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Original Article

Internal Medicine Section

Study of Aplastic Anaemia with Cyclosporine in Resource Poor Setting

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ABSTRACT

Introduction: Aplastic Anaemia (AA) is a syndrome characterized by peripheral pancytopenia with hypo-cellular marrow. Acquired idiopathic AA is the most common variety, probably of an autoimmune aetiology. Bone Marrow Transplantation (BMT) is the treatment of choice but cost is the limiting factor. Antithymocyte Globulin and Cyclosporine-A is an alternative to BMT. Cyclosporine alone has been tried as a single agent in resource poor setting.

Aim: The study was conducted with the aim to observe the treatment response in aplastic anaemia to Cycloserine-A.

Materials and Methods: Patients who were diagnosed as AA and opted for Cyclosporine with informed consent were included in the study. All the subjects were started on 5mg/kg of Cyclosporine and were followed up for three months to see the treatment response. This study had the approval from IEC.

Results: Twenty patients were enrolled in the study. Age of the patients ranged from 10 to 65 years. Maximum number (10/20) of patients was in the 2nd decade. Most of the patients presented with mucosal bleeds and breathlessness on exertion; the predominant sign was pallor. Eleven patients had severe AA, eight had non severe and one had very severe anaemia. Out of 20, three patients were lost to follow-up and one patient discontinued therapy due to renal dysfunction; finally sixteen patients' data was analysed. Out of 16 patients, 9 responded and 7 did not respond. Complete response was observed in three patients, partial response in six patients. Seven patients had drug toxicity in the form of acute renal failure and gum hypertrophy.

Conclusion: Cyclosporine seems to be a reasonable therapeutic option with good response rate and minimal side effects.

Keywords: Absolute neutrophil count, Antithymocyte globulin, Bone marrow transplantation

INTRODUCTION

Aplastic Anaemia (AA) is a syndrome characterized by peripheral pancytopenia with hypocellular marrow [1]. Acquired idiopathic AA is the most common variety, probably of an autoimmune aetiology primarily due to suppression of haemato-poiesis by autoreactive T-lymphocytes [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 and cyclosporine-A is an alternative to BMT [2]. Cyclosporine-A alone has been tried as single agent in resource poor setting [2,3].

Cyclosporine is a potent immunosuppressive agent, cheap, easily available and less toxic. It can be used alone or in combination with Antithymocyte Globulin. A potential practical advantage of Cyclosproine alone would be that it can be administered to outpatients, reducing the cost of treatment. Studies conducted on Cyclosporine therapy in India and most of the studies from other countries showed response rates of 30 to 50% [2-7].

In the early 1980s, Antithymocyte Globulin was shown to significantly improve the survival of patients with AA in comparison to supportive care alone [4]. Several studies have shown encouraging results with a combination of Antithymocyte Globulin and Cyclosporine [2]. Studies conducted from western countries on Antithymocyte Globulin and Cyclosporine combination therapy showed the response rate of 60 to 70%. Studies from India on combination therapy showed a lower response rate of 40% [5]. Antithymocyte Globulin is expensive and many patients cannot afford Antithymocyte Globulin [5].

In resource poor setting where patients cannot afford BMT or Antithymocyte Globulin, cyclosporine is the treatment option available.

AIM

The aim of this prospective study was to see the response of Cyclosporine-A in patients with aplastic anaemia in a resource poor setting.

MATERIALS AND METHODS

This was a prospective study conducted in the Department of General Medicine at Nizam's Institute of Medical Sciences (NIMS) which is a multi-specialty tertiary referral care centre located at Hyderabad in the state of Telangana. This study had approval from ethical committee of NIMS. The patient's consent was taken before participation and in case of children their parents consent was taken.

Inclusion Criteria

All diagnosed patients of acquired idiopathic anaemia without active infection were included, with age above 5 years of both gender and patients who are not eligible for BMT like: a) young patients who lacked an HLA – compatible sibling donor; b) patient who were more than 40 years of age were also included.

Exclusion Criteria

Patients with inherited AA like Fanconianaemia, dyskeratosis congenital underlying immunodeficiency state including HIV, infections not adequately responding to appropriate therapy, serum creatinine more than 2.5mg/dl, pregnancy or lactation or immunizing to take contraception, underlying major systemic illness and contraindication to cyclosporine A were excluded.

