A 10 month old girl with fever and cough

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Article Info

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Presentation of Case

Dr. Sifat-E-Moyen (Hematology resident): A 10 month old baby girl, hailing from Cumilla (a city in the Chattogram Division), Bangladesh was brought to the Department of Hematology, Armed Forces Institute of Pathology, Dhaka Cantonment, Dhaka, Bangladesh with the complaints of fever and cough for the last seven days. With these complaints, the parents initially took the child to a local child specialist. The child specialist gave initial supportive medications and advised to investigate with the complete blood count, urine routine and microscopic examination and Widal test.

After 2 days, the parents again took the baby to the child specialist for reviewing the child and to show the investigations result. The child and all investigation's results were reviewed by the child specialist and again advised to do hemoglobin electrophoresis. The parents did the hemoglobin electrophoresis and showed it to the child specialist. After reviewing the hemoglobin electrophoresis, the child specialist referred the case to a pediatric hematologist for further management.

With the medications prescribed by the child specialist of Cumilla, the child got cured but as the child repeatedly got sick after neonatal period, so the parents with anxiety and tension took the child to a pediatric hematologist in Dhaka for better management and treatment.

Pediatric hematologist requested the parents to repeat complete blood count and hemoglobin electrophoresis. Therefore, the parents did these tests in the Armed Forces Institute of Pathology where the concerned hematologist interviewed the parents and examine the baby. On general examination, mild anemia was found, her milestones of development were age proportional, jaundice, clubbing, koilonychia, angular stomatitis were absent and there were no bleeding manifestations. All other parameters were within normal limit. Systemic examinations revealed no abnormality as well as lymphadenopathy and hepatosplenomegaly were absent. Vaccination was done as per the national immunization schedule. The baby was delivered per vaginally normally and she is the only child of the non-consanguineous parents. Feeding history is also good and taking all types of solid and liquid foods. The parents are also apparently healthy, the father is working in an industry and mother is a housewife. Her maternal and paternal uncles and aunts are also apparently healthy. With such findings, I made a provisional diagnosis and for proper diagnosis, I advised to repeat complete blood count, peripheral blood film examination, hemoglobin electrophoresis and reticulocyte count.

Provisional Diagnosis

Anemia under evaluation

Differential Diagnosis

Dr. Mohammad Mizanur Rahman: While constructing the differential diagnosis, I considered the age of the child, recurrent mild illness during the neonatal period and childhood and socio-economic status. As there was no abnormality other than the mild anemia, the following differential diagnoses were considered.

Iron deficiency anemia

Among the global health problems, iron deficiency and iron deficiency anemia are the leading issues encountered in everyday clinical practice. The prevalence of iron deficiency anemia is decreasing gradually but still, it is the top-ranking cause of anemia worldwide and has the significant sequel on the lives of young children and post-menopausal women in both low-income and developed countries.[1] Iron deficiency anemia may occur at any age but in infancy and childhood, it is one of the most common forms of anemia. The greatest prevalence is in between the ages of 6 and 24 months but may occur up to the age of 5 years. Iron deficiency anemia is rather infrequent in school -going children.[2] Iron is an essential and pivotal element that is required in several biologic functions such as respiration, energy production, DNA synthesis and cell proliferation.[3] Human body has developed to preserve the iron in a number of ways such as the recycling of iron after the breakdown of red cells and

Table I

Hematological parameters of the patient and her parents

	Patient	Father	Mother
Hemoglobin (g/dL)	8.5	11.4	12.3
Red blood cell count (x10^{12}/L)	4.0	5.3	4.1
MCV (fL)	58.7	65.3	82.3
MCH (pg)	23.5	25.1	29.7
MCHC (g/dL)	21.1	24.8	31.5
Red cell distribution width–CV	14.8	15.2	14.9
Reticulocyte (%)	1.3	1.5	1.9
Hb A	75.6	75.5	97.3
Hb A2	1.4	1.9	2.7
HbF	-	-	-
Hb Bart's	23.0	22.6	-

