ABSTRACT. Objective. Diamond-Blackfan anemia is a rare congenital hypoproliferative anemia of infancy and early childhood. Treatment with corticosteroids is commonly used, but with limited success. Trials with cyclosporin-A (CSA) are not frequently reported. Therefore, in this study we analyzed our results in the management of this rare disease by different medical treatments.

Design. The results of 22 patients diagnosed at our Hematology Center in the New Cairo University Children’s Hospital during the period 1991–2001 were retrospectively analyzed. Our patients first received prednisolone (2 mg/kg/d) for different courses according to their response. Since the year 2000, the steroid nonresponders received CSA (3–12 mg/kg/d) for 6 months unless treatment complications developed.

Results. The age at the onset of the disease ranged from 1 to 24 months (median: 2.5 months). The mean values of the hemoglobin, the reticulocyte count, and the myeloid/erythroid ratio at the onset of the disease were 4.75 ± 1.79 g/dL, 0.14 ± 0.16, and 39.4 ± 27.8, respectively. Patients received prednisolone from 0.25 to 10 years (median: 2 years). Ten patients were nonresponders (45.5%), and 5 patients (22.7%) responded to corticosteroid therapy. Two of 5 responders are off treatment with a hemoglobin level of >9 g/dL, and 3 of 5 are currently corticosteroid-dependent. Of 10 patients not responding to steroids, 8 received CSA for 6 months. Four patients (50%) responded to CSA therapy. A significant positive association was found between CSA dose and response.

Conclusion. CSA therapy should be tried in steroid-resistant Diamond-Blackfan anemia patients before blood transfusion or corticosteroid therapy complications are instituted. Pediatrics 2002;110(4). URL: http://www.pediatrics.org/cgi/content/full/110/4/e44; Diamond-Blackfan anemia, cyclosporin-A, corticosteroids.

ABBREVIATIONS. DBA, Diamond-Blackfan anemia; CSA, cyclosporin-A.

Diamond-Blackfan anemia (DBA) is characterized by decreased or absent bone marrow erythroid precursor cells, severe anemia, and reticulocytopenia, and it is frequently associated with a variety of somatic malformations.1 It is a heterogeneous disorder in which several pathophysiological mechanisms may result in blocked erythropoiesis at different stages along the erythroid differentiation pathway. Affected individuals in the same family may vary dramatically as to the degree of anemia, response to corticosteroids, and the presence of congenital anomalies.2,3 The majority of patients with DBA achieve initial clinical remission with conventional doses of prednisone.4 Many require high-doses, long-term treatment, or experience progressive resistance to therapy.5 For patients who are steroid refractory and who require high daily doses that cause toxicity, chronic transfusion is instituted with the accompanying problems of iron overload and the risk of exposure to transfusion-transmitted disease.4 Alternative therapies are required for patients who are unsuitable for steroid therapy. Bone marrow transplantation has been used in DBA with reasonable results, but it is not available for every patient, is expensive, and may result in serious, life-threatening complications.6 Clinical trials have documented hematologic responses in DBA patients receiving recombinant human interleukin-3, androgens, cyclophosphamide, plasmapheresis, antithymocyte globulin, and high intravenous doses of methylprednisolone.7–10 Cyclosporin-A (CSA) has been reported in few patients by several investigators to produce remission in patients with DBA in whom standard prednisone doses had failed.11,12

PATIENTS AND METHODS

Patients

The 22 patients with DBA included in this study were seen at the Hematology Clinic of the New Cairo University Children Hospital during the period 1991–2001. Included patients presented in early childhood with normochromic, usually macrocytic anemia; reticulocytopenia (<1%); normocellular bone marrow with selective deficiency of red cell precursors (<0.5% mature erythroblasts), and normal leukocyte and platelet counts. Complete physical examination was conducted on all patients to detect other congenital abnormalities. Abdominal ultrasonography was done to detect suspecting anomalies.

Blood Studies

Complete blood count and reticulocyte count were estimated in all patients at the time of diagnosis and during the follow-up before blood transfusion.

