A 65–year–old male with moderate anemia, low–grade fever and weight loss

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Article Info

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Presentation of Case

Dr. Monirul Islam (Hematology Resident): A 65-year-old male hailing from Lolua, Tangail north bordering district of Dhaka, Bangladesh, reported to the Department of Hematology, Armed Forces Institute of Pathology on 28th January 2017 with the complaints of marked weight loss, generalized weakness, low-grade fever and anorexia. He was referred from Kurmitola General Hospital, Dhaka, Bangladesh for bone marrow aspiration and examination.

Before aspirating the bone marrow, a detailed history was taken which revealed that he had been suffering from the above mentioned symptoms for the last 10 months. During this time, he reported to the local physicians who investigated with routine laboratory tests such as complete blood count, peripheral blood film examination, blood grouping, serum iron profile, HBsAg, anti-HCV, VDRL, urine examination, random blood sugar, immunochromatographic test for malaria and kala-azar and serum creatinine.

Among these, the notable findings were low hemoglobin level ranging from 6 to 9.5 g/dL in different time, very high erythrocyte sedimentation rate of 100 mm at the end of 1st hour, combined deficiency anemia on peripheral blood film examination.

On the basis of these findings, the patient was transfused with seven units of packed red cell and given symptomatic treatment. As the patient was not either responding or improving, so he was referred to a tertiary level hospital, Dhaka. The patient then reported to Kurmitola General Hospital, Dhaka on 20th October, 2016. The duty medical officer attended the patient and took detailed history, performed physical examination, advised laboratory as well as radiological investigations. In the next day, the medical specialist in charge of the ward was briefed by the indoor medical officer during morning round that the patient was found to have moderate to severe anemia on general examination and moderate splenomegaly on examination of the gastrointestinal system. No other abnormality was detected on physical examination. Then the medical specialist advised to sent the patient to Armed Forces Institute of Pathology, Dhaka Cantonment, Bangladesh for complete blood count, blood film examination, hemoglobin electrophoresis, reticulocyte count, Coomb's test, random blood sugar, serum iron and protein profile, serum lactate dehydrogenase, liver and renal function tests, immunochromatographic test for malaria and dengue, HIV, anti-HAV, anti-HEV, anti-HCV, HBsAg, Widal test and also advised to do X-ray chest (P/A view), ultrasonography of the whole abdomen and endoscopy of upper gastrointestinal tract. After five days, results of all investigations were available which is shown in Table I. During endoscopy, gastric polyp was found and polypectomy was done and sent for histopathological examination which revealed antral polyp. After analyzing the results of all other investigations, the patient was diagnosed as hemoglobin E disease with early gastro-esophageal varices and gastric polyp. As the hemoglobin level was very low (5.8 g/dL), therefore the patient was transfused with three units of packed red cells and prescribed to take tablet folic acid (5 mg) daily for life long. The patient was discharged on 27th October 2016.

With this treatment his weakness had improved reasonably but he was persisting with the symptoms of fever, significant weight loss (about 20 kg in the last 10 months) and anorexia. So, he again reported to the same hospital on 24th January 2017 and got admitted, made a provisional diagnosis and referred to hematologist, Armed Forces Institute of Pathology, Dhaka Cantonment for bone marrow aspiration and examination.

Provisional Diagnosis

Hemoglobin E disease with fever of unknown origin

Differential Diagnosis

Dr. Mohammad Mizanur Rahman (Hematologist): Considering the presence of Hemoglobin E disease with fever of unknown origin,

Table I

Laboratory data[a]