retention of iron in the absence of an excretion mechanism. The physiology of iron homeostasis is now well-understood and involves a number of proteins including duodenal ferric reductase, divalent metal transporter 1, ferroportin, hephaestin, transferrin receptor 1 and 2, hemochromatosis factor and hemojuvelin. All such proteins are expressed at highest levels in the first part of the duodenum as well as pH and redox potential of this part of the intestine favors iron absorption. For the absorption of non-heme and heme iron, at least three pathways are known. Absorption of non-heme iron utilizes divalent metal transporter 1 (ferrous valence) and/or mobilferrin-integrin-paraferritin [ferric valence] and heme iron is absorbed through the third pathway utilizing heme oxygenase. Iron absorption depends on the body's need for iron. Active erythropoiesis and/or iron deficiency increases iron absorption but on the other hand, iron overload down-regulates its absorption. Iron absorption involves two notable steps: Mucosal uptake of iron and transfer of this iron from mucosal cell to the lamina propria where it enters into the plasma. Specific iron carrier proteins are required in both steps.[2] Excess free iron are toxic to the human body and participate in Fenton reaction to generate free radicals that cause oxidative cell damage, that's why its absorption is limited to 1–2 mg daily.

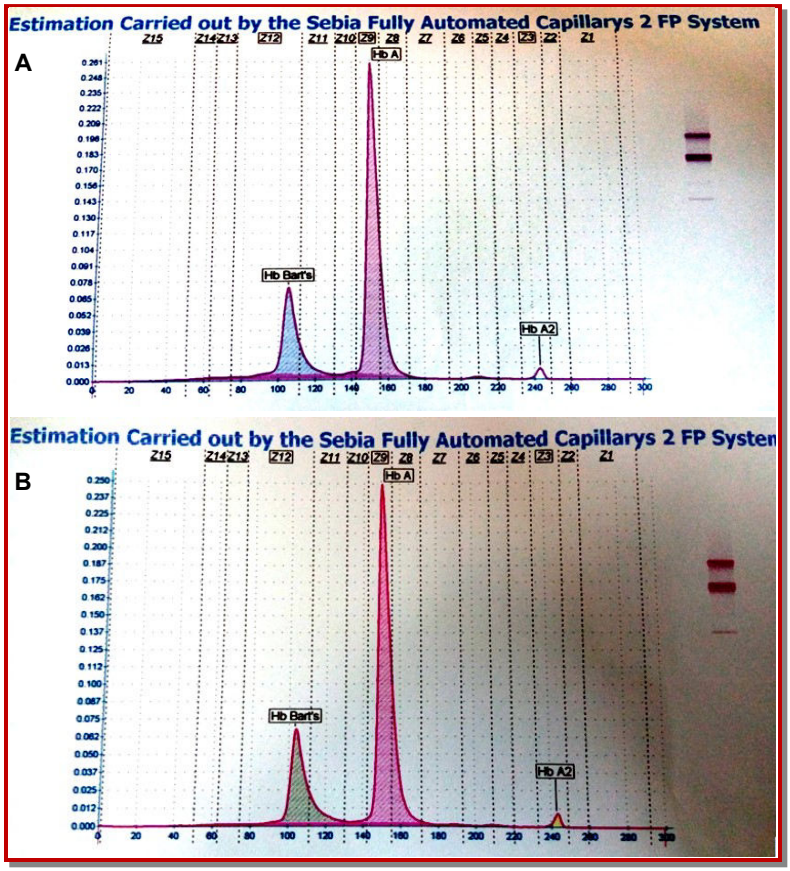

Figure 1: Hemoglobin patterns of the patient (A) and father (B)

The daily iron requirement is about 25 mg and most are provided through recycling by macrophages that phagocytes senescent erythrocytes.[4,5] Both iron absorption and release from the body stores are tightly controlled by a hormone, hepcidin.[3] Hepcidin is a hormone synthesized by the liver and plays a vital role in iron homeostasis. It also acts as an acute phase protein that acclimatizes the variation of plasma iron level. During iron metabolism, hepcidin binds to cell-surface ferroportein, causing its degradation in lysosomes. Therefore, hepcidin inhibits iron absorption, iron release from macrophages and iron transport across the placenta.[6] Raised plasma hepcidin level is found in hemochromatosis, patients with systemic inflammation or infections. Its level is reduced or undetectable in iron deficiency anemia, erythroid hyperplasia and tissue hypoxia. ELISA or mass-spectrometry-based techniques are employed to measure serum or urinary hepcidin.[7]

During the third trimester of pregnancy, 80% of the iron present in the newborn term infant is accumulated from the mother. Therefore, premature infants skipped this accretion of iron and are lack of total body iron stores. Besides this, several maternal factors such as anemia, maternal hypertension with intrauterine growth retardation and gestational diabetes also contribute to low iron stores in both term and pre-term infants.[8] The Institute of Medicine recommended the daily iron allowance for 7 to 12 months' completed age is 11 mg/day. From birth to 6 months' completed age, iron requirement is less in comparison to infants after 6 months' age because of rapid growth and increased demand.[9] Anemia is labeled for both male and female children aged 12 months through 35 months, when hemoglobin level is less than 11 g/dL as per the guidelines of US National Health and Nutrition Survey 1999–2002.