Bone marrow aspirate and biopsy were assessed for cellularity, state of various lineages, and erythroid/myeloid ratios.

Treatment Protocol

Prednisone Therapy

Prednisone therapy consisted of 2 mg/kg/d given orally immediately after diagnosis for 2 to 4 weeks, followed by gradual tapering over 3 to 6 months. Nonresponders received another course after 3 months.
CSA Therapy

CSA therapy started in the year 2000 for steroid nonresponders. It was given orally at 3 to 12 mg/kg/d for 6 months unless complications developed.

A physical examination, including weight and height determinations, was performed once monthly. Blood pressure was measured once weekly, and the parents were questioned about possible side effects such as headache, fever, temperament changes, and myalgias. Hematologic values and serum concentration of urea, creatinine, electrolytes, alanine aminotransferase, aspartate aminotransferase, and total bilirubin were determined every 2 weeks during CSA therapy.

Definition of Response Criteria

- Responders (either complete or partial): Achievement of a hemoglobin concentration >9 g/dL without prednisone requirement or with a low-dose alternate-day prednisone therapy.
- Nonresponder (failed): Failure to achieve any erythropoietic response as demonstrated by a lack of increment in the hemoglobin concentration and reticulocytic count.
- Relapse: Unequivocal decrease in the hemoglobin concentration or reticulocytic count below the previously achieved improvement or normal values, either during or after completion of therapy.

Statistical Methods

Data were summarized as mean ± standard deviation and as percentage and counts. Unpaired Student t test and χ² test was used.¹³

RESULTS

Our 22 patients were 13 males and 9 females, with age ranging from 1 to 12 years with a mean 5.22 ± 3.69. The age at the onset of the disease ranged from 1 to 24 months (median: 2.5 months). None of our cases were familial cases. There were associated congenital anomalies in 5 patients (22.7%) as polycystic kidney and carnovema of the portal vein in 1 patient, hypospadius in another patient, short stature in 1 patient, and microcephaly in 2 patients.

At the onset of the disease, the hemoglobin level ranged from 1.7% to 7.6% with a mean 4.75 ± 1.79; the mean cell volume ranged from 70.3 to 105.2 fl with a mean 84.6 ± 8.5; the mean of the mean cell hemoglobin was 28.8 ± 5.5. The reticulocyte count ranged from 0 to 0.6 with a mean 0.14 ± 0.16, and the myeloid:erythroid ratios in the bone marrow examination ranged from 5:1 to 78:1 with a mean of 39.4 ± 27.08.

All included patients received prednisolone therapy at diagnosis. The age of start of steroid therapy ranged from 1 to 26 months. The patients received the therapy over variable numbers of courses from 1 to 7 and over a period from 0.25 to 10 years. Ten patients were nonresponder to steroid therapy (45.5%), 5 patients (22.7%) responded, 1 patient was noncompliant, and 6 patients did not follow-up. Two of 5 responders are off treatment (for 4 years for 1 and 2 months for the other), and both are maintaining their hemoglobin levels >9 g/dL at last follow-up. Three of the responders are currently corticosteroid-dependent on 25% of the dose every other day.

Eight of 10 cases in whom corticosteroid therapy failed received CSA therapy. Four (50%) of 8 patients responded to CSA after variable time as shown in Table 1. One (12.5%) of 8 patients relapsed, so we increased the CSA dose, which lead to an initial improvement but relapsed with withdrawal of therapy, so we started another course of oral steroid therapy. One case (12.5%) failed to respond to CSA therapy for 6 months. Two patients (25%) discontinued the therapy after 2 months and 3 months because of urinary tract infection and hypertension, respectively. No other CSA complication was encountered in our treated patients.

We divided the patients into 2 groups: those who received an initial dose of CSA <8 mg/kg/d and those who received ≥8 mg/kg/d. One hundred percent of patients who received the lower dose did not improve, but 66.7% of patients who received the higher dose improved, and 33.3% did not respond. There was a significant positive association between response and the initial dose received with χ² test value 3.88 at degree of freedom, P < .05.