Variable	Patients' data	Reference range, Male ¶
Hemoglobin (g/dL)	5.8	13.0–17.0
Erythrocyte sedimentation rate (mm in 1st hour)	120	0–12
Hematocrit (L/L)	0.16	0.40–0.50
Red blood cell count (x10^{12}/L)	2.63	4.5–5.5
Mean corpuscular volume (fL)	66	83–101
Mean corpuscular hemoglobin (pg)	22.10	27–32
Mean corpuscular hemoglobin concentration (g/dL)	35.4	31.5–34.5
Red cell distribution width (%)	18.20	11.6–14.0
White cell count (x10^9/L)	1.70	4.0–10.0
Differential count (%)		
Neutrophil	59	40–80
Lymphocyte	35	20–40
Monocyte	05	2–10
Eosinophil	01	1–6
Basophil	00	0.02–01
Atypical cells	00	0
Platelet count (x10^9/L)	210	150–410
Peripheral blood film	Bicytopenia	
Hemoglobin electrophoresis		
Hemoglobin F (%)	6.4	
Hemoglobin E (%)	87.3	
Hemoglobin A_2 (%)	6.3	
Reticulocyte count (%)	3.0	
Serum lactate dehydrogenase (U/L)	515	225–480
Liver function tests	Normal	
Renal function tests	Normal	
Random blood sugar (mmol/L)	5.4	
Serum ferritin (ng/mL)	1744.0	28.0–397.0
Serum protein profile		
Total protein (mg/dL)	100	
Albumin (mg/dL)	19	
Globulin (mg/dL)	81	
A:G ratio	0.2:1	
Coomb's test (direct and indirect)	Negative	

¶ Reference values are affected by many variables, including the demographics and the laboratory methods used. The ranges used at Armed Forces Institute of Pathology (AFIP) are for adults who are not pregnant and do not have medical conditions that could affect the results. They may therefore not be appropriate for all patients

significant weight loss, massive splenomegaly and persistence of generalized weakness despite the transfusion of sufficient amount of packed red cells, I thought about the following differential diagnosis and at the same time decided to perform bone marrow study on 28th January 2017.

Hemoglobin E disease with chronic myeloid leukemia

Hemoglobin E disease is one of the most common type of hemoglobinopathies which comprise an obligatory etiological factor for anemias in childhood and adult.[1] The defect in hemoglobin E disease lies in the beta-globin chain of hemoglobin where a single point mutation leads to a change in the amino acid from glutamic acid to lysine at the position 26 (E26K).[2]

According to the WHO statistics, the common hemoglobinopathies are hemoglobin S, hemoglobin C, hemoglobin D-Punjub and hemoglobin E. Among thalassemia, beta-thalassemia occurs in a malleable frequency throughout the world.[3]

As per the order of discovery, hemoglobin E is the 4th abnormal hemoglobin variant after hemoglobin S, hemoglobin C and hemoglobin D-Punjub. Hemoglobin E was first discovered by Itano, Bergren and Sturgeon in 1954 in a Guatemalan origin with Spanish and Hindu ancestry.[4]

A few studies regarding hemoglobinopathies and thalassaemias had been conducted in Bangladesh. Among these, a study on Bangladeshi school children and tribal school children encompassing six divisions of this country revealed the prevalence of hemoglobin E trait 16.5% in Rajshahi, 8.1% in Barishal, 5.2% in Dhaka, 4.2% in Sylhet and 2.9% in Chattogram division. The overall prevalence of hemoglobin E trait throughout Bangladesh is 6.1%. On the other hand, the prevalence of hemoglobin E trait in tribal school children 41.7%.[5] In Southeast Asia, hemoglobin E disorders are the most common hemoglobinopathies, estimating 30 million persons suffering from either heterozygous or homozygous hemoglobin E disorders. In the mainland of Southeast Asia, around 80% individuals are carrying hemoglobin E gene. Like Bangladesh, study in India showed highest prevalence of hemoglobin E disorders in Assam and neighbouring states.[1] Hemoglobin E may be either heterozygous (genotype AE), homozygous (genotype EE) or compound heterozygous state such as hemoglobin E-beta thalassaemia (genotype E/β thal) or sickle cell-hemoglobin E disease (genotype SE).[6] Expression of β gene is affected in hemoglobin E disorders as a result of βE mutation which produce an alternate splicing site in the mRNA at codons 25–27 of the β-globin gene. As a consequence, there is mild deficiency of normal mRNA, resulting in the creation of abnormal β mRNA. Therefore, in various hemoglobin E disorders there is reduced rate of synthesis of βE. Because of this pathology, heterozygous, homozygous and compound heterozygous of hemoglobin E show some β thalassemic features. For this reason, hemoglobin E may be regarded as β^+thalassemic hemoglobinopathy.[7] Hemoglobin E trait is a heterozygous condition inheriting one normal adult hemoglobin β