When the amount of iron in the body is not sufficient to preserve normal physiologic function, then the state is defined as iron deficiency. Iron deficiency may or may not be accompanied by iron deficiency anemia. Anemia associated with iron deficiency is called iron deficiency anemia.[10] At present, there is no national statistics regarding the incidence of iron deficiency and iron deficiency anemia among infants before the age of 12 months. According to the Institute of Medicine report, the prevalence of iron deficiency at 6 months of age is 4% and increased to 12% at 12 months of age.[11] Anemia is one of the most important pointers of iron deficiency or iron deficiency anemia. Nevertheless, without anemia, the iron deficient state may develop and in such situation, tissues may suffer from hypoxia. Iron deficiency anemia develops in three stages: Pre-latent stage which is marked by reduced iron stores due to less intake of iron. At this stage hemoglobin level may remain normal for a while indicating the presence of iron deficiency in the absence of anemia. During this time, serum iron is normal but only plasma ferritin level and plasma transferrin saturation are reduced. This is followed by a latent stage where iron stores are depleted but still blood hemoglobin level is normal. Biochemical parameters such as plasma ferritin level and plasma transferrin saturation are reduced, total iron binding capacity is increased. The next stage is the development of iron deficiency anemia which is marked by low hemoglobin level and negative iron balance indicated by low plasma iron, plasma transferrin saturation and increased total iron binding capacity. Iron deficiency anemia is the most common nutritional deficiency anemia in infancy and in adolescents who have menstruation but those having increased growth rate and cannot meet their requirements are at increased risk of developing iron deficiency anemia.[2, 12] Maternal iron crossing through the placenta is the sole source of iron for the fetus in utero. Full-term fetus contains iron in about 75 mg/kg body weight in the last trimester of pregnancy and such iron stores are adequate enough for erythropoiesis in the initial 6 months of life. In children, the etiologies of iron deficiency anemia are insufficient intake with rapid growth, low birth weight and gastrointestinal losses due to exorbitant consumption of cow's milk. In low birth weight infants, the iron stores are exhausted earlier as they are smaller. In such cases, the risk of iron deficiency anemia can be reduced by delayed clumping of the umbilical cord after delivery.[13] Iron content in breast milk is highest in the first month and then reduces gradually in the ensuing periods and reaches to 0.3 mg/L in about fifth month.[14] However, iron content in breast milk cannot be increased by providing iron-rich foods to the mother but breast milk's iron absorption is high (50%) in comparison to food iron. Also, absorption of iron in breast milk is reduced if given with other foods to infants. Therefore, though absorption of iron in breast milk is high in comparison to the growth of the child, it is inadequate. During the first 6 months, infants use the iron from their iron stores and then additional iron is required from foods for normal erythropoiesis in the subsequent period.[15] Solid foods rich in iron should be given after 6 months to maintain normal erythropoiesis. Foods such as meat, fish, eggs and green leafy vegetables are rich in iron and such foods should be given to infants after the age of 6 months to meet the iron requirement. Excessive consumption of cow's milk by the infants may cause chronic blood loss due to heat-sensitive protein present in the cow's milk and presence of calcium as well as casein phosphopeptides in cow's milk impairs iron absorption resulting in iron deficiency anemia.[12] However, a commonly accepted generality is that dietary deficiency alone is not sufficient to cause a clinically significant iron deficiency, therefore attempt should always be made to find out a source of blood loss as a cause of iron deficiency after the age of 1 year.[16] In general, anemia produces non-specific pallor of the mucous membrane. Specific to iron deficiency anemia are epithelial tissue abnormalities such as esophageal webbing, koilonychias, glossitis, angular stomatitis and gastric atrophy. The relationship between iron deficiency anemia and such epithelial changes is not clear.[17] History and physical examination may elicit the etiology of iron deficiency anemia but laboratory investigations such as complete blood count, peripheral blood film study, serum iron, total iron binding capacity and serum ferritin are usually sufficient to establish an uncomplicated iron deficiency anemia. Evaluation of hemoglobin electrophoresis, measurement of hemoglobin A_2, red cell distribution width (RDW) and hemoglobin content of reticulocyte are helpful to differentiate from other microcytic anemia.[18] Age of the patient and presence of anemia favors the diagnosis of iron deficiency anemia in this child but age proportional development, the absence of features such as angular stomatitis, koilonychia and history of taking iron-rich foods are the points against the diagnosis of iron deficiency anemia.

