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Original article

Anti Thymocyte globulin therapy for treatment of Aplastic Anemia- A retrospective Study

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ABSTRACT

Background: Anti thymocyte globulin therapy is considered as standard first line immunosuppressive therapy for patients with acquired aplastic anemia. Aim of this study is to evaluate the response of anti thymocyte globulin (ATG) therapy for treatment of aplastic anemia (AA). **Methods and Materials:** In this retrospective analysis of 104 aplastic anemia patients**.** All patients were treated with anti-thymocyte globulin (ATG)(40 mg/kg/day for 4 days) plus Cyclosporin A (CSA) (6 mg/kg/day for 6 months). Blood component support was given up to 3 to 6 months depending on the response and comprehensive measures were provided to prevent treatment-related side effects. **Results:** Full course of anti-thymocyte globulin (ATG) of 4 days was successfully administered in 103 patients out of 104 patients (age 7-63). At 24 weeks, one patient achieved complete remission(CR) and 50 patients could achieve partial remission(PR). At 24 weeks overall response (OR) was observed in 51 out of 103(49.51%) patients. 44 out of 85 (51.76%) adult patients achieved overall response(OR) at 24 weeks whereas 7 out of 18 (38.88%) pediatric patients could achieve overall response (OR).Relapse of aplastic anemia did not occur in any patient during 6 months. One patient died due to infection on 3rd day. All adverse events were successfully managed symptomatically. **Conclusion:** Horse Anti-thymocyte globulin and cyclosporine therapy produces response rates around 50% at the end of 6 months. It gives reasonably good response. Anti-thymocyte globulin combined with cyclosporine remains the standard immunosuppressive therapy for transplant ineligible patients of aplastic anemia.

KEYWORDS: Anti-thymocyte globulin, aplastic anemia, immunosuppressive therapy

INTRODUCTION

Aplastic anemia (AA) is an immune related disorder in which bone marrow failure leads to deficiency of all three types of blood cells (pancytopenia)[1]. Bone marrow failure occurs due to the destruction of hematopoietic stem cells (HSCs). Allogeneic bone marrow transplantation (BMT) is the first line therapy for treating this disorder in younger age group(≤40 years).[1] Lack of histocompatible donors restricts most of the patients treated by this procedure and BMT in patients more than 40 years of age are associated with high morbidity and mortality.[2]Immunosuppressant therapy (IST) is the first line treatment in aplastic anemia patients who are ineligible for BMT and have shown similar response rates.[3] These two strategies have been compared and have shown equivalent long term survival.[3,4] In addition, combining anti-thymocyte globulin (ATG) and cyclosporine as IST have improved survival.[5]

ATG is a monomeric immunoglobulin G (IgG) which is obtained from the serum of horses or rabbits immunized with human thymus lymphocytes and acts on circulating lymphocytes to reduce their number.[6] Cyclosporine is a cyclic non ribosomal peptide which reduces the activity of the immune system by interfering with the activity and growth of T cells.[6]Earlier studies with ATG and cyclosporine as immunosuppressive therapy have shown

response rates around 60-70% and survival around60% to 90% [4,5,7,8] .

Patients receiving ATGAM should be treated in facilities equipped and staffed with adequate laboratory and supportive medical resources. Hypersensitivity testing is mandatory before administering ATG. In our report we have explain in detail method of administering ATG that we follow in our institute.

There are published studies from India on the response rates of ATG, however to our best of knowledge there are no studies from Odisha region. Most of the studies have reported on the efficacy of ATG however none have reported the side effects occurring due to the ATG. In our study we have reported the response rates with ATG along with the method of administering it and the side effects in aplastic anemia patients.

MATERIALS AND METHODS

Patients: Patients admitted to Clinical Hematology Department S.C.B Medical College & Hospital, Cuttack, Odisha with a diagnosis of primary aplastic anemia between January 2012 and June 2013 were included for retrospective analysis. The study was approved by the institutional ethics committee. Referrals were from hematologists, pediatricians or Physicians in private practice or at academic institutions throughout India.