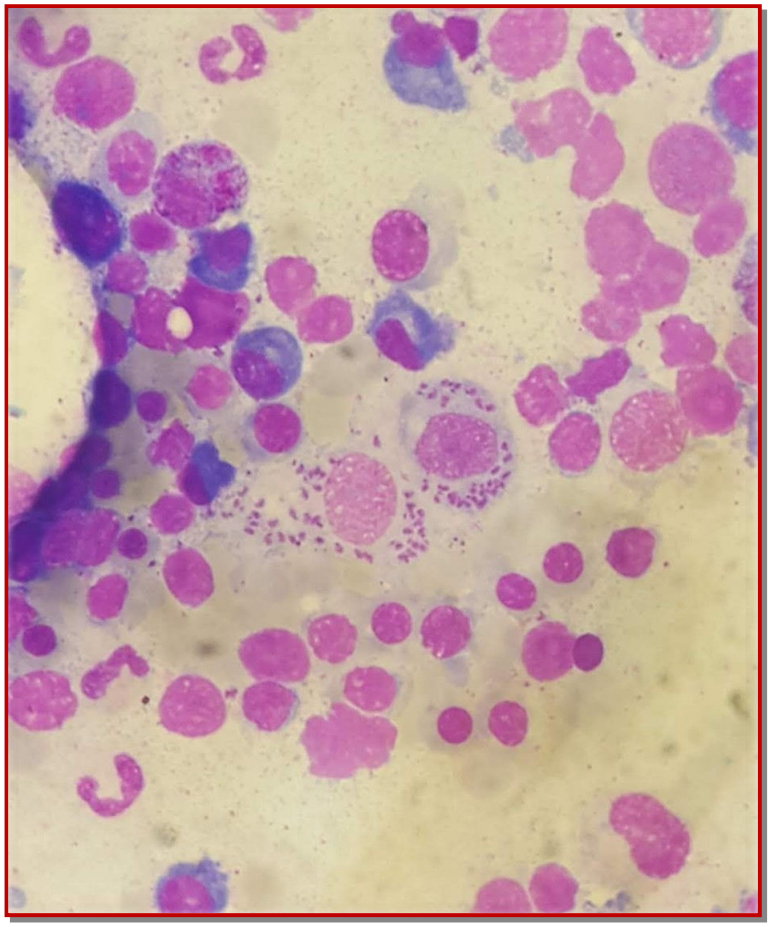

Figure 1: Bone marrow showing intracellular LD bodies

Figure 2: Immunochromatographic test for kala-azar showing positive result

gene from one parent and one variant hemoglobin E β gene from another parent. Clinically patients with hemoglobin E trait are usually asymptomatic and do not produce any health problem.[8] In the laboratory, hemoglobin level is usually found normal or slightly reduced and there is decreased red cell indices notably mean corpuscular volume and mean corpuscular hemoglobin. Blood film usually shows mild hypochromia, microcytosis, target cells and schistocytes. Capillary hemoglobin electrophoresis demonstrates the hemoglobin E. In hemoglobin E trait, the abnormal hemoglobin usually comprises 33% or less of total hemoglobin A. Individuals in whom capillary hemoglobin electrophoresis showed hemoglobin E less than 30% of total hemoglobin almost always have co-existing alpha thalassemia trait.[9] Hemoglobin E disease is characterized by the co-existence of βE alleles (homozygous state EE). At birth, babies homozygous for hemoglobin E allele are asymptomatic due to the presence of fetal hemoglobin. Usually hemoglobin F starts to disappear after one month of birth and the amount of hemoglobin E increases, so the individuals start to develop symptoms of anemia and mild splenomegaly.[10] Diagnosis of hemoglobin E disease may be suspected from the findings of microcytosis, hypochromia, target cells and schistocytes in the blood film with or without anemia. Capillary hemoglobin electrophoresis demonstrates hemoglobin E in about 90-95%.[11] Treatments of hemoglobin E disorders depend on the nature and severity of symptoms. It usually involves regular follow up of hemoglobin level and folic acid supplement. In a few cases, especially compound heterozygous disorders, blood transfusion may be required to maintain the hemoglobin level of about 10 g/dL and this can be achieved by transfusion of concentrated red cells at an interval of 4–6 weeks.[12] Though hemoglobin E is an inherited disorder but it can be effectively prevented by providing genetic counseling to the affected individuals. Besides this, detection of persons carrying gene for inherited hemoglobin disorders can be made by screening program through measurement of red cell mean corpuscular volume using an electronic cell counter.[13]