Acquired hemolytic anemia

Increased red cell destruction may result from either inherited or acquired disorders. Hemolytic anemia results when compensatory increased erythropoiesis by the bone marrow is unable to recompense increased red cell destruction. The common causes of acquired hemolytic anemia from infancy to toddlerhood are: Antibody-mediated hemolysis, hemolytic uremic syndrome, drug-induced hemolysis and disseminated intravascular hemolysis.[19] Antibody-mediated hemolysis is the most important etiology of acquired hemolytic anemia. Two types of antibodies such as auto-antibodies and alloantibodies are usually responsible for the development of antibody-mediated hemolytic anemia. Autoantibodies are produced by

the patient's own immune system and directed against epitopes of the patient's own red cell antigens.[20] Characteristic features of the auto-antibody determine the type of AIHA. Ig class of the autoantibody and thermal features i.e., the optimal temperature of the reaction at which autoantibody reacts with autologous red cells divided AIHA into three types such as warm AIHA, cold hemagglutinin disease (CHAD) and mixed forms.[21] AIHA may also be classified as primary (idiopathic), in which red cell destruction occurs in the absence of any other coexisting disorder, or as a secondary form in which hemolysis occurs secondary to an underlying disease. Warm AIHA is usually idiopathic or secondary to other autoimmune disorders. Acute CHAD are mainly secondary to infections while chronic CHAD is predominantly associated with lymphoproliferative or neoplastic diseases.[22] Genetic predisposition and environmental factors are important in the pathogenesis of autoimmunity. Several autoimmune diseases have HLA association but still no HLA linkage has been identified in AIHA. Mechanisms involving the breakdown of central and peripheral tolerance in the pathogenesis of AIHA are substantially unknown.[23] Another mechanism of autoimmunity is molecular mimicry involving self-antigens and foreign pathogens. Most probably this mechanism is mainly responsible in the pathogenesis of AIHA secondary to various bacterial, mycoplasma or virus infections likely due to the presence of common antigenic epitopes on red cell proteins or carbohydrates. Polyclonal lymphocyte activation caused by several viral infections and the emergence of "forbidden clones" in lymphoproliferative disorders are more other mechanisms of development of AIHA.[24] Another form of AIHA is drug-induced AIHA where environmental agents or drugs take part in the development of neo-antigens which stimulate antibody production against red cells culminating the destruction of RBCs until the autoimmune response is destroyed and removed from the body.[25] Autoantibodies against red cells have also been detected in about 30% of individuals who previously received red cell transfusion for any reason where transfused red cells elicit the generation of autoantibodies.[26] Many other hypotheses about autoimmune/inflammatory diseases have come to the attention in the recent years but the most agreed mechanism is the loss of supperssor mechanisms and breakdown or alteration of self-tolerance.[23]

IgG is the most common antibody formed against red cells in AIHA and causes extravascular hemolysis through antibody-dependent cell mediated cytotoxicity (ADCC) in the reticuloendothelial system especially in the spleen. Red cell breakdown is influenced by the subclass of IgG; IgG1 together with IgG3 shortens the half-life more effectively than IgG2 and IgG4. These autoantibodies are directed against the epitopes of Rh system and react at 37°C, liable for the warm type of AIHA. Occasionally IgA auto-antibodies are generated and usually associated with IgG, rarely operative as the single etiology of AIHA.[27] IgM autoantibodies are also formed in this disease causing mainly intravascular hemolysis as these antibodies are pentameric, capable of fixing complement more efficiently but extravascular hemolysis mainly in the liver occurs when it is C3d mediated. Autoantibodies of these type are directed against I/i blood group system and react optimally at 4°C, categorizing it as a cold form of AIHA. Nonetheless, the range of the thermal amplitude of IgM autoantibodies is 0–34°C. If the thermal amplitude of IgM autoantibodies is close to physiological temperature (Warm IgM), then the severe form of AIHA ensures in which cases the mortality rate may be 18–25%.[25] Intravascular hemolysis in AIHA is more detrimental and severe than extravascular hemolysis as the rate of RBC destruction is 200 mL per hour in case of intravascular hemolysis which is 10 times more than extravascular hemolysis.[22]

