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Research Article

EFFICACY OF LOW-DOSE PULSE CYCLOPHOSPHAMIDE IN THE MANAGEMENT OF SEVERE REFRACTORY AUTOIMMUNE HEMOLYTIC ANEMIA: A PROSPECTIVE STUDY

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ABSTRACT

Autoimmune hemolyticanemia (AIHA) is a rare condition characterized by the destruction of red blood cells due to autoantibodies. The management of severe, refractory AIHA remains challenging, particularly for patients who do not respond to steroids or other immunosuppressive therapies. This study evaluates the efficacy of low-dose pulse cyclophosphamide therapy (1g/month for four months) as an alternative to splenectomy and rituximab. A total of 34 patients (20 males, 14 females) aged 21–53 years were included. Hemoglobin levels significantly increased, and reticulocyte counts decreased following treatment. By the fourth cycle, 83% of patients achieved partial remission (PR), while 42% maintained hemoglobin levels ≥10 g/dL without transfusion. Six months post-treatment, complete remission (CR) was observed in several cases. No severe adverse effects were reported. These findings suggest that low-dose pulse cyclophosphamide may be a promising and cost-effective option for refractory AIHA, warranting further large-scale studies.

Keywords :- Autoimmune hemolyticanemia, Cyclophosphamide, Steroid-refractory AIHA, Immunosuppressive therapy, Hemolytic crisis.

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INTRODUCTION

Autoimmune hemolyticanemia (AIHA) is rarely reported, with a population-based study estimating an incidence of 0.8 per 10,000 individuals and a prevalence of 17 per 1,000 [1]. Primary (idiopathic) AIHA is less frequent, whereas secondary AIHA requires identification and management of the underlying condition(s) [2]. Laboratory investigations remain the primary diagnostic tool, with significant advancements in diagnostic techniques. AIHA is typically characterized by decreased haptoglobin levels, elevated lactate dehydrogenase (LDH) levels, and broad-spectrum antibodies targeting immunoglobulins and complement. However, in secondary AIHA cases, some characteristic laboratory findings may be absent [3]. Various factors aid in diagnosing secondary AIHA, such as disease onset, recent infections, prior blood transfusions, vaccinations, and indicators of immune disorders (e.g., arthritis). It is particularly crucial to exclude drug-induced hemolytic anemia, as discontinuation of the causative medication is the most effective treatment approach. Clinical history, physical examination, and the antibody profile guide further investigations. Additional diagnostic procedures relevant for treatment decisions include abdominal computed tomography, immunoglobulin level assessment, lupus anticoagulant testing (if warm antibodies are detected), and bone marrow analysis [4]. Most AIHA cases respond to glucocorticoid therapy; however, relapses are frequent. For patients who are

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refractory to steroids or unresponsive to treatment, splenectomy serves as a second-line intervention [5]. Various immunomodulatory agents are employed as salvage therapies, including intravenous immunoglobulin, danazol [6], cyclophosphamide, azathioprine, cyclosporine, and vincristine at minimal doses [5]. Rituximab is another second-line treatment option, generally administered at a dosage of 375 mg/m² on days 1, 8, 15, and 22. Rituximab offers an optimal short-term benefit-to-risk ratio for patients ineligible for or unwilling to undergo splenectomy. However, due to the limited patient selection, heterogeneity of the study population, and insufficient long-term data on safety and efficacy, its broader applicability remains uncertain. In cases where complete remission (CR) is achieved with rituximab, splenectomy may be postponed or avoided [7]. Both azathioprine and cyclophosphamide function as immunosuppressants, decreasing autoantibody production. If steroid therapy fails, maintenance doses exceeding 20 mg/day or steroid tapering should be considered.

Cyclophosphamide (100 mg/day) or azathioprine may be administered either as monotherapy or in combination with steroids. Due to their myelosuppressive effects, routine peripheral blood cell monitoring is recommended, with dose adjustments as required. Before rituximab was introduced, azathioprine and cyclophosphamide were commonly used as second-line treatments; however, their use has significantly declined due to their limited effectiveness and associated adverse effects [4]. Patients undergoing long-term high-dose steroid therapy often develop resistance to multiple treatments. Studies have explored high-dose cyclophosphamide therapy in AIHA patients unresponsive to conventional treatment [8]. Pulse cyclophosphamide therapy has demonstrated efficacy in managing lupus nephritis associated with systemic lupus erythematosus [9]. This regimen suppresses both T and B lymphocytes in autoimmune conditions, reducing autoantibody production [10]. In allogeneic bone marrow transplantation, cyclophosphamide exerts potent immunosuppressive effects on transplanted cells [11]. Cyclophosphamide-resistant lymphocytes contain aldehyde dehydrogenase, an enzyme that provides protection against its cytotoxic effects [12]. In severe aplastic anemia, high-dose cyclophosphamide has been shown to induce long-term remission without requiring continued treatment [13]. This approach has also been effective in treating other autoimmune diseases [14] and in eliminating alloantibodies [15]. Patients with severe refractory AIHA, unresponsive to steroids, were treated with pulse cyclophosphamide (1g/month) over a four-month period.