Chronic myeloid leukemia is one type of chronic myeloproliferative neoplasm and clonal stem cell disorder characterized by markedly increased proliferation of granulocyte cell line without the loss of capacity to differentiate.[14] It is one of the few malignancies recognized to be caused by a single, specific genetic mutation. In more than 90% of chronic myeloid leukemia cases, there is a balanced reciprocal translocation between long arm of chromosome 9 and chromosome 22, resulting in the formation of an abnormal 22 chromosome called Philadelphia chromosome named after the city of discovery.[15] Such translocation results in the formation of BCR-ABL chimeric gene which encodes a constitutively active oncogenic BCR-ABL tyrosine kinase. Through an unknown process, this oncogenic protein having strong tyrosine kinase activity leads to the development of chronic myeloid leukemia phenotype.[16] In a patient with classical clinical features of chronic myeloid leukemia, the presence of BCR-ABL rearrangement is diagnostic of chronic myeloid leukemia.[17]

The etiology or inciting factor for the development of chronic myeloid leukemia is unknown but retrospective analysis among the survivors of atomic bomb blast in Hiroshima and Nagasaki showed increased incidence of chronic myeloid leukemia indicating exposure to ionizing radiation may be an initiating factor. Other agents, such as benzene are other possible causes.[18] Regarding the prevalence of chronic myeloid leukemia in Bangladesh, no authentic data is available but in Western countries it constitutes about 15 to 25% of all adult leukaemia.[14] The clinical presentation of chronic myeloid leukemia depends on the phase of the disease it presents.[19] More than 90% patients are often asymptomatic, diagnosed incidentally during the chronic phase. In this stage, patient usually presents with weakness, elevated white cell count

and splenomegaly. There are two other phases of chronic myeloid leukemia, accelerated and blast phase. In chronic phase, patient may also report to the physician with fatigue, weight loss, loss of energy, reduced exercise tolerance, low-grade fever, and excessive sweating from hypermetabolism, patient develops early satiety and decrease food intake due to pressure on the stomach by splenomegaly. The patient may also complain of dragging pain in the left upper quadrant of abdomen. Without treatment, the disease may progress to accelerated phase or blast phase where patient presents with bleeding, petechiae and ecchymoses, bone pain and fever, increasing anemia, thrombocytopenia, basophilia, and a rapidly enlarging spleen.[20] The diagnostic approach consists of complete blood count with differential, peripheral blood smear, bone marrow study and karyotyping as well as molecular investigation. About 85% patients are in the chronic phase at the time of diagnosis. The duration of chronic phase varies and depends on how early the disease was diagnosed and treatment stated.[21]