AIHA may occur after allogeneic hemopoietic stem cell transplantation. The 3 year cumulative incidence in such a population is about 4.4%.[28, 29] AIHA is rare with an estimated incidence of 0.2 per million individuals below 20 years of age.[30] Peak incidence is found in less than four years of age.[31] The mortality rate is approximately 11%.[32] Most patients with AIHA presents their clinical manifestation insidiously but some patients have severe acute symptoms. Symptoms may range from asymptomatic compensated reticulocytosis with mild hyperbilirubinemia to acute fulminant hemolysis leading to jaundice, hepatosplenomegaly, tachycardia and angina. They are also determined by the causative factor responsible for AIHA and degree of hemolysis which again depends on the type of autoantibody. IgM warm reactive AIHA presents with life-threatening acute intravascular hemolysis with high mortality rate compared to patients with cold type AIHA where the symptoms are usually mild.[33] Bone marrow compensatory ability also determine the degree and severity of anemia. Those patients with AIHA who respond their anemia by reticulocytosis usually presents with less severe clinical symptoms than patients who do not respond by reticulocytosis rather develop reticulocytopenia. Such reticulocytopenia are observed in 20% of adult and 38% of children with AIHA.[34] Laboratory diagnosis depends on the detection of autoantibody either by direct antiglobulin test (DAT) or column agglutination or flow cytometry. Positive DAT is found with anti-IgG (usually in warm AIHA) and/or anti-C3d (usually in cold AIHA) antisera. About 10% of all cases of AIHA due to anti – IgA, low-affinity IgG or RBC-bound IgG below the detection threshold of the test may yield false negative DAT. Therefore, not all

AIHA cases show positive DAT results. To diagnose the AIHA with false negative DAT, more sensitive reagents such as mono-specific anti-IgA antisera/ low ionic strength solutions (LISS) and techniques such as column agglutination test or flow cytometry can be considered. Supportive laboratory investigations for AIHA may include estimation of serum lactate dehydrogenase (LDH), reticulocyte count and examination of blood film which shows a significant number of spherocytes.[35] In addition to traditional treatment by corticosteroids and immunosuppressive drugs as first-line therapy or splenectomy as second-line therapy, recently few emerging treatment modalities are considered which include administration of rituximab (anti-CD20 monoclonal antibody), erythropoiesis-stimulating agents, other immunosuppressive agents such as cyclosporine A (CsA), danazol (synthetic anabolic steroid) and showed meaningful success. These therapies especially rituximab can be applied in patients who are refractory to corticosteroid/splenectomy or relapse after splenectomy. Corticosteroid as the first-line treatment for patients with warm AIHA provides a response rate of 70–85%. Rest of the patients still needs second-line therapy. On the other hand, due to the presence of co-existence morbid conditions such as diabetes, uncontrolled hypertension, obesity, osteoporosis, peptic ulcer or children where treatment with corticosteroid is relatively contraindicated and in such cases second-line therapy i.e., splenectomy is thought to be the most effective and produce high response rates up to 82%. Rituximab (so-called "medical splenectomy") is another effective second-line therapy, providing high initial response rates up to 85% and prolonged disease-free survivals of 55% up to 2 years with reduced adverse reactions.[36] In this patient, age, presence of anemia and possible viral respiratory tract infections favors the diagnosis of AIHA but chronic as well as less severe anemia for a longer duration are the points against the diagnosis of AIHA.

