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STUDY OF COMPARING RESPONSE TO TREATMENT WITH CYCLOSPORINE-A (CSA) ALONE VERSUS THE COMBINATION OF ANTITHYMOCYTE GLOBULIN (ATG) AND CYCLOSPORINE-A IN PATIENTS WITH APLASTIC ANEMIA

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ABSTRACT

Introduction: Aplastic anemia (AA) is a syndrome characterized by peripheral pancytopenia with hypocellular marrow. Acquired idiopathic aplastic anemia is the most common variety, and its treated with cyclosporine-A, or Antithymocytic globulin or with Bone marrow transplantation. **Aim:** Study of comparing response to treatment with CSA alone versus the combination of ATG + CSA in aplastic anemia patients. **Materials and Methods**: This study was a prospective randomized study, conducted after getting approval from Institution ethical committee and conducted over 18 months. All patients underwent a detailed clinical examination and laboratory investigations. Diagnosed patients of acquired idiopathic aplastic anemia without active infection, otherwise not eligible for bone marrow transplantation were recruited. Patients were randomized to receive either CSA alone or the combination of ATG and CSA according to their choice. Assessment of response to therapy was made at the end of 3 months. **Observations and Results:** After the diagnosis, 31 patients were enrolled. The age of the patients ranged from 10 to 65years. Male to female ratio was 1.8:1. The average duration of symptoms before presentation was 8 months. Almost all the patients were requiring regular component support with packed red cells and platelets before starting therapy. 20 patients were randomized to receive CSA. Out of 20, 3 patients were lost to follow up due to financial constraints and 1 patient discontinued therapy due to renal dysfunction, finally 16 patients were analyzed. In this group 9 out of 16 (56.5%) responded to treatment. 11 patients were assigned to ATG & CSA group. 3 patients died within two months of therapy, these were excluded from the study, and finally 8 patients were analyzed. In this group 5 out of 8 (62.5%) responded. **Conclusion:** CSA treatment is as efficacious as ATG+CSA combination therapy for acquired idiopathic aplastic anemia, otherwise not eligible for BMT from HLA-identical siblings.

KEYWORDS: Cyclosporin A, Hypocellularmarrow, Packedredcelltransfusion, Thrombocytopenia, Bone marrow transplantation, Neutropenia.

INTRODUCTION

Aplastic Anemia (AA) Is A Syndrome Characterized By Peripheral Pancytopenia With Hypocellular marrow.[1] Acquired Idiopathic Aplastic Anemia Is The Most common variety, probably of an autoimmune etiology primarily due to suppression Of hematopoiesis by Autoreactive Tlymphocytes[1], but the precise pathogenic mechanism remains unclear. Bone marrow transplantation (BMT) from HLA matched sibling donor is the treatment of choice but because of non-availability of matched donors and cost of treatment this approach is limited. Immunosuppression with antithymocyte globulin (ATG) and cyclosporine-A(CSA) is an alternative to bone marrow transplantation.[2]

In the early 1980s, ATG was shown to significantly improve the survival of patients with AA in comparison to supportive care alone.[3] Response rates vary between 40% and 70% and long-term survival after ATG based immunosuppressive therapy is similar to that in unselected patients treated with bone marrow transplantation.[4] Studies from India and other countries on ATG showed around 30 to 50% of response rate.[5,6,7,8] studies conducted from western countries on ATG and CSA combination therapy showed the response rate of 60 to 70%9,[10] Studies from India on combination therapy showed a lower response rate of 40%[11] which is almost equal tothe response rates achieved with CSA monotherapy in studies from India

and other countries.[2,7,12] Randomized Prospective multicenter European study concluded that the combination of ATGand CSA is superior to CSA alone in terms of the hematological response, the quality of response and early mortality.[13]

Though the individual studies conducted on CSA monotherapy and ATG and CSA combination therapy from India did not show much difference in terms of response rate[2,5,6,7,8,11], there were no studies comparing CSA alone with ATG and cyclosporine-A combination therapy from India and there were very few studies from other countries in this context. Thus, we have undertaken the study to determine difference between ATG and CSA combination therapy with CSA monotherapy.