All patients diagnosed as primary (idiopathic) aplastic anemia and not eligible for allogenic bone marrow transplantation (BMT)were included in the study.104 AA (age between 7-65 years) patients were enrolled. The details regarding medical history, physical examination, complete blood count, bone marrow aspirate and biopsy were retrieved. Ongoing infections were ruled out. Fanconi's anemia was ruled out in all pediatric patients (≤14 years) using stress cytogenetics. Paroxysmal nocturnal hemogobinuria(PNH) screening was performed by flow cytometric determination of CD55/59. Additional tests, including liver and renal function tests, viral markers study (HbS Ag, anti-HCV, HIV), coombs test (Direct and Indirect) and serum ANA and anti-ds DNA were also performed in all cases. Pretreatment characteristics of the patients are given in table1.

Disease severity: Patients were classified according to the established severity criteria [9] . AA was considered severe aplastic anemia (SAA) if marrow cellularity was <25% and at least two of the following criteria were met: absolute neutrophil count (ANC) $<0.5\times10^9$/l, platelet count $<20\times10^9$/l, reticulocytes $<20\times10^9$/l. AA was considered very severe (VSAA) if the above criteria for SAA were fulfilled, and the ANC was $<0.2\times10^9$/l. Patients not fulfilling the criteria for severe or very severe aplastic anaemia were included as non severe AA (NSAA) patients. Number and percentage of patients according severity of the disease have been added in the table1.

Table 1: Pretreatment Characteristics of Patients with Aplastic Anemia

Characteristics	observation	
	Percentage	Mean SD
Age(Years), Mean ± SD	-	32.7 ±16.64
Male N(%)	61(58.7)	-
Female,(N%)	43(41.3)	-
Adult	86 (82.69)	-
Child	18(17.3)	-
Age(years) at Diagnosis, Median(Range)	30.0(7.0-63.0)	-
Severe Aplastic Anemia	62 (59.61)	-
Very Severe Aplastic Anemia	21 (20.19)	-
Non Severe Aplastic Anemia	21 (20.19)	-
Duration of disease(months), Median(Range)	7.5(1.0-36.0)	-
Bonemarrow findings, N(%)Hypoplastic	103(99.0)	-
Symptoms, N(%)	Weakness95(91.3)	-
	Fever77(74.0)	-
	Bleeding83(79.8)	-
Liver function test(LFT) SGOT (units/litre)	-	45.0 ± 14.81
Liver function test(LFT) SGPT(units/litre)	-	45.6 ± 13.46
Liver function test(LFT) Bilirubin (mg/dl)	-	0.9 ± 0.26
Kidney Function test(KFT) Urea (mmol/L)	-	33.2 ± 9.97
Kidney Function test(KFT) Creatinine (mg/dl)	-	3.0 ± 19.71
Serum Iron (ng/ml)	-	97.6 ±233.32
Hb (g/dl)	-	4.9 ±1.02
Absolute neutrophil count (x109/L)	-	286.6 ± 99.92
Platelet(x109/L)	-	23.6 ± 5.47
Reticulocyte (x109/L)	-	10.4 ± 2.87

The study's primary end point was to determine hematologic response at 6 months, defined as no longer meeting criteria for severe aplastic anemia.[9]Secondary end point was to evaluate the safety of horse ATG.

Treatments: Patients were treated at the indoor of Clinical Hematology department of SCB Medical College & Hospital, Cuttack. All patients were treated with equine ATG (e-ATG; ATGAM;Pfizer), which was administered intravenously through peripheral venous access at a dose of 40 mg/kg/day as slow infusion with 0.9% normal saline for consecutive 4 days. On day 1, immediate hypersensitivity to anti-thymocyte globulin was assessed by skin test before infusion by administering a test dose of 0.1 ml (diluted with normal saline) subcutaneously.

If there was no reaction / anaphylaxis observed after 30 minutes, ATG infusion was started at 20 micro drop/ min for initial 30 minutes, and gradually increased to 60-80 micro drop / min. Total duration was 10-16 hours. If non-hemolytic transfusion reaction reappeared, then rate was decreased and adjusted accordingly until the completion of the ATG.

