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Original Research Article

Study of Response to Treatment with Antithymocytic Globulin (Atg) +Cyclosporine (Csa) In Aplastic Anemia

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Abstract: Aplastic anemia is disorder of Bone marrow failure causing peripheral cytopenias and causing huge morbidity and mortality. It is treated with Cyclosporine (CSA), Antithymocytic globulin (ATG) and Bone marrow transplantation. Our aim of the study is study the response to Antithymocytic globulin plus Cyclosporine in Aplastic Anemia. All patients who opted for treatment with ATG+CSA were recruited. This study was conducted over a period of 5 years, Total 60 patients were recruited, but finally 55patients were included with mean age of 29years,and male to female ratio of1.6 to1.and followed up for 3 months. The commonest symptom was bleeding gums 75%, shortness of breath 41.66% and the commonest sign was pallor in66.66%. Out of 60 patients 58 required component support before treatment with ATG+CSA. There 61.8% patients responded, among them, complete response was seen in 22(66%), and partial response was seen in 11 (33%). 3 patients died, 1 patient had renal failure, and 35 (63.63%) had serum sickness.
Keywords: Aplastic anemia, Antithymocytic globulin, Cyclosporine, Bone marrow transplantation, Neutropenia, Hemoglobin

INTRODUCTION

Aplastic anemia patients have Bone marrow failure, causing peripheral pancytopenia, and mortality occure due to bleeding, infections or due to complications of anemia [1]. Commonest variety of Acquired aplastic anemia probably of an autoimmune etiology due to suppression of hemopoiesis due to T lymphocytes [2]. Presently allogenic bone marrow transplantation produces high cure rates and is the treatment of choice, but only few have donors [3]. Alternative to bone marrow transplantation immunotherapy with Cyclosporine (CSA) or Antithymocytic globulin (ATG) +cyclosporine is the choice [4]. Cyclosporine-A which block T –lymphocyte function has shown good response in Indian and other studies ranging from 30% to 50% [4, 5] when cyclosporine has been combined with Anti thymocytic gloubine the response rates were 60% to 70% [6-8] from western studies, but studies from India showed lower response of around 40% [9]. Since there are very few studies from India on ATG+CSA therapy, hence we have under taken the study on ATG+CSA.

MATERIAL METHODS:

This study was a prospective study conducted in the department of General Medicine at Nizam's Institute of Medical Sciences (NIMS) which is a multispecialty 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 5 years from 2011 to 2016 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, Dyskeratotic 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 & Purpurea 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 Bacigalupo *et al.;* in 1988 [8].

Severe AA (SAA): Bone marrow Cellularity <25% or 25–50% with <30% residual hemopoietic cells and two out of three of the following:

1. Absolute Neutrophil Count (ANC) <0.5x 10^9/L,
2. Platelets <20 x 10^9/L,
3. Reticulocyte count <20 x 10^9/L

Very severe AA (VSAA): As for severe but ANC <0.2 x10^9/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 Antithymocytic 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:

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, and Hyderabad for 3 months. 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 /Cefoperazone + sulbactum and Amikacin. Whenever 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 60 (Table-1) patients were enrolled in the study. The age of the patients ranged from 10 to 55 years with mean age of 29 years. Male to Female ratio was 1.6:1. Majority of the patients presented with bleeding gums 75%, weakness 50% and breathless in 41.66% and predominant sign was pallor in 66.66%. The average duration of symptoms before presentation was 7-8 months. Non-severe (NSAA), Severe SAA, Very severe aplastic anemia (VSAA). Patients were included in the study. Almost all the patients requiring regular component support with packed red cells and platelet before starting therapy.