Clinical Examination

All patients presenting with symptoms of anaemia, petechiae, bruises and mucosal bleeds underwent a detailed clinical

examination for the presence of pallor, petechiae and purpurae and features of inherited AA like Short stature, Café au lait spots, Skeletal anomalies, Leucoplakia, Nail dystrophy and pigmentation of the skin along with the systemic examination.

Laboratory Investigations

Haemoglobin (Hb), Total Leukocytes Count (TLC) and differential Counts (DC), platelet count, reticulocyte count, 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 40 years of age to exclude inherited AA.

Diagnosis

Patients were diagnosed as AA based on the peripheral cytopenias along with hypocellularity on the bone marrow.

The patients were divided into Non Severe (NSAA), Severe (SAA) and Very Severe (VSAA) according to the classification given by Camitta et al., and Bacigalupo et al., [4,6].

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) <0.5x 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 anaemia.

After diagnosis based on the selected criteria, patients were explained about the treatment options available (BMT, Antithymocyte Globulin, Cyclosporine). Twenty patients who opted cyclosporine were enrolled during the one and half year study period. Written informed consent was taken from all the patients.

Treatment Protocol and Dosages

Cyclosporine 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.

Follow-up

Patients were followed at 2 weekly intervals in the out-patient clinic. Assessment of response to therapy was made by regular haemograms. Record of blood and blood product transfusion, infective and hemorrhagic complications was maintained. Patients were also monitored for side-effects of cyclosporine therapy with urea and creatinine levels in blood during each follow up visit. Blood levels of cyclosporine were not monitored in these patients.

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 three months of therapy.

2. *Complete Response:* Transfusion independence and an absolute neutrophil count (ANC) of >1.5x10^9 /L platelet count >150x10^9 /L and haemoglobin >11gm/dl after three months of therapy.

3. *Non-Responders:* No haematological response and transfusion dependence after three months of therapy.

STATISTICAL 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 pre-treatment 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 20 patients [Table/Fig-1] were enrolled in the study. The age of the patients ranged from 10 to 65 years. Maximum number (10/20) of patients was present in the 2nd decade. Male to female ratio was 1.8:1. Majority of patients presented with mucosal bleeds and breathlessness on exertion and the predominant sign was pallor. The average duration of symptoms before presentation was 8 months. Non Severe (NSAA), Severe (SAA), Very Severe Aplastic Anaemia (VSAA) patients were included in the study. Almost all the patients were requiring regular component support with packed red cells and platelets before starting therapy.

Twenty patients received cyclosporine. Out of them three patients were lost to follow-up due to financial constraints and one patient discontinued therapy due to renal dysfunction, finally 16 patients were analysed.

Eighteen patients presented with bleeding, among 16 patients who were analysed 14 had bleeding in first three months and most of them presented with bleeding gums. Seventeen patients presented with symptomatic anaemia and predominant complaint was breathlessness on exertion. Seven patients presented with fever suggesting symptom related to leucopenia. Fifteen patients presented with both bleeding and breathlessness on exertion. Symptoms related to all three cytopenias were seen in six patients. Average duration of symptoms before presentation was 8 months (Std. deviation ±13.2). Majority of patients presented with pallor 13. Six patients presented with petechiae / purpirae and one with echymoses. Mean Hb was 5.1gm/dl , TLC – 3.2 x 10^9/ L, ANC – 1.06 x 10^9/L, platelet count – 23.2 x 10^9/L, reticulocyte count – 0.5% , marrow cellularity – 24% . Eleven of the 20 had severe aplastic anaemia, eight had non severe and one had very severe anaemia.

Out of 20 patients component support was required in 19 patients before treatment. Nine patients received packed red cell transfusions and 7 patients required platelet transfusions after treatment.

Out of 16 patients who completed the study, Complete Response (CR) observed in 3 patients (NSAA-2 and VSAA-1), partial response (PR) in 6 patients (NSAA-2, SAA-4). Total 9 out of 16 (NSAA-4, SAA-4, VSAA-1) responded. Seven patients did not respond.