METHODOLOGY

A prospective study was conducted wherein intravenous pulse cyclophosphamide (1 gram/month) was administered to individuals with severe refractory warm autoimmune hemolyticanemia (AIHA) who had not responded to conventional treatments such as steroids, azathioprine, intravenous immunoglobulin, and oral cyclophosphamide. These patients were unable to reduce their prednisone dosage below 10 mg/day.For patients with suspected secondary AIHA, the diagnosis of warm AIHA was established based on clinical symptoms, physical examinations, and a comprehensive blood profile, which included reticulocyte count, direct antiglobulin test (DAT) positivity, unconjugated hyperbilirubinemia, elevated lactate dehydrogenase (LDH), antinuclear factor (ANF), and anti-double-stranded DNA (anti-dsDNA) antibodies.Before obtaining verbal consent, participants were informed about the study objectives and methodology and were invited to enroll. Monthly laboratory evaluations were performed, which included complete blood counts, DAT levels, bilirubin levels, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels.

The treatment response was classified as follows:

- Complete Response (CR): Hemoglobin (Hb) ≥ 12 g/dL
- Partial Response (PR): Hemoglobin (Hb) ≥ 10 g/dL or an increase in Hb by at least 2 g/dL
- No Response (NR): Failure to meet the criteria for either CR or PR.

STATISTICS

Statistical analysis was conducted using the SPSS software, with a significance threshold set at $P < 0.05$.

RESULTS

This study comprised 20 males and 14 females, ranging in age from 21 to 53 years (average: 35.52 × 10). Among them, 26 individuals were diagnosed with primary warm AIHA, while four female participants had secondary warm AIHA. Over the past year, patients who received packed RBC transfusions had baseline hemoglobin levels (g/dL) and reticulocyte counts (%) of 6.6 ± 2.6 and 14.23 ± 8.29, respectively, before initiating cyclophosphamide therapy.During four consecutive months of pulse cyclophosphamide treatment (1 g/month), hemoglobin levels (g/dL), direct antiglobulin test (DAT) results, and reticulocyte counts were monitored. By the completion of the fourth cyclophosphamide cycle, 83% of patients exhibited a partial response (PR), while 26 experienced no response (NR). Seven patients (42%) achieved hemoglobin levels

of at least 10 g/dL without requiring a blood transfusion, whereas 16 (48%) were able to maintain a prednisone dosage of less than 10 mg/day without transfusion support. Additionally, 4 patients (12.7%) had hemoglobin values ranging between 8 and 8.4 g/dL.Six months post-cyclophosphamide treatment, complete remission (CR) was observed, with patients sustaining prednisone doses below 10 mg/day and achieving transfusion independence (Table 1). Hemoglobin levels significantly increased after the first, second, third, and fourth months of cyclophosphamide therapy, while reticulocyte percentages showed a notable decline. Hemoglobin levels progressively rose after each cyclophosphamide cycle, reaching their peak by the fourth cycle (Table 2). Similarly, the reticulocyte count (%) continued to drop significantly following each cycle, eventually reaching its lowest point. Throughout the study, no abnormalities were detected in white blood cell (WBC) count, platelet count, or renal function parameters.

Table 1: Patient demographics before and after cyclophosphamide treatment.

Age (years) and Patients sex	Types of AIHAa	Therapy prior to pulse cyclophosphamide	HB (g/dL)	RC %	DAT	HB (g/dL)	RC %	DAT	Type of response	HB (g/dL)
21/Male	Primary	Steroids+ azathioprine	6	34	+	10.9	8	_	112	PR
31/Female	Primary	Steroids+ azathioprine	8	24	+	11.3	7	_	13.3	PR
41/Female	Secondary (SLE)	Steroids+ azathioprine Intravenous immunoglobulin + oral cyclophosphamide	10	13	+	12.2	5	_	13.7	PR
51/Female	Primary	Steroids+azathioprine+oral cyclophosphamide	4.5	15	+	11.7	7	_	PR	14
31/Male	Primary	Steroids+azathioprine	7.3	13	+	10.7	7	+	PR	11.8
32/Male	Primary	Steroids+oral cyclophosphamide	6.3	24	+	11.2	8		PR	12.5
24/Female	Primary	Steroids + azathioprine + Intravenous	5.7	19	+	10.4	6	+	PR	13.3
		immunoglobulin + oral cyclophosphamide								
36/Male	Primary	Steroids+ azathioprine	7.8	15	+	10	5	+	PR	11.5
23/Female	Secondary (SLE)	Steroids+ azathioprine + oral cyclophosphamide	5.5	8	+	11.2	5		PR	13.3
47/Male	Primary	Steroids + azathioprine	5.6	9	+	9.9	6	+	PR	10.2
53/Male	Primary	Steroids + azathioprine	8.3	8	+	10.3	5	_	NR	12.2
44/Female	Secondary (SLE)	Steroids+ azathioprine + oral cyclophosphamide	7.4	8	+	11.6	7	_	PR	13.6
45/Male	Primary	Steroids + azathioprine + oral cyclophosphamide	9.3	13	+	10.4	8	_	NR	14.3
28/Female	Secondary (SLE)	Steroids + azathioprine + oral cyclophosphamide	6.7	9	+	10.3	7	_	PR	13
36/Male	Primary	Steroids + azathioprine	7.2	13	+	10	7	+	PR	12.1
35/Male	Primary	Steroids + azathioprine	9.3	9	+	10	7	+	NR	13.9
34/Male	Primary	Steroids + azathioprine	5.5	8	+	8.4	7	+	PR	10