If infusion-related toxicity was noted during ATG infusion, it was stopped and restarted at a slower rate after administration of antihistamines. Tablet paracetamol (15mg/Kg) and injection dexamethasone (0.1 mg/kg) was given intravenously 30 minutes before ATG infusion as premedication and was repeated at the interval of 4-6 hours till the completion of ATG infusion. Oral prednisolone (1 mg/kg/day) was administered in divided doses from day 6 of ATG administration to prevent or ameliorate serum sickness syndrome (SSS) and was withdrawn by day 25 while paracetamol (15mg/Kg orally) was used whenever required.

Cyclosporine (CsA) was administered orally from day 26of anti-thymocyte globulin (ATG) treatment at a dose of 6 mg/kg/day in two divided doses and was gradually increased up to a maximum of 10mg/kg/day in case of unsatisfactory response. While in case of adverse eventsdue to cyclosporine the dose was reduced to 2-4 mg/kg/day. The maximum dose was continued for 6 months and was gradually reduced till complete stoppage or minimum effective required dose by 1 to 1 ½ years. In case of hepatotoxicity occurrence in patients, the CsA dose was reduced to 25–50% of initial dose and then slowly increased over 6–12 weeks to maximal tolerable level. In patients who developed nephrotoxicity, CsA was withheld until serum creatinine levels returned to normal, then reintroduced at 25% of the initial dose and gradually increased to maximal tolerable dose over 6–12 weeks.

Oral co-trimoaxazole prophylaxis was given twice a week for prevention of *Pnemocystiscarini i*nfection, oral acyclovir (8mg/kg twice daily) for prevention of viral infection and oral hygiene was maintained by Chlorhexidine mouth washes. Blood component support (Irradiation Red cell/ platelet) was given up to 3 to 6 months depending upon the response.

Supportive care was provided to all patients. Infections were diagnosed on clinical evaluation and judgment. Whenever there was no response to antibiotic therapy fungal therapy was started. Broad spectrum antibiotics were used to control infections whenever required. Platelets were transfused prophylactically. Total platelet count was raised to attend

minimum level ($\geq 10x10^9$ /l) by platelet support (group-specific units of single donor platelets/ Random donor matched platelets). In case of muco-cutaneous bleed platelet support was continued in order to achieve haemostasis. Red blood cell transfusions were given for symptomatic anemia and Hb was to maintain at 10gm% by leuko-reduced red cell support. The blood products used in all the cases were irradiated. Adverse drug reactions (ADRs) were noted in the CDSCO (Central Drug Standard Control Organization) format. Evaluation and Response criteria

Hematology parameters were assessed regularly in all the cases at the interval of 2 weeks. The response was assessed 6 months after ATG administration. The response was either considered as complete hematological response (CHR), partial response (PR) and no response (NR). Complete hematological response is defined as haemoglobin normal for age neutrophil count $>1.5 \times 10^9$/l platelet count $>150 \times 10^9$/l. Partial response is defined as transfusion independent, 2/3 of the following: neutrophil count $<0.5 \times 10^9$/l Platelet count $<20 \times 10^9$/l Reticulocyte count $<20 \times 10^9$/l.[9]Responses not fulfilling either of these criteria were considered as NR. Relapse was defined as the decline in patient's peripheral blood cell count to levels meeting the definition of AA.

Statistical analysis: Response rates were calculated as percentage. Overall response was taken as complete and partial response. Summary statistics, including means, medians and range were used to describe the patient's baseline characteristics. Statistical analysis was performed using SAS statistical software package.

RESULTS

Patient characteristics

From January 2012 to June 2013, a total of 104 aplastic anemia patients were enrolled in the study with a mean age of 32.7 years with preponderance of males (58.7%). Patient characteristics are shown in Table 1. Among 104 patients (age range 7-63), Sixty two (59.6%) patients had severe aplastic anemia (SAA), 21(20.2%) patients had very severe aplastic anemia (VSAA) and 21(20.2%) patients had non-severe aplastic anemia (NSAA). One patient was lost to follow up. And one died on day 3 of ATG administration. The percentage of patient to receive one full course of treatment was 99.03% (103/104). One patient died due to infection on 3rd day of ATG administration.

Response to ATG treatment

Complete course of ATG of 4 days was successfully given to 103 patients out of 104 patients (age 7-63). Response to ATG treatment is given in table2.Relapse of AA did not occur in any patient during 6 months.