Subcutaneous erythropoietin at 200 IU/kg for 20 doses was used in 5 of our patients, but no patients improved on this line.

Intavenous methylprednisone was used for 1 patient at 20 mg/kg for 7 days then tapered, but this patient showed no improvement on this line table.²

Because of complications with packed red cell transfusion, 5 patients (23%) of 22 developed hemosiderosis with serum ferritin >1000 ng/dL. Three patients (14%) of 22 were infected by hepatitis C virus.

DISCUSSION

Congenital red cell aplasia was initially reported by Diamond and Blackfan in 1938.¹⁴ An incidence of 4 to 5 cases per million live births was reported.¹–¹⁵ Although most cases are sporadic, inheritance is observed in 10% of patients, with a dominant or, more rarely, recessive pattern.⁸–¹⁶ Abnormalities in chromosome 19q13.2 have been identified in 25% of familial and sporadic cases of DBA, in another subset of patients another locus on chromosome 8p has identified in association with DBA.¹⁷ The pathophysiology of DBA remains controversial and may reflect multiple causes. An intrinsic defect in the erythroid progenitor cell,³ the dysfunction in T-lymphocyte,¹⁸ and it is anticipated that additional molecular studies will lead to a better understanding of this complex disease.¹⁷ Chronic parvovirus infection is a rare cause of pure red cell aplasia in an immunologically normal host. Studies of parvovirus B19 were not performed in our patients, yet we believe it should be excluded in our future studies.

All cases reported fulfilled the diagnostic criteria of DBA. Congenital anomalies are present in 22.7% of our cases. Other studies reported that 25%, 30%, and 40% of DBA patients have at least 1 associated physical anomaly.⁵–⁸,¹⁷

Despite encouraging early responses to corticosteroid, this therapy eventually fails in up to 50% of patients with DBA, necessitating the institution of a chronic transfusion program. Risks of transfusion therapy, such as iron overload, have rekindled interest in alternative treatment modalities.₁² Similarly, in our studied cases, corticosteroid therapy fails in 45.5% of cases. All corticosteroid nonresponders received periodic prednisone trials over variable courses (ranging from 1–7 courses) to hopefully
<table>
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<th>Number</th>
<th>Age (Years)</th>
<th>Of Onset (Months)</th>
<th>Duration (Years)</th>
<th>Number of Courses</th>
<th>Steroid Therapy</th>
<th>Response</th>
<th>Others</th>
<th>CSA Therapy</th>
<th>Age of Onset (Years)</th>
<th>Dose in mg/kg/day</th>
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<tr>
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<td>11</td>
<td>3</td>
<td>10</td>
<td>7</td>
<td>NR Stopped</td>
<td>Transfusion-dependent Chelation Splenectomy SC-EPO</td>
<td>8</td>
<td>3</td>
<td>NR HCV → active Stopped after 3 mo Hypertension 4.8/89</td>
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<td>2</td>
<td>10</td>
<td>2</td>
<td>9</td>
<td>6</td>
<td>NR Tapered</td>
<td>50 PRBCT Chelation</td>
<td>8.6/12</td>
<td>10</td>
<td>GR after 1 mo Stunted Tapered 10: 100</td>
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<tr>
<td>3</td>
<td>8</td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>NR Tapered</td>
<td>Transfusion-dependent SC-EPO</td>
<td>7.9/12</td>
<td>8 then 12</td>
<td>NR Initial response then relapse after 1 mo, so increase the dose. Tapered after 6 min relapse with withdrawal 6.8; 83</td>
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<td>4</td>
<td>7</td>
<td>1</td>
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<td>5</td>
<td>Dependent then NR Tapered</td>
<td>25 PRBCT</td>
<td>5.10/12</td>
<td>11</td>
<td>GR after 1 mo Tapered Hypertension 9: 102</td>
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<tr>
<td>5</td>
<td>6</td>
<td>14</td>
<td>2</td>
<td>3</td>
<td>NR Stopped</td>
<td>60 PRBCT Chelation Splenectomy</td>
<td>5</td>
<td>8</td>
<td>GR after 4 mo Tapered after 6 mo Stopped 10: 93</td>
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<td>Transfusion-dependent</td>
<td>2.9/12</td>
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<td>NR Tapered</td>
<td>5 PRBCT</td>
<td>7/12</td>
<td>10</td>
<td>GR after 1 mo Tapered after 4 mo Stopped HCV → active 11: 96.9</td>
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</tbody>
</table>