Concurrent infections

Anemia is one of the most noticeable features of infection. The severity of anemia in infection ranges from a mild, asymptomatic form to an acute life-threatening complication of infection. Anemia of chronic disease is defined as the anemia seen in patients with chronic infections. In addition to infections, anemia is also seen in other chronic diseases including cancer and chronic inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease and renal failure. This anemia is referred to as anemia of inflammation. In acute inflammation, anemia is directly related to bone marrow suppression and acute hemolysis but in chronic inflammation, several factors are involved in the pathogenesis of anemia. Inflammatory cytokines, hepcidin and bacterial toxins play a significant role. The anemia in chronic inflammation is usually normocytic normochromic and refractory to therapy with hematinics.[37] The dominant underlying mechanism is impaired response of the bone marrow to erythropoietin (EPO) due to disturbance of iron metabolism which is mediated by a recently discovered peptide hormone called hepcidin.[38-40] Hepcidin is synthesized in the liver, present in plasma and excreted through urine and functions as a major regulatory hormone for iron transport.[41] Hepcidin controls the activity of ferroportin 1, a transmembrane domain protein present on the surface of enterocytes, hepatocytes and macrphages. The movement of iron through these cells' wall is regulated by ferroportein 1. By binding with ferroportein 1, hepcidin inhibits the function of ferroportein 1 and thereby prevents the transportation and exit of iron through the cell membrane. As a consequence, mobilization from hepatocytes and release of sequestered iron from macrophages is impaired and result in a decrease in the availability of iron for erythropoiesis. In inflammatory states, hepcidin synthesis is increased mainly by the cytokine IL- 6 and thus preventing the body from more iron absorption and inhibiting iron transportation. Hence, the response of anemia of inflammation to EPO and EPO stimulating agents is suboptimal.[42] Anemia of inflammation is an anemia that develops within 1–2 months of the onset of illness. Anemia persists without increasing in severity. Chronic pulmonary infections such as pulmonary tuberculosis, pulmonary abscess, subacute bacterial endocarditis, pelvic and urologic infections, osteomyelitis, chronic fungal disease, meningitis and human immunodeficiency virus infections are the predominant clinical conditions which predispose to the development of anemia of inflammation.[43-45] The anemia is usually mild and morphologically either normocytic normochromic or microcytic hypochromic anemia. The clinical features are those of the underlying disorders.[46] Anemia of inflammation is diagnosed by the presence of anemia in association with inflammatory or infectious disease. Laboratory investigations revealed low serum iron, decreased soluble transferrin receptors and reduced transferrin saturation with iron. Reticuloendothelial iron stores are increased depicted by Perl stain of bone marrow sample and increased serum ferritin.[47] Anemia of inflammation is usually treated by alleviating the underlying disorders. Iron therapy is unsatisfactory unless there is a concomitant iron deficiency. Administration of EPO may provide mild or suboptimal response.[37]

This girl has been suffering from upper respiratory tract infection and fever which favor the diagnosis of anemia of inflammation but presence of weakness from toddlerhood; less playful and persistent mild anemia are the points against the diagnosis of anemia of inflammation.

Congenital hemolytic anemia

Premature destruction of red cells is called hemolysis. Mild hemolysis is not manifested as anemia but if the erythrocyte loss is more than the compensatory activity of the bone marrow then only anemia develops which may be of varying severity.[48] There are a wide range of causes, both congenital and acquired, responsible for hemolysis.[49, 50] Red cell membrane, enzyme and hemoglobin defects are the main etiologies of congenital hemolytic anemia. In congenital hemolytic anemia the genes that master the production of red cells are defective. On the basis of the affected defective genes, different types of congenital hemolytic anemia develop. In each type, abnormal erythrocytes are produced in the body and destroyed prematurely.[51] Hemolytic anemia in children should be suspected in any child with anemia, jaundice and splenomegaly. In severe cases, skeletal deformities especially of the skull and facial bones due to erythroid hyperplasia and cardiomegaly due to high cardiac out may be demonstrated by radiographs. A few patients may also develop pigment gallstones after the first decade of life. Children with mild hemolytic anemia may have nearly normal hemoglobin levels if reticulocyte production increases sufficiently for the increased rate of RBC destruction. However, concurrent infectious illness may aggravate the anemia during which mild hemolytic cases can be identified. Hemoglobin level falls during infectious illness due to oxidative stress and depression of bone marrow activity.[52] Examination of blood film is the mainstay in the evaluation of hemolytic anemia and in some cases, the morphology of red cells may point to the diagnosis of a typical congenital hemolytic anemia. However, it is also important to keep in mind that similar morpho-logical features may be found in acquired hemolytic anemia such as spherocytes which are found not only in hereditary spherocytosis but also in immune hemolytic anemias, ABO incompatibility in neonates and other forms of oxidant damage to the red cell membrane.[53] After a critical evaluation of blood film, further investigations such as reticulocyte count, hemoglobin electrophoresis, estimation of HbA_2, G6PD estimation, osmotic fragility test, hemoglobin insolubility test, sickling test and Coomb's test may be advised to confirm the diagnosis.[54]