Table 2: Hemoglobin levels before and after cyclophosphamide treatment at 1, 2, 3 and 4 months.

Hemoglobin level (g/dL)	P value
Prior to cyclophosphamide therapy (6.6 ± 2.6) vs. After 1 month	**
2 months later	***
3 months later	***
4 months later	***
One month later (8.1 ± 2.2) vs.	
Two months later	**
3 months later	***
4 months later	***
Two months later (9.1 ± 0.9) vs.	
Three months later	**
Four months later	***
Three months later (9.8 ± 0.9) vs.	
After 4 months (10.6 ± 0.10)	**

DISCUSSION

Managing patients with severe autoimmune hemolyticanemia (AIHA) that is resistant to steroids presents significant challenges, particularly when individuals are unable to tolerate the maximum steroid dose or reject adjunct therapies such as azathioprine, intravenous immunoglobulin, or cyclophosphamide. Additionally, patients often prefer to avoid surgical interventions like splenectomy, while the limited availability of compatible blood products, including washed red blood cells, and financial restrictions imposed by healthcare funding authorities further complicate treatment.Our study demonstrated promising outcomes with pulse cyclophosphamide therapy, showing no detectable risks. AIHA typically has an acute onset but is generally considered a chronic condition. Long-term remission or cure rates for primary AIHA remain low, making symptom management and the prevention of "hemolytic crises" the primary treatment goals while minimizing both short- and long-term adverse effects. Surprisingly, AIHA management continues to be largely based on clinical experience rather than standardized protocols, which is an unfortunate reality. Although some phase 2 prospective studies exist, no randomized trials have been conducted. Furthermore, there is no universally accepted definition for partial remission (PR) or complete remission (CR).The optimal treatment approach for AIHA patients who fail corticosteroid therapy and for whom splenectomy is not an option remains uncertain. These patients are often treated with a combination of low-dose cytotoxic therapies, danazol, and intravenous immunoglobulins. However, many individuals exhibit only partial responsiveness to these treatments and continue to require glucocorticoids. Despite ongoing research, treatment advancements have been slow, and no definitive guidelines have been established.For AIHA patients who do not respond to glucocorticoids, splenectomy remains the primary second-line treatment. However, in cases of secondary AIHA, splenectomy has a lower success rate and is associated with increased complications. The efficacy and safety of rituximab for AIHA remain uncertain, and its use requires repeated administration every 1–3 years, heightening the risk of infections, including progressive multifocal leukoencephalopathy.The selection of second-line treatment for warm AIHA (WAIHA) patients is largely influenced by the clinician's expertise, patient's age, comorbidities, drug availability, cost, and individual preferences. Safety is the key consideration in choosing any medication, as no existing treatment has a high cure rate, and the risks associated with therapy may outweigh the benefits. In clinical practice, hematologists engage in detailed discussions with patients to make personalized treatment decisions.Previous studies have supported the efficacy of cyclophosphamide in treating AIHA, although they lacked specific patient data. Further research is needed to explore this therapeutic approach for refractory AIHA. Our study yielded results similar to those of a previous study [8] but with a lower dose and without the use of mesna. Unlike high-dose cyclophosphamide, our patients did not experience transient alopecia, nausea, vomiting, or neutropenia. To further validate these findings, a comparative study involving a larger patient population and different cyclophosphamide regimens is recommended.

CONCLUSION

For patients with severe refractory AIHA, low-dose pulse cyclophosphamide has shown effectiveness in inducing remission, offering a viable alternative to splenectomy and the associated risks, as well as to rituximab, which comes with higher costs. However, a

larger patient cohort would be required to thoroughly evaluate the efficacy and feasibility of this treatment approach.

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