Total 60 patients received ATG + CSA, 1 was lost to follow up and 3 died. 2 had mild renal dysfunction and 2 had gum hypertrophy. But 1patient discontinued due to renal dysfunction, finally 55 patients were analysed. 45 patients presented with bleeding, among them 35 patients had bleeding in first 3 months and most of them had bleeding gums. 30 patients had weakness and 25 patients had shortness of breath among patients of anemia. 8 patients presented with fever suggesting symptoms related to neutropenia. 30 patients presented with both bleeding and breathlessness, and symptoms related to all Cytopenias were present in 8 patients. Average duration of symptoms was 7.6 months, standard duration ±12.98. Majority of the patients presented with pallor in 40patientsand 16 patients had petechial/purpurea and 8 had ecchymoses. Mean Hb was 6.8 gm/dl, TLC was 3.1 x 10^9/L, ANC is 1.10 x 10^9/L, platelet count was 20 x 10^9/L. Marrow cellularity was 28% and Reticulocyte count was 0.5%.

Table-1: Clinical features of the patients

Symptom	ATG+CSA GROUP Numbers (%)
Shortness of Breath	25 (41.66%)
Bleeding Gums	45 (75%)
Fever	8(13.33%)
Weakness	30 (50%)
Epistaxis	15 (25%)
Malena	4 (6.66%)
Bleeding per vagina	4 (6.66%)
Signs Pallor	 40 (66.66%)
Petechiae/purpurea	16 (26.66%)
Ecchymoses	8 (13.33%)

25 of the 55 patients had NSAA, 22 patients had SAA and 13 patients had VSAA. Out of the 60 patients 58 had received component support before treatment. 30 patients received PRC transfusion and 12 required platelet transfusion after treatment. Out of the 55 patients (Table-2) who completed the study complete response was observed in 22 10 in NSAA group, in SAA group 8, and in VSAA group 4patients. Partial response was seen in 11patients among them 5 in NSAA group, in SAA group 3patients, and in VSAA group 3 patients. 22 patients did not respond. Total 33 out of 55 patients responded.

Table-2: Response according to severity of Aplastic Anemia.

Severity	NSAA	SAA	VSAA	total
Responders	16	12	5	33
Non responders	9	9	4	22
Death	Nil	1	1	2

2 patients had renal dysfunction mainly due to CSA, and majority had serum sickness in 35 patients due to ATG.

DISCUSSION:

Acquired Aplastic anemia is treated with Bone Marrow transplantation, if not available or the patient is not eligible they are treated with ATG + CSA, or with CSA alone who cannot afford [4]. The total number of patients is present study were 60, but only 55 patients were evaluated which is less than S.Rosenfeld *et al.;* [10] and Mahapatra *et al.;* [11] (Table-3) who had 106 and 97 patients respectively but more than Sharma *et al.;* [12] Patel AB *et al.;* [12] Frickhofen *et al.;* [6] who had patients ranging from 8 to 40 patients in their study. In the present study, the study period was 5 years only. Sharmal *et al.;* [12] had 9 years, Mahapatra *et al.;* [11] had 8 years, Marlenee *et al.;* [14] had 15 years, but S.Rosenfeld *et al.;* [10] 7 years had longer follow up. But all the other studies had few years of follow up: Jagadish Chandra etal[15] 4 years, Frickhofen *et al.;* [6] 3 years, Patel AB *et al.;* [12] had 2 years.

The median age in our study was 29years which is almost similar to Mahapatra *et al.;* [11], S.Rosenfeld *et al.;* [7] but Sharma *et al.;* [12], Patel AB *et al.;* [13], Marlene *et al.;* [14] had median age of 8 to 10 years. Most of the patients were Males when compared to female in our study which is also seen in other studies Mahapatra *et al.;* [11] Sharma *et al.;* [12] Patel AB *et al.;* [13], Marlene *et al.;* [14]. Most of our patients presented with bleeding gums 75% followed by shortness breathe 41%. Most of the studies conducted in the past have similar pattern [10, 13]. In present study, average duration of symptoms before presentation was 7.6 months which is longer compared to M.Rai *et al.;* [4], Mahapatra *et al.;* [11].