SIDE EFFECTS

Renal Dysfunction: Mild renal dysfunction was seen in 4 patients. 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 4 patients while on therapy and they did not require decrease in drug dosage.

DISCUSSION

In the present study 20 patients [Table/Fig-2] received cyclosporine. In this study the median age was 27 years which is in agreement with the previous studies done by Rai et al., (19 years) at Banaras Hindu University, Varanasi in the year 2000, Subhash Varma et al., (21years) at Chandigarh from India in the

S. N	Age (yr)	Sex	Before therapy			At 3 months after therapy			Response	Side effects
			Hb (g/dl)	ANC ($x10^9$/L)	Platelets ($x10^9$/L)	Hb (g/dl)	ANC ($x10^9$/L)	Platelets ($x10^9$/L)		
1	14	M	7	1.2	1.0	4	0.46	10	NR	GH
2	20	F	7	1.2	30	10.2	1.8	140	CR	-
3	45	M	9	2.5	50	7.1	2.1	30	PR	ARF,GH
4	30	F	3	0.94	30	5.1	1.48	25	PR	-
5	40	M	9	0.54	38	10.6	2.8	18	CR	-
6	29	M	4	2.2	9	4.4	1.24	12	NR	-
7	11	M	3	0.25	10	5.4	0.26	20	NR	-
8	16	M	4	0.72	10	4	1.0	30	NR	ARF
9	37	M	5	0.43	10	7.4	0.42	60	NR	GH
10	65	M	8	0.5	4.5	5.9	0.62	20	NRDT	ARF
11	15	M	5	1.3	5	9.8	2.6	30	PR	-
12	11	M	3	0.17	15	8.5	1.3	150	CR	GH
13	12	M	5	1.6	15	8.2	1.7	15	PR	-
14	26	F	2	0.46	30	5.2	2.7	70	PR	-
15	10	M	3	1.26	50	-	-	-	LF	-
16	19	M	2	2.1	30	-	-	-	LF	-
17	51	F	7	0.57	40	6	0.15	80	LF	-
18	17	F	3	0.71	20	5.3	0.7	20	NR	-
19	67	F	7	2.0	60	5.4	2.3	70	NR	-
20	17	M	6	0.58	14	7	2.3	10	PR	ARF

[Table/Fig-1]: Clinical summary of patient characteristics in CYCLOSPORINE group.
CR-Complete response, PR-Partial response, NR-Non responder, LF-Lost to follow up, ARF- Acute renal failure, GH-gum hypertrophy,DT-Discontinued Therapy

Studies	Shaheena Hanif [7]	Jameel Al ghazaly [9]	Macshan [8]	Rai et al., [2]	Subhash varma [5]	Mahapatra.m et al., [3]	Present study
Year of study	2000-2003	2001-2004	1999	2000	1998-1999	2007-2014	2008-2010
Study period (y)	3	4	Retrospective	NA	1	7	1.5
Study area	Karachi	Saudi	Moscow	India	India	India	India
Sample size	40	20	66	26	15	158	20
Mean age (yrs)	9.3	NA	10.5	19.4±7.8	21	25	27
Gender (M:F)	3.4:1	4:1	NA	11:1	1.8:1	7:3	1.8:1
Follow-up (months)	18	6	2	6	3	6	3
Deaths	11	3	Nil	5	3	Nil	nil
Lost to follow up	3	4	Nil	Nil	2	5	4
No of patients evaluated	26	13	66	12	15	62	16
No of responders	20	7	30	5	6	20	9
Response (%)	76	50	45	41.6	30.8	32.2	56.2
Side effects	Hirsutism, ARF, deranged LFT	ARF, HTN, GH	NA	Myalgia, Hypertrichosis, Parasthesia, rise in bilirubin	GH	Gastro Intestinal upset	Renal dysfunction GH

[Table/Fig-2]: Comparison of the Cyclosporine group in present study with the previous studies done on Cyclosporine therapy [2,3,5,7-9].
NA-not available, ARF-acute renal failure, HTN-hypertension, GH-gum hyperplasia, LFT-Liver function tests.

year 2005 and Mahapatra et al., at AIIMS, New Delhi 2007-2014 (25 years) [2,5,3]. Other studies done on cyclosporine – A therapy by Shaheena Hanif et al., (9.3 years) at the National Institute of Child Health, Karachi in 2003 and Maschan et al., (10.5 years) in a retrospective study from Moscow in 1999 have included only children [7,8].

