Original Article

Diamond–Blackfan Anemia with Mutation in \textit{RPS19}: A Case Report and an Overview of Published Pieces of Literature

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\textbf{Introduction:} Diamond–Blackfan anemia (DBA), one of a rare group of inherited bone marrow failure syndromes, is characterized by red cell failure, the presence of congenital anomalies, and cancer predisposition. It can be caused by mutations in the \textit{RPS19} gene (25\% of the cases). \textbf{Methods:} This case report describes a 10-month-old boy who presented with 2 months’ history of gradually increasing weakness and pallor. \textbf{Results:} The patient was diagnosed as a case of DBA based on peripheral blood finding, bone marrow aspiration with trephine biopsy reports, and genetic mutation analysis of the \textit{RPS19} gene. His father refused hematopoietic stem cell transplantation for financial constraints. Patient received prednisolone therapy with oral folic acid and iron supplements. \textbf{Conclusion:} Hemoglobin raised from 6.7 to 9.8g/dL after 1 month of therapeutic intervention.

\textbf{KEYWORDS:} Diamond–Blackfan anemia, hematopoietic stem cell transplantation, \textit{RPS19} gene, Prednisolone, Bone Marrow, Anemia, Genetic and Rare Disease

\textbf{INTRODUCTION:} "Diamond–Blackfan anemia (DBA; Mendelian Inheritance in Man #105650), one of a rare group of inherited bone marrow failure syndromes (IBMFSs), is characterized by red cell failure, the presence of congenital anomalies, and cancer predisposition.\textsuperscript{[1]} DBA is also characterized as a developing cluster of illnesses identified as ribosomopathies.\textsuperscript{[2,3]} DBA is a scarce inherited diverse clinical and genetic erythroblastopenia\textsuperscript{[4]} because IBMFS\textsuperscript{[5]} resulted in red cell aplasia with physical malformations.\textsuperscript{[9]}

\textbf{REVIEW OF LITERATURES:} Brief history

DBA was first stated in 1936 by Dr. Hugh W. Josephs of Johns Hopkins University, Maryland, USA. Later, in 1938, a more detailed description was given by Dr. LK Diamond and Dr. KD Blackfan; initially, it was considered as congenital hypoplastic anemia. The diagnostic principles for DBA were published in 1976 and continued as an acceptable standard.\textsuperscript{[7-9]}

DBA initially presented with anemia before the age of 12 months. The signs include either typical or a little reduced neutrocytes counts, inconstant platelet counts, reticulocytopenia, macrocytosis, and healthy bone marrow cellularity with a scantiness of red cell precursors.\textsuperscript{[9]}

\textbf{Pathology:} The molecular biology of DBA is comprehensively researched, and, in more than 50\% of cases, the syndrome appears to result from haploinsufficiency of either a small or large subunit-associated ribosomal protein (RP).\textsuperscript{[6,10]} However, the exact process by which RP haploinsufficiency consequences in erythroid failure, in addition to the other clinical symptoms and signs, remains indeterminate.\textsuperscript{[6]} Macrocyclic anemia

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is a protuberant characteristic of DBA, but the illness is also pigeonholed by physical developmental impedance, and at least 40% of DBA cases had inherited inconsistencies. [11] DBA is correlated with single, monoallelic, and inactivating mutations in RP genes. In DBA, modifications or bulky deletions in RP genes include RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPL5, RPL11, RPL26, and RPL35A. These mutations have been confirmed by approximately 60% of DBA patients. [12] Additionally, multiple studies revealed that equally have large and small subunits of RP genetic inconsistencies observed in RPL5, RPL11, RPL35A, RPS7, RPS10, RPS17, RPS19, RPS24, and RPS26, but also affects and mutated many other genes. [12-14] Furthermore, several research studies reported that outlying cases and patients of DBA have been recognized with an increased number of mutated RPs genes, which include RPL3, RPL7, RPL9, RPL14, RPL19, RPL23A, RPL26, RPL35, RPL36, RPS8, RPS15, RPS27A, RPL18, and RPL35. [11,15,16] The molecular pathology of DBA has been extensively studied in the last few years. These research studies revealed that the cause for the anemia observed in both DBA and in del (5q) myelodysplastic syndrome is haploinsufficiency of RPs, [17,18] leading to nucleolar function, translational surveillance, erythroid failure, cancer predisposition, and physical disabilities. [19,20]