NR indicates nonresponder; GR, Good response; HCV, hepatitis C virus; SC-EPO, subcutaneous erythropoietin; PRBCT, packed red blood cell transfusion.
achieve the response even at a later date. All of our steroid responder DBA cases (22.7%) responded after 1 or 2 courses of prednisone therapy. Although we do not have an explicit interpretation, it has been suggested that as the interval between diagnosis and initiation of glucocorticoid therapy expands, the response to glucocorticoids decreases.

Alternative medical therapies are required for patients who are unsuitable for steroid therapy. Several investigators have reported at least partial responsiveness in their cohorts to high dose intravenous methylprednisolone, bone marrow transplantation, recombinant human interleukin-3, androgen, cyclophosphamide, and antithymocyte globulin. Studies suggest a role for T-cell suppression of erythroid stem cells in this disease, making an agent such as CSA a logical therapeutic choice. CSA has been shown to inhibit apoptosis by modulation of Fas and through downregulation of γ-interferon production, which may play a role in the pathophysiology of this disease.

In our center, 8 patients received CSA therapy (Table 1). Four cases (50%; Case no. 2–4–5–8) responded to CSA and remained transfusion-independent for 12-months follow-up. Two cases (25%; Case no. 1–6) developed complications necessitating stoppage of therapy. One case (12.5%; Case no. 3) showed transient response but relapsed after 1 month, so we increased the dose that lead to an initial response but relapsed with withdrawal of CSA. One case (12.5%; Case no. 7) did not respond to CSA. Raghavachar reported that CSA is effective in the treatment of steroid resistant acquired pure red cell aplasias in ~65% of cases. Alessandri et al reported that 18.2% of patients with DBA from different studies have experienced a sustained remission or partial response to CSA. Although long-term spontaneous remission occurs in ~20% of DBA patients, our responders to CSA were on steroid therapy for at least 2 courses and did not improve, yet did improve after CSA therapy.

The complications of CSA therapy in our patients were hypertension and urinary tract infection; they were reversible or treatable on cessation of therapy and compare favorably with the potential toxicity of a transfusion program. None of our cases were infected seriously while they were on CSA therapy, although they were not isolated during therapy so we did not investigate their immunologic status. The lack of serious side effects in our patients is encouraging and probably relates to the relatively short duration of therapy and relatively low dose required to sustain remission if needed. Similarly, CSA has been used in the long-term treatment of young patients after solid transplant, and the risks of CSA are generally reversible on cessation of therapy. The well-documented side effects to CSA therapy include renal dysfunction, hypertension, hypomagnesemia, and an increased risk of Epstein-Barr virus-related lymphoproliferative disease. Although these risks are potentially serious, they are generally reversible or treatable on cessation of therapy and compare favorably to the potential toxicity of a transfusion program or matched unrelated bone marrow transplantation. CSA has also been shown to increase the incidence of secondary malignancies so physicians should be aware of this complication and it should be discussed with the patients. As is true with every situation, the risks and benefits have to be weighed in deciding particular forms of therapy.

**CONCLUSION**

CSA therapy is a useful agent in a subset of DBA patients and should be tried in those resistant to corticosteroid therapy. It should be tried before blood transfusion or corticosteroid therapy complications.

**ACKNOWLEDGMENTS**

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**REFERENCES**


http://www.pediatrics.org/cgi/content/full/110/4/e44
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