Presence of respiratory tract infections, fever and absence of splenomegaly disfavor the diagnosis of congenital hemolytic anemia but the presence of weakness from toddlerhood, less playful, persistent mild anemia after recovery from respiratory tract infection and age of the patient favor the diagnosis of congenital hemolytic anemia.

Dr. Moin's Diagnosis

Congenital hemolytic anemia

Dr. Moin: Keeping in mind the diagnosis of congenital hemolytic anemia, especially thalassemia and hemoglobinopathies in this girl, I advised the parents to do some laboratory investigation of the whole family members (patient, his father and mother) such as complete blood count, blood film examination, reticulocyte count and capillary hemoglobin electrophoresis. The results of all investigations are shown in Table I. Capillary hemoglobin electrophoresis showed a most remarkable finding and revealed 23.0 and 22.6% Hb Bart's in zone 12 of the electrophoretic graph of the patient and father respectively which is shown in Figure 1. After getting such finding I advised to do HbH inclusion test to differentiate from HbH. But the test turned out as negative in both the cases which almost confirms that it is Hb Bart's not HbH.

Discussion

Dr. Rahman: Recent introduction of capillary hemoglobin electrophoresis in the diagnosis of congenital hemolytic anemia facilitates the precise detection of different types of abnormal hemoglobin than by gel hemoglobin electrophoresis used in the recent past. By exploiting this capillary hemoglobin electrophoresis method, it enables us to detect Hb Bart's in zone 12 of electrophoretic graph in these two patients which is absolutely distinct from hemoglobin H which appears in zone 15 of the graph. Hb Bart's is detected in a small amount of 1–2% in newborn infants with alpha thalassemia trait and 5–6% in alpha thalassemia carrier.[55]

Genetically the different types of alpha thalassemia occur on the basis of the number of alpha thalassemia gene(s) a person is missing. Missing one alpha thalassemia gene (αα/α -) indicates silent alpha thalassemia carriers or two alpha thalassemia genes (α-/α– or αα/- -) indicates alpha thalassaemia trait. Individuals with either silent alpha thalassemia carrier or alpha thalassemia trait are asymptomatic with normal laboratory features. On the other hand, the number of dysfunctional genes is reflected by the percentage of Hb Bart's seen on the newborn screen by capillary hemoglobin electrophoresis. Presence of less than 25% Hb Bart's indicates loss of one (silent carrier) or two (alpha thalassaemia trait) genes. Genetically deletion or dysfunction of three of the four alpha globin genes manifests as HbH disease and deletion or dysfunction of all of the four alpha globin genes leads to Hb Bart's hydrops foetalis where the percentage of Hb Bart's is 80% or more and the affected infants are either born dead or die within a few hours of birth.[55]

Alpha thalassemia is caused by mutations or deletions affecting either one or more α–globin genes, leading to decreased or absent α–globin chain production from the affected gene(s).[56] Insignificant hematologic findings are usually the

result of deletion or inactivation of only one α–globin gene. When two α–globin genes deleted or inactivated, either both on the same chromosome 16 (*in cis*) or on one each of the two chromosomes 16 (*in trans*), the affected person is well but has borderline anemia, as well as microcytic hypochromic red blood cells.[57]

Deletions removing one α–globin gene are results of misalignment crossovers during meiosis. This single α–globin gene deletion is known as silent alpha thalassemia carrier. In silent alpha thalassemia, the remaining normal three α–globin genes can compensate nearly completely and only a low level of Hb Bart's is detected. These individuals are likely to be clinically and hematologically normal.[56] Silent alpha thalassemia carrier is very common and found in 30% of African Americans and up to 60 to 80% of people living in parts of Saudi Arabia, India, Thailand and Papua New Guinea.[58]

Alpha thalassemia trait typically results from the dysfunction of two α–globin genes, either due to gene deletions or a specific change in α–globin gene that produces elongated alpha globin. It produces moderate level of Hb Bart's. These conditions are usually benign although mild to moderate microcytic anemia is common. The patient in the present case presentation and his father had also moderate level of Hb Bart's (23.0 and 22.6%) and mild to moderate anemia (8.5 and 11.4 g/dL) with microcytic hypochromic red cells. Therefore, the amount of Hb Bart's in the peripheral blood may be used to predict the genotype of α–thalassemia.[59]

Dr. Lutfunnahar Khan: What is the cut off value of Hb Bart's for diagnosing alpha thalassemia trait or carrier?