MATERIAL METHODS

This study was a prospective randomized study conducted in the department of General Medicine at Nizam's Institute of Medical Sciences (NIMS) which is a multispeciality tertiary referral care centre located at Hyderabad in the state of Andhra Pradesh. After getting approval from ethical committee of NIMS, it was conducted over a period of 2 years after taking consent from the patients.

INCLUSION CRITERIA

1. Diagnosed patients of acquired idiopathic aplastic anemia without active infection.
2. All the patients above five years of age and of both gender are included in the study.
3. Patients who are not eligible for bone marrow transplantation like.

a). Young patients who lack an HLA- compatible sibling donor.
b) Patients who are more than 40 years of age.

EXCLUSION CRITERIA

1. Diagnosis of inherited AA like Fanconi anemia, Dyskeratosis congenita.
2. Infections not adequately responding to appropriate therapy.
3. Underlying immunodeficiency state including AIDS.
4. Serum creatinine more than 2.5 mg/dl.
5. Current pregnancy or lactation or unwillingness to take contraceptives.
6. Patients with underlying major systemic illness
7. Contraindication to ATG and Cyclosporine-A.

METHODOLOGY

Clinical Examination

All patients presenting with symptoms of anemia, Petechiae, bruises and mucosal bleeds underwent a detailed clinical examination for the presence of pallor, Petechiae & Purpurae and features of inherited aplastic anemia like Short stature, Café au lait spots, Skeletal anomalies, Leucoplakia, Nail dystrophy and Pigmentation of the skin along with the systemic examination.

Laboratory investigations

Hemoglobin (Hb), total leukocytes count (TLC) and differential counts (DC), platelet count, reticulocyte count (Reticount), red cell indices and peripheral smear were done in all these patients. Bone marrow aspiration (BMA) and trephine biopsy was done in all patients. Renal function tests (RFT), liver function tests (LFT) and screening for hepatitis B, C and HIV were undertaken in every patient. Chromosomal breakage studies were carried out in all those below 40yrs of age to exclude inherited aplastic anemia.

Diagnosis

Patients were diagnosed as aplastic anemia based on the peripheral cytopenia, which may be monocytopenia or bicytopenia or pancytopenia along with hypocellularity on the bone marrow biopsy.

The patients were divided into non severe (NSAA), severe (SAA) and very severe (VSAA) according to the classification given by Camitta et al[3] (1976) and Bacigalupo et al[4](1988).

Severe AA (SAA)
Bone marrow Cellularity <25% or 25–50% with <30% residual haemopoietic cells and two out of three of the following:
1. Absolute Neutrophil Count (ANC) $<0.5 \times 10^9$/L,
2. Platelets $<20 \times 10^9$/L,
3. Reticulocyte count $<20 \times 10^9$/L

Very severe AA (VSAA): As for severe but ANC $<0.2 \times 10^9$/L.

Non-severe AA (NSAA): Patients not fulfilling the criteria for severe or very severe aplastic anemia.

After diagnosis based on the selected criteria, thirty one patients were enrolled during the one and half year study period. Written informed consent was taken from all the patients. They were explained about the treatment options and cost of Antithymocyte globulin (ATG) and cyclosporine-A (CSA). Patients were randomized to receive either CSA alone or the combination of ATG and CSA according to their choice. Eleven patients were assigned to ATG & CSA group and twenty patients were assigned to CSA alone group.

Treatment protocol & Dosages

In CSA alone group CSA was administered orally from day 1 to day 90 at a dose of 5 mg/kg/d in two divided doses, with subsequent adjustment according to two weekly serum urea and creatinine levels.

Patients were treated with horse ATG and CSA in ATG and CSA combination group. Horse ATG was administered at a dose of 15mg/Kg/day for 5 days or 40mg/kg/day for 4 days as a slow intravenous infusion through central venous line over 4-6 hours. All the patients were given a test dose of ATG (10 mg of horse

ATG in 100 ml of normal saline intravenous over 1 h) before each course of ATG. Premedication with hydrocortisone and pheniramine maleate was given before each daily dose of ATG. For the prevention of serum sickness, prednisolone (1-2 mg/Kg/ day) was administered orally on days 1 to 14 and the dose was tapered to end on day 28. Following ATG, CSA (5mg/Kg/day orally) was started and continued at least for three months.