In the present study, most of the patients were males with male to female ratio being 1.8:1. Most studies conducted in the past have reported a similar pattern [3,10,11]. Our patients predominantly presented with symptoms due to anaemia and thrombocytopenia as was noted in the study conducted by Shaheenahanif et al., and other studies [7]. In the present study, average duration of symptoms before presentation was 8 months, which was longer when compared to the previous studies like M Rai et al., Mahapatra et al., [2,3].

The present study included patients with VSAA, SAA and NSAA same as in the study done by Subhash Varma et al., Shaheena Hanif et al., and Maschan et al., [7,5,8] whereas study by M Rai et al., included the patients only with SAA [2].

In the present study out of 20 patients who were started on cyclosporine therapy, three patients were lost to follow-up because of financial constraints and one patient discontinued cyclosporine due to renal dysfunction which was not responding with the reduction in the dosage, after excluding these patients from the study, finally 16 patients were analysed. Response rate with cyclosporine in the present study was 56.2% at the end of 3 months which is in agreement with the previous studies by Jameel Al Ghazaly et al., (50%) [9] from Al-Jomhori Educational Hospital, Yemen in 2005 at the end of 6 months, by Maschan et al., (45%) [8] in their retrospective data, Mahapatra et al., (32.2%) [3] and Rai

et al., (41.6%) [2] at the end of six months. This is in contrast to Shaheena Hanif et al., (76%) [7] which showed greater response at the end of 18 months.

In the present study, no significant difference in the response was noted between patients with SAA (30%) and NSAA (24.8%). Thus, our study did not find any correlation between the severity and the rate of response. This is in contrast to previous studies which showed better response in patients with NSAA. In the study done by Shaheena Hanif et al., out of 76% of total responders most of the them were with NSAA [7], study by Maschan et al showed, out of 45% of total responders 85% were of NSAA [8] and in the study done by M Rai et al., included only patients with SAA showed a response rate of 41.6% [2] and in the study done by Mahapatra et al., the response was 32.2% but there is no mention about specific groups [3].

In the present study, the transfusion requirement decreased even in the non responders at the end of 3 months, similar observation was noted with Shaheena Hanif et al., [7].

In the present study, the side effects due to cyclosporine were mild, renal dysfunction was noted in four patients, three patients had transient elevation of creatinine which recovered within 2 weeks of decreasing the dose of cyclosporine, only one patient required discontinuation of cyclosporine, four patients developed gum hypertrophy which was asymptomatic. Thus, in our study nephrotoxicity, gum hypertrophy were the common side effects as was noted with the study by Maschan et al., (Nephrotoxicity) Jameel Al Ghazaly et al., (Nephrotoxicity, gum hypertrophy) and Subhash Varma et al., (gum hypertrophy) [8,9,5]. Others have found liver toxicity to be the most common. Upper GI symptoms were common in the study by M Raietal., Mahapatra et al., and hirsutism was noted commonly in the study by Shaheenahanif et al., [2,3,7].

There were no deaths in cyclosporine group during the follow -up period where as previous studies done by Shaheena Hanif et al., have reported 11/40 deaths in the first six months of starting therapy mostly due to infection and haemorrhages [7], M. Rai et al., (5/12) during 6 months of follow-up [2] and Subhash Varma et al., (3/15) during 3 months of follow-up period [5] and Jameel Al Ghazaly et al., reported 2/20 deaths within 1 month of therapy [9].

Thus, patients in our study had better survival and minor side effects and comparable response with the previous studies.

LIMITATIONS

1. Our study was limited by small sample size.
2. Duration of follow-up is short when compared with previous studies.

CONCLUSION

In developing countries like India, majority of the patients with aplastic anaemia cannot afford Antithymocyte Globulin, bone marrow transplantation. Cyclosporine-A seems to be a reasonable therapeutic option in such cases with good response rate and minimal side effects.

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