**Diagnostic principles**

The diagnostic principles have extended intensely as a result of detail insight of gene and upgraded data regarding DBA epidemiology. [6,22,23] The frequency of DBA is about six per million live birth. [24] DBA usually presents around the age of 1 year with “macrocytic, or occasionally normocytic, anemia with reticulocytopenia, essentially normal neutrophil and platelet counts, and a normocellular bone marrow with a lack of erythroid precursors.” [6] Research studies showed that 25% of the DBA cases are associated with a mutation in the RPS19 gene. [25-27] Other studies showed that approximately 43% of patients with DBA are related to mutations in six more RP genes including RPS24, RPS17, RPL35A, RPL5, RPL11, and RPS7. [28] Many nonclassic patients of DBA have been recognized. These pediatric cases often have mild blood-related disorders or DBA-associated hereditary abnormalities or totally healthy. [6] Furthermore, patients with DBA sometimes present beyond 1 year of age into late childhood, adolescence, or even adulthood. [4,29,30] The number of autosomal-dominant recessive inherited patients has been assessed, for at least one genotype, to be approximately 45%, with the recognition of these non-classic DBA phenotypes. [6,31]

The preliminary laboratory investigations regarding DBA diagnosis comprise a total “blood count, reticulocyte count, and if possible, fetal hemoglobin (HbF) level and an erythrocyte adenosine deaminase (eADA) activity.” [6] Patients with DBA who possess repeatedly physical abnormalities or family history of physical deformities, found to have hypoproliferative anemia, unclarified macrocytosis, raised HbF, and even devoid of anemia, should be inquired further. [16,32] Most of the patients with DBA have macrocytosis, with an elevation in HbF. [33,34] A bone marrow aspirate is required for diagnosis of DBA and usually shows a normocellular marrow with normal myeloid maturation, adequate megakaryocytes, and a discerning deficient of red cell precursors. A bone marrow histopathology is likewise suggesting considering cellularity. Cellularity has been noted to decrease inexplicably to the usual age-related decrement. [35] A routine karyotype is necessary to identify any significant chromosomal abnormalities. Indeed, two of the “DBA genes” have been identified through the evaluation of a translocation involving the gene encoding RPS19 and a large deletion on chromosome 3q involving the coding region for RPL35a, respectively. Although not pronounced, the presentation of this disease in childhood is conceivable. If the bone marrow morphology is unswerving with giant, multinucleated erythroblasts and pronormoblasts, or if there is no genetic indication supportive of the diagnosis of DBA, predominantly in atypical presentations, viral titers and assessment for viral genome are carried out to rule out parvovirus B19 as the reason. [6,36,37]

**Inherited bone marrow failure syndromes**

The clear understanding of molecular pathogenesis of IBMFS is essentially and urgently required for proper diagnosis and treatment, although these disease incidence rate is quite low. [38] IBMFS patients often have several complications, involving many major systems. Hematopoietic stem cell transplantation (SCT) resolves some issues, avert others, but causes some new pathology. [39] Quite a few propositions have appeared within each subcategory of IBMFS including the ribosomopathies that comprise both ribosome assembly and ribosomal ribonucleic acid (RNA) processing. [38] A recent study reported that ribosomopathies are involved in DBA, Fanconi anemia (FA), Shwachman–Diamond–Bodian syndrome (SDBS), dyskeratosis congenita (DC), and cartilage hair hypoplasia. These all disease conditions have mutations in RPs and in proteins responsible for processing of ribosomal RNA. [40] The respective pathologic pathways involve deoxyribonucleic acid (DNA) repair (FA), telomere biology (DC), and ribosome biogenesis (DBA and
A lot of patients with IBMFS present with hematologic symptoms and signs, such as single-cell or pancytopenia, MDS, or leukemia, particularly acute myeloid leukemia (AML). The diagnosis of an IBMFS often exposed during assessment for the hematologic indexes, due to the reflection of specific clinical phenotypes or screening tests for syndrome-specific or genomic studies. The principal diagnostic feature for FA includes increased chromosome breakage in lymphocytes cultured with a DNA cross-linker; for DC it includes short telomeres by lymphocyte flow cytometry and fluorescent in situ hybridization; for DBA it includes elevated red cell adenosine deaminase; and for SDS it includes low levels of serum trypsinogen and isoamylase.