Follow up

Patients were followed at 2 weekly intervals in the out-patient clinic in the Department of General Medicine, NIMS, Hyderabad. Assessment of response to therapy was made by regular measurements of hemoglobin, total leucocytes, neutrophils and platelet counts. Record of blood and blood product transfusion, infective and hemorrhagic complications was maintained. Patients were also monitored for side-effects of CSA therapy with urea and creatinine levels in blood during each follow up visit. Blood levels of CSA were not monitored in these patients.

Supportive therapy

Throughout the period of administration of ATG hemoglobin, neutrophil and platelet counts were monitored on a daily basis and prophylactic packed red cell transfusion (PRCs) were administered to maintain Hb >8g/dL, platelet transfusions (PRPs) were given to keep the platelet count above $20x10^9$/L. Infections were investigated and treated with broad spectrum parenteral antibiotics Ceftazidime /Cefaperazone + sulbactum and Amikacin. When ever needed antifungal treatment was initiated with parenteral Amphotericin-B.

Measurement of outcome

1. **Partial Response:** Neutrophil count (ANC) over 0.5×10^9/L, platelet count over 30×10^9 /L and achievement of transfusion independence and maintenance after 3months of therapy.
2. **Complete Response**: Transfusion independence and an absolute neutrophil count (ANC) of $>1.5x10^9$ /L platelet count $>150x10^9$ /L and hemoglobin >11gm/dl after 3months of therapy.
3. **Non-Responders**: No hematological response and transfusion dependence after 3months of therapy.

Statistical methods and data analysis

Descriptive statistics is expressed as frequencies with percentages for categorical data. Continuous variables are expressed as median values with inter quartile range (IQR Q1 to Q3) as the sample size was small. Categorical data were compared between the groups using Chi-Square test and Fisher's exact test when the expected frequencies were less than 5. A p value of <0.05 was considered as significant difference between the groups. Continuous variables were compared between the groups using non parametric method Mann-Whitney U test and pretreatment and post treatment values were compared within the group using Wilcoxon Signed Ranks test. A p value of <0.05 was considered significant.

RESULTS

Total 31 patients (Table -1)were enrolled in ths study. The Age of the patients ranged from 10 years to 65 years, with median age in CSA group was 27 years and ATG+CSA group was 32 years. Male to Female ratio of 1.8:1.Most common symptom in both groups was breathlessness, in CSA group 70% and in ATG +CSA group54.5%, followed by bleeding gums in CSA 45%and ATG+CSA group 27.3%. The average duration of synptoms were 8 months.

Table: 1 Showing clinical feautres

	Treatment Group		Total
	CSA+ ATG (%)	CSA (%)	
Male	7(63.6%)	13(65%)	20(64.5%)
Female	4(36.4%)	7(35%)	11(35.5%)
Symptoms Breathlessness	6(54.5%)	14(70%)	20(64%)
Easy fatigability	0(0%)	1(5%)	1(3.2%)
Generalized weakness	3(27.3%)	3(15%)	6(19.4%)
bleeding gums	3(27.3%)	9(45%)	12(38.7%)
Epistaxis	1(9%)	4(20%)	5(16%)
Malena	1(9%)	3(15%)	4(13%)
Bleeding PV	0(0%)	1(5%)	1(3.2%)

Very severe aplastic anemia (VSAA), Severe aplastic anemia (SAA), Non severe aplastic anemia (NSAA) were included in both CSA and ATG+CSA groups. NSAA was common in 40% in CSA group and 54.5% in ATG+CSA group. Almost all patients were requiring packed red cell and platlet transfusions before starting the treatment.

Median Hemoglobin (Table-2) was lower in CSA group than ATG+CSA Group at presentation 4.7g/dl to 7.2g/dl. The rise of hemoglobin in CSA group was statistically significantin post treatment group, but there was no difference was observed with ATG+CSA group from pretreatment to posttreatment.

There was no change in the ANC counts in CSA and ATG+ CSA group, but there was mild increase in

platelet count in CSA pretreatment group to Posttreatment group but it was not statistically significant. But in ATG + CSA group, there was not much change pretreatment and to post treatment platelet count. There is definite decrease in requirement of packed red cell transfusion in both groups, but more in ATG + CSA group compared to CSA group.