Adverse Events

Gastrointestinal toxicity and hepatotoxicity followed by nephrotoxicity were the most common adverse effect. The

various adverse events occurred in the study are provided in Table 3. Total number of adverse events according to severity of aplastic anemia have been provided in table 4. Cyclosporin A therapy was well tolerated, and it was continued in all patients for six months.

Table 2: Response to anti-thymocyte globulin treatment

	Number of Patients (n)	Number of patients with overall response at 24 weeks (%)
Adult	86	44 (51.16)
Pediatric	18	7 (38.88)
Severe aplastic anemia	61	31 (50.81)
Very severe aplastic anemia	21	7 (33.33)
Non severe aplastic anemia	21	13 (61.9)
Total	104	51 (49.03)

Table 3 Adverse Events Occurred in the Study Population

Adverse event	Day1	Day 2	Day 3	Day 4
Rigor, N(%)	54(52.42)	6(5.82)	5(4.85)	4(3.88)
Fever, N(%)	27(26.21)	90(87.37)	41(39.8)	17(16.5)
Urticira, N(%)	18(17.47)	3(2.91)	24(23.3)	4(3.88)
Myalgia, N(%)	3(2.91)	1(0.97)	15(14.56)	5(4.85)
Muco-cutaneous bleed, N(%)	2(1.94)	1(0.97)	1(0.97)	8(7.76)
Arthralgia, N(%)		1(0.97)	8(7.76)	18(17.47)
Serum Sickness Syndrome, N(%)		1(0.97)	7(6.79)	15(14.56)
Upper Respiratory Tract Infections, N(%)		1(0.97)	2(1.94)	5(4.85)
Lower Respiratory Tract Infections, N(%)				10(9.7)
Sum of episodes, N(%)	104(100.97)	104(100.97)	103(100)	86(83.49)

Table 4: Total Adverse Events according to severity of Aplastic anemia

Severity	Total number of AEs
Severe aplastic anemia	231 (58.18%)
Very severe aplastic anemia	82 (20.65%)
Non severe aplastic anemia	82 (20.65%)

DISCUSSION

In this retrospective study the sample size was reasonably large. The dose of anti-thymocyte globulin used in this study was 40 mg/kg/day.The supportive treatment used was efficient in controlling adverse events during the treatment period and none of the patient discontinued the anti-thymocyte globulin therapy due to adverse events. The combination of cyclosporine (CSA) with anti-thymocyte globulinhas provedto be effective in increasing the response rate (60–70%) and long termsurvival probability (70–80%).[10,12]and is now considered as standard first lineimmunosuppressive therapy for severe aplastic anemia (SAA) for patients more than 40 years of age.[13,14]

In a recently published trial authors reported 43.5% response to immunosuppressive therapy.[15] Similarly, an earlier series indicated 40% response to immunosuppressive therapy at 6 months and 45% response to immunosuppressive therapy at one year.[16]In a study conducted on adult patients of aplastic anemia also showed overall response rate of 42.20%. In the same study overall response rate of 54.63% was achieved at 6 months. This implies that our results are comparable to these results at 49.51% at 6 months. To our knowledge there is no data on immunosuppressive therapy usage on aplastic anemia patients from Eastern region of India, especially Odisha. This fact gives significant importance to this study.

Out of 104 patients 18 were pediatric. Out of 18, 7 responded.Response rate in children was 38.88% whereas in adults 51.16% response rate was seen. Response rate was higher in adults as compared to pediatric patients. This pediatric response is comparable to response (43.5%) given by pediatric patients in another study.[17]It is not very clear why children had lower response rate as compared to the adults. One of the reasons could be that children may be suffering from other forms of inherited bone marrow failure

which have been missed because of no overt manifestations. One limitation of the study is that it is based on a retrospective data and also we have not collected long term data limiting us to give survival results.

CONCLUSION

Anti-thymocyte globulin and cyclosporine treatment gives 50% response rates at 6 months in our set up. We were able to administer anti-thymocyte globulin in almost all patients with the method explained in our paper. There were several side effects due to anti-thymocyte globulin which were effectively managed and we were able to give anti-thymocyte globulin to almost all patients. Anti-thymocyte globulin plus cyclosporine remains the standard immunosuppressive treatment for acquired aplastic anemia.

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Conflicts of Interest: NIL

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