WHO and M.D. Anderson Cancer Center set some criteria whether the patient is landing to accelerated phase or blast phase.[22-25] According to WHO criteria, the accelerated phase is said to be present when ≥1 of the following criteria is present: persistent or high white cell count (>10x10^9/L) unresponsive to therapy, persistent or increasing splenomegaly unresponsive to therapy, persistent thrombocytosis (>1000x10^9/L) unresponsive to therapy, persistent thrombocytopenia (<100x10^9/L) unresponsive to therapy, ≥20% basophils in the peripheral blood, 10 –19% blasts in the peripheral blood and/or bone marrow, additional clonal chromosomal abnormalities in Philadelphia (Ph) chromosome (a second Ph chromosome, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype and abnormalities of 3q26.2, any new clonal chromosomal abnormality in Ph+ cells that occurs during therapy.[26]

The importance of detecting accelerated phase is important. It dictates that the disease is progressing and metamorphosis to blastic phase is impending.[25] Blast phase is the ultimate destination of chronic myeloid leukemia and its demeanor is like acute leukemia and diagnosed by the following criteria: >20% blasts in the blood or bone marrow and the presence of an extra-medullary proliferation of blasts.[27] Initially the median survival for chronic myeloid leukemia was 3-5 years from the time of diagnosis. At present, the median survival is 5 years or more. Improvement in this survival rate among patients with chronic myeloid leukemia is the after effect of early diagnosis, use of new targeted therapy and bone marrow transplantation as well as highly skilled supportive care. Analyzing different variables, patients are classified into three groups: good risk (average survival of 5-6 years), intermediate risk (average survival of 3-4 years) and poor risk (average survival of 2 years). Sokal score, one of the widely accepted prognostic score is measured for patients in the age between 5 to 84 years by: Hazard ratio = exp 0.0116 (age - 43) + 0.0345 (spleen size [cm below costal margin] – 7.5 cm) + 0.188 [(patient platelet count/700)^2 – 0.563] + 0.0887 (% blasts in blood – 2.1). On the basis of Hazard ratio, three groups of Sokal scores are described: Low-risk – score <0.8, intermediate risk – score 0.8 – 1.2 and high-risk >1.2. The importance of Sokal score is that it correlates with the probability of attaining total/ complete cytogenetic response: Low-risk patients – 91%; Intermediate risk – 84% and high-risk patients – 69%.[28] Besides Sokal score, in 1990s and 2000s, two other chronic myeloid leukemia prognostic scores have been evolved: the Hasford score and EUTOS (European Treatment and Outcome Study). Hasford score is like that of Sokal score categorizing patients into low-, intermediate- and high-risk groups. However, the Hasford group included peripheral blood eosinophils and basophils as a percentage of total white cell count to provide more accuracy in differentiating low-risk and intermediate-risk chronic myeloid leukemia and thereby the Hasford score is more useful in predicting molecular response to initial TKI (tyrosine kinase inhibitors) treatment of patients with chronic phase chronic myeloid leukemia.[29] To define the poor-prognostic criteria, a collective prognostic model has been constructed and stages the chronic myeloid leukemia patients as: Stage 1: 0 or 1 characteristic; Stage 2: 2 characteristics; Stage 3: 3 or more characteristics and Stage 4: blast phase at diagnosis. The clinical and laboratory characteristics of poor-prognosis are older age, symptomatic presentation, poor performance status, African-American descent, hepatomegaly, splenomegaly, negative Ph chromosome or BCR/ABL, anemia, thrombocytopenia, thrombocytosis, decreased megakaryocytes, basophilia and myelofibrosis. Therapy related factors that indicate poor prognosis are longer time to achieve hematologic remission with myelosuppression therapy, short duration of remission, high total dose of hydroxyurea or busulfan and poor suppression of Ph-positive cells by chemotherapy or interferon alfa therapy. At the same time if the patient with chronic myeloid leukemia has two or more additional chromosomal abnormalities accord inferior survival but the presence of trisomy 8, -Y and extra copy of Ph chromosome are associated with relatively good prognosis.[30-32]

In this patient, fever, weight loss, generalized weakness and anorexia favors the diagnosis of hemoglobin E disease with chronic myeloid leukemia but absence of splenomegaly, significant weight loss, severe to moderate anemia are the points against the diagnosis of hemoglobin E disease with chronic myeloid leukemia.