But pretreatment platelet transfusion requirement was higher in ATG + CSA group when compared to the CSA group. But there is decline in platelet transfusion in both groups. But it is not statistically significant (p=0.08). Total number of patients completing 3 months follow up were 24 and 16 in CSA group, 8 in ATG + CSA group. And in ATG + CSA group was 62.5% responded, non-responder were 37.5% death or discontinuation was 27.3%. and CSA group 56.25% were responded,43.7% were nonresponders and death or discontinuation was 20%.

Table 2: Comparison of pre-treatment and post-treatment values

	ATG+CSA	p*	CSA	p*	p**
Pretreatment Hb(g/dl)	7.2(4.0 to 8.8)		4.7(3.15 to 7.15)		0.21
Post treatment Hb	6.8(5.5 to 8.8)	0.37	6(5 to 8.6)	0.05†	0.52
Pre treatment Reticount (%)	0.5(0.5 to 0.5)		0.5(0.5 to 0.5)		0.58
Post treatment Reticount	0.5(0.5 to 0.87)	0.06	0.5(0.5 to 0.5)	0.56	0.6
Pretreatment TLC($x10^9$/L)	3.300(2.1to 4.1)		2.45(1.95 to 4.15)		0.9
Post treatment TLC	3.1(2.7 to 3.8)	0.6	3.6(2.25 to 4.6)	0.10	0.27
Pretreatment ANC($x10^9$/L)	0.8(0.42 to 2.9)		0.83(0.5 to 1.52)		0.85
Post treatment ANC	1.22(0.54 to 1.9)	1.0	1.4(0.58 to 2.3)	0.08	0.52
Pre treatment Platelet count($x10^9$/L)	20(10 to 30)		15(10 to 38)		0.9
Post treatment Platelet count	20(10 to 70)	0.26	30(18.75 to 72.5)	0.008††	0.61
Transfusion requirement of PRBC	5(4 to 10)		6.0(4.0 to 10.0)		0.8
Transfusion requirement of PRBC after treatment	0(0.0 to 8.5)	0.20	4.0	0.2	0.14
Transfusion requirement of PRP	10(4 to 14)		2.0(0.0 to 10.0)		0.02
Transfusion requirement of PRP after treatment	0(0 to6)	0.08	.0	0.18	0.4

P*= difference between pretreatment and post treatment values within the group

P**= difference between pretreatment and post treatment values between the group

Significant difference between pretreatment and post treatment Hb (p=0.05) † and in platelet count (p=0.008) †† in CSA group.

SIDE EFFECTS

CSA related: Renal Dysfunction

Mild renal dysfunction was seen in 4 patients (CSA group-3, ATG group-1). Three patients recovered after decreasing dose from 5mg/kg to 3mg/kg in 2 weeks. These 3 patients later tolerated the cyclosporine-A when the dose was increased to 5mg/kg. One patient could not tolerate the drug.

Gum hypertrophy

Asymptomatic gum hypertrophy was noted in 8 (CSA-6, ATG+CSA-2) patients while on therapy and they did not require decrease in drug dosage.

ATG Related side effects: Serum sickness

Developed in 4 of 11 patients after 3^{rd} -4^{th} dose. They presented with erythematous pruiritic rash all over body and polyarthralgias. Symptoms subsided after Inj. Hydrocortisone 100mg IV tid for 3 days followed by oral prednisolone 1-0.5 mg/kg. One patient developed urticarial rash which subsided with oral steroid and chlorpheneramine.

DISCUSSION

Aplastic Anemia is treated with cyclosporine or ATG or both if they cannot get Bone Marrow Transplantation treatment.[2]

The number of patients in our study were 31 (Table -3) 20 in CSA gropup and 11in ATG+CSA group, which is less than Mahapatra etal[7] who had 158 patients in CSAgroup, 113 patients in ATG + CSA group but JMarsh et[13] had 35 and 29 respectively, The number in ATG+CSA group was less mainly due to financial constraints. In present study CSA group was younger than ATG+CSA group with Median age of 27 and 32 years. This is in contrast to study by J Marshetal[13] showed ATG + CSA group had younger patients than CSA group, with Median age of 35 vs 29, p=0.04,. But Mahapatraetal[7] had almost similar age in both group. With Median Age in CSA group 25 years, ATG + CSA group 27 years. The duration of follow up in our study was 18 months were as in Mahapatra etal[7] was 4 years and in J Marshaletal[13] Was 4 years.