**Epidemiology**

Three hundred fifty-four DBA patients’ were enrolled in the Diamond Blackfan Anemia Registry of North America (DBAR) in 2001, and another research study reported additional six hundred DBA patients’ were registered by 2012. DBAR was commenced in 1991 for a wide-ranging evaluation of the clinical epidemiology and pathophysiology of DBA. The DBAR is a professional and charitable organization that registered patients after necessary informed consent is obtained in agreement with the Helsinki Declaration.

**Morbidity, mortality, and treatment options**

Multiple studies from several countries showed that approximately 40% of patients with DBA were transfusion-dependent as these cases were not responding to corticosteroids, although 40% were corticosteroid-dependent and 20% were transfusion-independent with additional medication. A group of patients improved and maintained adequate hemoglobin levels with initial corticosteroid therapy. Nonetheless, a small number of patients do not respond to corticosteroid, and remission occurs after prolonged blood transfusion. The DBAR defines “remission” as a stable, physiologically acceptable hemoglobin, lasting for at least 6 months, independent of corticosteroids, transfusions, or other therapy. Among DBA cases, 72% of them had remission within the first 10 years of life, and the majority have remission maintained, and 20% DBA cases have remission by the age of 25 years. Nevertheless, among pregnant woman hormonal stress increases and contributes as a significant aspect for relapse, which usually fades away after childbirth. Although the cancer risk among patients with DBA is quite low, approximately 4.14% (29 patients of 700 DBA cases) develop AML. The median age for the development of cancer among DBA cases was 15 years (range 1–43 years), considerably earlier than the median of 68 years in the general population. Glucocorticoids persist for almost 70 years, as the primary choice of treatment for DBA. Although glucocorticoid efficacy regarding the management of DBA was first described in 1951, the mechanism of action glucocorticoids in DBA is still shadowy and under search. Nonetheless, approximately 80% of patients with DBA improve to a preliminary treatment schedule of glucocorticoids.

Researchers relentlessly work to develop more effective and safer alternative medicine to cure DBA. Thereafter, several new therapeutic alternative appear in the market including high-dose corticosteroids, intravenous immunoglobulin, high-dose erythropoietin, interleukin-3, and androgens. Immunosuppressive therapy with cyclosporine A has been investigated in patients with DBA with no theoretic origin. Consequently, the efficacy of cyclosporine A in the treatment of DBA was not well established. In the treatment of DBA the efficacy of antithymocyte globulin had not obtained much successful intervention. Another study showed metcloplamide (a dopamine antagonist) to be effective at least 33% to produce hematologic response among DBA cases. It is believed that metcloplamide encourages the release of prolactin from the pituitary gland, and prolactin possibly recovers and expands erythropoiesis. Nevertheless, such positive findings of erythropoiesis were not observed in research studies in the USA and Europe. These studies found only a 10% improvement. Recent studies used in limited scale leucine and lenalidomide for DBA and had remission.