Hemoglobin E with pulmonary tuberculosis

Tuberculosis, caused by *Mycobacterium tuberculosis* which was first identified by Dr. Robert Koch on 24th March 1882.[33] That's why World Tuberculosis day is celebrated on 24th March every year. For this discovery Dr. Koch was awarded with the Nobel Prize in physiology or medicine in 1905.[34] About 25% of the world's population has been infected with *M. tuberculosis*[35] with new infections occurring in about 1% of the population each year.[36] Nevertheless, most of the infections with *M. tuberculosis* do not cause tuberculosis and 90–95% of infections remain asymptomatic.[37-39] Tuberculosis is one of the most important threat in Bangladesh and it ranks six among the 22 high burden countries of tuberculosis by World Health Organization.[40] In developing countries, especially in many Asian and African countries, tuberculin test is positive in 80% of the population but in USA, it is positive in only 5-10% of the US population.[41] Due to a number of factors such as difficulty of developing an effective vaccine, the expensive and time-consuming diagnostic process, the requirement of many months of treatment, the increase in HIV-associated tuberculosis and the emergence of drug-resistant cases, it has become difficult to eradicate or control the disease.[42] In Bangladesh including Southeast Asia, rates per 100,000 people is 278.[43] *M. bovis, M. africanum, M canetti* and *M microti* constitutes the *M. tuberculosis* complex.[44] Tuberculosis should be suspected when a patient presents with signs of lung disease or constitutional symptoms persisting for more than two weeks.[45] Two initial tests which are usually used to diagnose tuberculosis are chest X-ray, multiple sputum for microscopy and cultures for acid-fast bacilli. In most developing countries, two other methods are also widely used: a) Tuberculin skin tests and b) Interferon gamma release assays.[46] However, the definitive diagnosis of tuberculosis depends on the identification of *M. tuberculosis* in a clinical specimen, such as sputum, pus or tissue biopsy. Transmission of *M. tuberculosis* occurs to human being from a people with active tuberculosis through cough, sneeze, sing or split; they eject infectious aerosol droplets of 0.5 to 5.0 µm. About 40,000 droplets can be unlocked by a single sneeze and each one of these droplets is capable of transmitting the disease as the infectious dose of tuberculosis is very small (the inhalation of fewer than 10 bacteria may cause an infection).[47] A tuberculosis patient may infect 10-15 (or more) other people per year if remain untreated and approximately 22% individuals may be infected if remain in prolonged, frequent or close contact with tuberculosis patient.[47, 48] If an active tuberculosis patient starts taking the anti-tubercular drugs, such patients if non-resistant to conventional anti-tubercular drugs generally do not remain infectious after about three to four weeks of starting treatment.[42] Signs and symptoms of tuberculosis vary with the variants and stages of tuberculosis as well as the organs involved by the mycobacterium. Most commonly affected organ is lungs but extra-pulmonary tuberculosis may also occur or both may coexist. Initially patients present with constitutional symptoms such as fever, chills, night sweats, loss of appetite, weight loss, fatigue and nail clubbing.[50] Patient with active pulmonary tuberculosis usually present with prolonged cough producing sputum, hemoptysis and chest pain in about 90% of cases, however, 25% patients may be asymptomatic.[45] Upper lobes of the lungs are more commonly affected by tuberculosis than the lower ones but the reason for this upper lung lob affiliation is not clear. It seems to be due to either better air flow or poor lymph drainage within the upper lungs.[47, 48] Extra-pulmonary tuberculosis occurs in about 15-20% of active cases. Young children and people who have impaired immune system are more vulnerable to develop extra-pulmonary tuberculosis.[51] The noteworthy extra-pulmonary sites are pleura (tuberculous pleurisy), the central nervous system (tubercular meningitis), the lymphatic system (in scrofula of the neck), the genitourinary system (urogenital tuberculosis) and bones and joints (Potts disease). Besides this disseminated or miliary tuberculosis comprise 10% of extra-pulmonary tuberculosis.[52]

Presence of fever, weight loss, generalized weakness and moderate anemia favors the diagnosis of tuberculosis but absence of cough of longer duration, sputum production, hemoptysis and chest pain disfavors the diagnosis of tuberculosis.

