epitope of RBC antigens, thereby stimulating the formation of autoantibodies. Additionally, leucocyte antigens are an important source of immune stimulation [13-15]. Leuco-depletion, preferably pre-storage, is therefore imperative to reduce allo and autoimmunization in patients with thalassemia. Significantly higher CD4, CD8 and IgA levels in splenectomized subjects, as seen in this study have also been reported previously [8,16].

In conclusion, with appropriate transfusion and chelation therapy, the immunological alterations in transfusion-dependent children with thalassemia can be minimized, thereby preventing morbidity and mortality due to infections.

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