Table 3: Comparison of present study with the previous studies done on ATG+CSA therapy

Authors	**Stephen Rosenfeld *et al* [10]**	**Sj. Rosenfeld *et al*[7]**	**Frickhofen *et al.;* [6]**	**Jagdishchandra *et al.;* [15]**	**Mahapatra *et al.;* [11]**	**Marlene *et al.;* [14]**	**Patel AB *et al.;* [13]**	**Sharma *et al.;* [12]**	**Present study**
Year of Study	1991-1998	1989-94	1986-89	2001-05	2007-14	1996-2011	2011-2012	2001-2009	2011-2016
Place of Study	USA-multicenter	Bethesda-single center	Germany	India	India	Brazil	India	India	India
Study period (Yrs)	7	5	3	4	8	15	2	9	
Sample size	122	51	43	23	103	26	18	35	60
Mean Age(Yrs)	35	28	NA	8	27	8.1	9	10	29
Gender (M:F)	1.14:1	1.4:1	NA	2.8:1	2.3:1	1.3:1	2:1	4:1	1.6:1
Follow-up(months)	3 & 6	3 & 6	4	6	3,6	6	6,12	12,40	3
Deaths	15	5	4	3	6	6	1	7	3
No. of patients evaluated	106	48 & 46	39	20	97	26	16	28	55
No. of patients responded	64	34 & 36	30	9	42	9	8	10	33
Response(%)	60 & 61	67 & 71	70	40	58	34.6	50	50	61.81
Side effects	NA	HTN, Seizures, gynecomastia, GH	ARF, HTN, hepatitis, GH Hypertrichosis, edema, cramps	Serum sickness, ARF, GH, HTN, hepatitis	Fever, Serum sickness, GI upset	NA	NA	NA	Renal dysfunction, GH, Serum sickness

NA- not available, ARF-Acute Renal Failure, HTN-Hypertension, GH-Gum hyperplasia

In our study, we included patients with VSAA, SAA and NSAA, same as in the study done by, S.Rosenfeld *et al.;* [10], Mahapatra *et al.;* [11], Sharmal *et al.;* [12], Marlene *et al.;* [14], but Patel AB *et al.;* [13], had included patients only with SAA. In present study of 60 patients who were started on ATG + CSA. 1 patient was lost to follow up, 1 discontinued due to renal dysfunction and 3 patients died, after excluding

them, 55 patients remained in the study they were followed up for 3 months for the response rate and for side effects if any. There are other studies which had similar follow up like S.Rosenfeld *et al.;* [7, 10] Mahapatra *et al.;* [11] but other studies had longer follow like Mahapatra *et al.;* [11] Marlene *et al.;* [14] for 6 months, but Sharmal *et al.;* [12] had follow up of 12 months and 40 months follow up, Similarly, Patel AB *et al.;* [13] had follow up of 6 and 12 months.

The response rate in the present study was 64% at the end of 3 months which is similar to Rosenfeld *et al.;* [10] Mahapatra *et al.;* [11] but less than Frickhofen *et al.;* [6] had 70% response and other studies like by L.S.Rosenfeld [7] and J.chandra *et al.;* [15] had 40%, Patel AB *et al.;* [13] and Sharma *et al.;* [12] had 50% response. In present study 3 patients died within 2 months of ATG, among them one patient had developed febrile neutropenia with right knee septic arthritis, another had sepsis, and third patient had febrile neutropenia with intracranial bleed with mass effect. Similar observations were seen by Jagdish Chandra *et al.;* [15], but others Rosenfeld *et al.;* [10] Mahapatra *et al.;* [11] sharma *et al.;* [12] Patel AB *et al.;* [13] had more mortality. Clinically apparent side effects of CSA were mild mainly renal dysfunction and gum hypertrophy which is similar to Mahapatra *et al.;* [11] sharma *et al.;* [12] Patel AB *et al.;* [13], Marlene *et al.;* [14] other studies. But with ATG main side effects were serum sickness which similarly seen by Mahaptra *et al.;* [11], J Chandra *et al.;* [15].

CONCLUSION

ATG+CSA are a better alternative to patients who don't have donors, and patients not eligible for bone marrow transplantation.

Limitations

Present study had short period of follow up, and there was no data regarding relapse, and long term side effects to ATG and CSA.

Conflict of interest –NIL,

Financial Disclosure -Nil

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