In our study Male to Female ratio was 1.8;1 which is less compared to Mahapatra etal[7] 2.5;1 but more than J Marsh etal[13] 1.5;1.

In present study clinical symptoms of breathless, weakness bleeding gums were present for average 8 months in both groups before presentation which is longer compared to Mahapatra et al[7] andJ Marsh etal.[13]

The mean hemoglobin in present study was 5.1g/dl in CSA group, 5.7g/dl in ATG+CSA group which is similar to Mahapatra etal[7] but less than J Marsh etal[13] which had 9.7 g/dl in CSA group, 11.8g/dl in ATG+CSA group. Absolute Neutrophic count was same in Mahapatra etal[7] and J Marsh etal[13] group. But platelet count was 23.5 x 10^9/l in CSA group and 18.9 x 10^9/l in ATG+CSA group in present study which is more than Mahapatra etal[7] study there platelet count was 10x10^9/dl but J. Marsh etal[13] had in CSA group 29x10^9/l and in ATG + CSA group 84x10^9/l.

Table 3 Comparison of present study with previous study

Studies	**J.Marsh et al[13]**		**Mahapatra et al[7]**		**Present study**	
Group	**CSA**	**ATG+CSA**	**CSA**	**ATG+CSA**	**CSA**	**ATG+CSA**
No of patients	61	54	158	113	20	11
Median Age in years	35	29	25	27	27	32
GenderMale:Female	1.5:1	1:1.25	2.25:1	2.3:1	1.8:1	1.7:1
Response	28	46	68	42	9	5
Hemoglobin(g/dl)	9.7	11.8	5.9	4.7	5.1	5.7
Absolute Neutrophil count(x10^9/L)	1.5	1.4	1.2	1.4	1.0	1.3
Plateletcount(x10^9/L)	29	84	17	10	23.5	18.9
Early Death	4	2	5	6	Nil	3
Lost to follow up	1	Nil	NA	NA	4	Nil

NA=Not Available

The response rate in present study in CSA group was 56.2% and in ATG + CSA group was 62.5% which is more than J. Marsh etal[13] who had 46% response in CSA group and in ATG + CSA group 74% response but Mahapatra etal[7] had 32.2% response in CSA group and 58.7% response in ATG + CSA group. The number of non responders in the present study were 51.5% in CSA group and 37.5% in ATG + CSA group which is almost similar to J.Marsh etal[13] who had 54%. Non responders in CSA group and 26% in ATG + CSA group, but Mahapatra etal[7] had 68% Non-responder in CSA group and 42% in ATG + CSA group.

There were early death of 3 patients in present study ATG + CSA group and none in CSA group in present study but in J. Marsh etal[13] study there were 4 patients in CSA group and 2 patients in ATG + CSA group who had early death in but in Mahapatra etal.[7] 5 patients in CSA group and 6 patients in ATG + CSA group had early death. Thus CSA group seems to be well tolerated agent.

The gum hypertrophy and Acute Renal failure were noted in both groups, there was no much difference in percentage of incidence, which is similar in Mahapatra etal[7] and J Marsh et al[13] But in ATG + CSA group serum sickness was major side effect 15% which is also seen in J Marsh etal[13] and Mahapatra etal[7] 25.9%.

The present study showed overall response rate of 62% in ATG + CSA group and 56% in CSA group. Mahapatra etal[7] showed 56.2% in CSA group and 58% in ATG + CSA group but J. Marsh etal[13] showed significant differences in both group. 74% response in ATG + CSA group and 46% in CSA alone group (R=0.02).

CONCLUSION

ATG+CSA is better option for patients who cannot afford Bone marrow transplantation or who don't have donors, but CSA can be an alternative to both ATG+CSA or BMT who can't afford both.

LIMITATIONS

Our study was limited by small sample size. Duration of follow-up is short when compared with previous studies. Small number of study patients in ATG group is largely due to cost involved.

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