Dr. Moin: Two types of information regarding the quantity of Hb Bart's are available. However, if the Hb Bart's level is less than 25%, the condition is likely to be either alpha thalassemia carrier or alpha thalassemia trait. Here I want to mention that in most published literates such cut off value of Hb Bart's is given for neonates but here we found such amount of Hb Bart's both in the adult patient and his 10-month old daughter.

Dr. Shourov: Here the HbA_2 level is low. What are the conditions where HbA_2 level is low?

Dr. Monirul Islam: Low HbA_2 level is found mainly in iron deficiency anemia and alpha thalassemia. Delta beta thalassemia and hereditary persistence of fetal hemoglobin are other conditions which may cause low HbA_2.

Dr. Zannat: Did low HbA_2 level in this case support the diagnosis?

Dr. Shahidul Islam: In this case low HbA_2 level along with the presence of moderate amount of Hb Bart's strongly suggest the presence of either alpha thalassemia carrier or alpha thalassemia trait.

Dr. Imana: Did you exclude iron deficiency anemia in this case as low HbA_2 indicate the possibility of iron deficiency anemia?

Dr. Monwar Tark: In this patient blood film showed no specific features such as elongated and pencil-shaped cells which are characteristic of iron deficiency anemia. Moreover, serum iron profile was done and it was within the reference limit.

Dr. Durdana Mahin: What is the prognosis of this patient and how will follow up this case?

Dr. Rahman: The patient will remain asymptomatic unless affected by any conditions which suppress the bone marrow. She should be followed-up by doing hemoglobin level monthly and serum ferritin six monthly.

Dr. Arif Ahmed Khan: How will you confirm the diagnosis and have you done it?

Dr. Moin: By doing alpha thalassemia mutation study, we can confirm the diagnosis. However, family screening and pedigree study (Figure 2) showed the presence of Hb Bart's in her father and these findings almost confirm the diagnosis of either alpha thalassemia carrier or trait. I have not done the molecular diagnosis as it is not available in our country at present.

Dr. Elias: By quantifying Hb Bart's by capillary hemoglobin electrophoresis, how can we diagnose different types of alpha thalassemia?

Dr. Rahman: Presence of Hb Bart's less than 25% indicates the presumptive diagnosis of either alpha thalassemia carrier or alpha thalassemia trait. Hb Bart's ≥ 25% indicates the diagnosis of HbH disease in neonates.[58] In Hb Bart's hydrops fetalis, its amount is approximately 80–85% which is incompatible with life.[55] Here, I like to mention that there is no conclusive data regarding the amount of Hb Bart's when it is in between 25–80%, then what should be nature of the condition. Therefore we recommend studying and analyzing alpha thalassemia gene in more cases to resolve the queries when the amount of Hb Bart's is in between 25 to 80% as no conclusive data is available in the

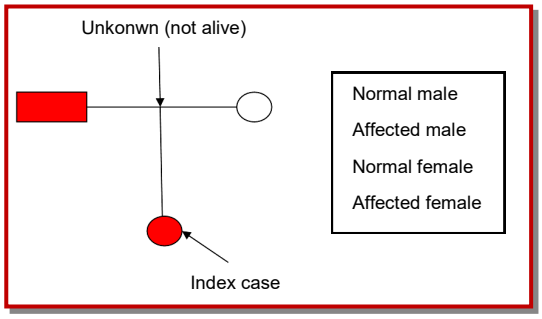

Figure 2: Pedigree chart of the patient

literature as well as published recognized books about the presence of Hb Bart's in the mentioned range.

Dr. Debashish Saha: What type of advice should be given to the parents?

Dr. Rahman: Genetic counseling is most important advice in such inherited disorders as such counseling reduces the reproductive risk of the family.

Final Diagnosis

Hemoglobin Bart's (silent alpha thalassemia carrier/alpha thalassemia trait)

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