**Case Report**

A 10-month-old male baby named Rafi Hossain, with his father, attended a district-level hospital, Borura, Comilla, Bangladesh, with 2 months’ history of gradually increasing weakness and pallor. Complete blood count showed severe anemia, treated with iron therapy and blood transfusion. There was no improvement, and the baby again developed weakness and pallor; repeat complete blood count showed severe anemia, treated with red blood cell transfusion. As there was no improvement, baby with his father came to hematology outpatient department in Apollo Hospitals, Dhaka, Bangladesh on June 27, 2019 (patient ID: Apollo UHID 842273). The baby had one elder brother who was 13 years old and led a healthy life. There was no history of consanguinity. On examination, he was severely anemic, but there was no icterus. The baby weighted 9 kg, pulse 150 beats/min, temperature 97°C, and respiration 24 breaths/min. Complete blood
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count showed hemoglobin 5.6 g/dL, hematocrit 16.6%, mean corpuscular volume 77.9 fL, mean corpuscular hemoglobin 26.3 pg, and mean corpuscular hemoglobin concentration 33.7%. Total leukocyte count was 11.82 × 10^9/L and platelet count was 401 × 10^9/L. Peripheral blood film showed normocytic anemia [Figure 1] with eosinophilia. Hemoglobin electrophoresis showed HbA: 80.04%; HbE: 16.60%; and HbF: 3.36%. The red cells were normocytic, and HbE was increased. These two findings can be attributed to the patient receiving 11 units of packed red blood cell transfusions before coming to our hospital, and because of the same reason research team could not perform erythrocyte adenosine deaminase (eADA) estimation. Reticulocyte count was 0.24%. Bone marrow aspiration showed suggestive of pure red cell aplasia [Figure 2]. Erythropoiesis markedly depressed with the block in maturation, and occasional proerythroblasts were observed. Bone marrow trephine biopsy showed normocellular marrow with consistent with significantly depressed erythropoiesis. This patient has karyotype 46, XY. Polymerase chain reaction (PCR) for viral DNA showed negative of parvovirus B19, cytomegalovirus, Epstein–Barr virus, Herpes simplex virus 1 and 2, Varicella-zoster virus, enterovirus, and human herpesvirus 6 and 7. Direct coombs test was negative and lactate dehydrogenase 305 U/L. Findings of serum iron profile, liver function, and renal function tests were normal. Chest X-ray, echocardiogram, and ultrasonogram of the abdomen did not show any abnormality. The genetic study was performed for screening the mutations in the RPS19 gene of the baby. The genetic analysis was carried out in the Medgenome Labs, Bangalere, Karnataka, India, after obtaining the informed consent of the parents. The genetic study was conducted based on internationally reputed methods.[75-79] The patient possesses the frameshift mutations in intron 3 of the RPS19 gene [Figure 3]. Genetic study of patients’ parents could not be performed as they dissent. Parents’ argued they are having a healthy and quality life for so years. Thus, viewed they do not need such expensive laboratory test for their child treatment. Research team made a diagnosis of DBA since the baby presented with anemia at near 1 year of age, reticulocytopenia, normal white cell count and platelet counts, elevated HbF, and normal marrow cellularity with markedly depressed erythropoiesis, supporting criteria of gene mutation in RPS19 gene, PCR for viral DNA of parvovirus B19 not detected and there was no evidence of any other bone marrow failure syndrome. The research team could not consider the possibility of hemopoietic SCT due to their financial crisis. The baby was started on prednisolone therapy with oral folic acid and iron supplements. After 1 month, hemoglobin raised from 6.7 to 9.8 g/dL.

DISCUSSION

The current case of DBA primarily presented in district-level hospitals (secondary level public hospital) when the patient was in less than 1 year of age, later in specialized hospital reticulocytopenia, normal marrow cellularity with a lack of erythroid precursors and supporting criteria RPS19 mutation established the diagnosis of DBA. These findings were like studies and reports.[8,21-23,80] Elevated levels of HbF and negative PCR for viral DNA of parvovirus B19 are supporting minor criteria.[81] All the aforementioned criteria of the current case established that it was a nonclassical DBA.[22] There are mounting pieces of evidence of diagnosing of the nonclassical presentation of DBA and generates more apprehension.[82,83] In the current case, the focal evidence for the determination of DBA was the persistent isolated anemia, and normal marrow cellularity with markedly depressed erythropoiesis was in the same as mentioned earlier study report.[84] DBA

Figure 1: Peripheral blood film showing normocytic anemia

Figure 2: Bone marrow smear showing scanty of erythroid precursors
patients with late-onset anemia or a relatively mild course have been reported. Multiple previous studies showed that a meager number of patients were identified as DBA cases after 12 months of age, with insignificant anemia requiring treatment or intervention at all. The present case, along with those previously reported, highlights the phenotypic heterogeneity of DBA and the prerequisite to contemplate this illness even in patients with no evident congenital glitches and only a distinctly drop of red cell precursors in the bone marrow. Incorporation of genetic studies assists an accurate diagnosis of patients with DBA, especially in cases with the nonclassical presentation.

**Conclusion**

To the best of our knowledge, this is probably the first case of DBA with RPS19 mutation confirmed by a genetic study in Bangladesh. The case had no physical abnormalities, but the detection of RPS19 mutation confirmed the diagnosis. This case reminds clinicians about DBA as a cause for anemia in infants and helps in further diagnosis of persistent isolated normocytic anemia of infants and children approximately 1 year of age.

**Declaration of Patient Consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that name and initials will not be published, and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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**Figure 3:** Gene analysis showing the mutation of the RPS19 gene
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