Compound heterozygous hemoglobin disorders

An individual is said to have compound heterozygous for the hemoglobin disorder when two of the globin genes are affected such as HbSC, HbSD, hemoglobin E-beta thalassaemia, HbS-beta thalassaemia. Compound heterozygous hemoglobin disorders are most common in people from Africa, Middle-East, India and the Mediterranean Basin.[53] Compound heterozygote is caused by inheriting one defective allele from one type of disorder and by inheriting a second defective allele from another disorder. The clinical presentation of patients with compound heterozygous disorder varies from mild to severe clinical signs and symptoms.[54] Inherited hemoglobin disorders are a growing health problem in the world due to impressive success in the control of infectious diseases. Approximately 320,000 babies are born each year with a clinically significant hemoglobin disorder and about 80% of these births in developing country.[55] In certain malaria endemic regions of the world including Africa, all Mediterranean countries, the Middle East, the Indian subcontinent and Southeast Asia, hemoglobinopathies are more common. Over 50,000 new patients are born each year with severe form of thalassemia such as thalassemia major or hemoglobin E-beta thalassaemia.[56] In the Southeast Asia, especially the Indian subcontinent and

Bangladesh, there are frequent co-inheritance of beta thalassemia and Hemoglobin E.[57] Regarding the prevalence of thalassemia in Bangladesh, the information is very limited. Though the incidence of anemia in Bangladesh is very high but all the anemias are not due to iron deficiency. Different study showed that the nationwide incidence of anemia (33.0% in children under five years of age and 25.0% in women) which is three times higher than iron deficiency anemia in children (10.5%) and women (7.0%).[58] From this data, it can be concluded that other factors such as hereditary hemolytic anemia, secondary anemia, micro-nutrient (dietary iron, vitamin A, E, folate, Vit B_{12} and zinc) deficiency play significant role in the development of anemia.[59] It was observed in a recent study that 28% of assessed rural women have beta thalassemia or Hemoglobin E disorder.[60] Though Bangladesh lies in the thalassemia and hemoglobinopathies belt, but the actual data regarding the incidence of thalassemia, hemoglobin E, HbS, and HbD is dearth.[61] As the common cultural and socio-economic sharing with West Bengal of India, the incidence of inherited hemoglobin disorders is almost similar to Bangladesh. In West Bengal, large population-based study revealed that about 6.5% and 2.8% individuals carry the gene of beta thalassemia and Hemoglobin E.[62, 63] Considering all these factors, it concludes that the incidence for beta thalassemia trait is in the range of 3-6% and Hemoglobin E trait is 3-4% in Bangladesh. Also it is estimated that the number of patients suffering from thalassemias (beta thalassemia major and Hemoglobin E-beta thalassemia) is about 60,000 to 70,000 in Bangladesh.[64] There is strong association between malaria and sickle cell anemia, especially in Africa but not in Asia or America and that's why in Bangladesh sickle cell hemoglobin is rare though malaria is endemic in Bangladesh.[65] Compound HbS-beta thalassemia is very rare in Bangladesh but Hemoglobin E-beta thalassemia is relatively common (7.4%) which was observed in a study upon 700 patient with hereditary hemolytic anemia.[66] Hemoglobin E is caused by a base substitution at codon 26 of the beta globin gene, GAG–AAG, which results in the substitution of lysine for glutamic acid. This mutation also activates a cryptic splice site that causes abnormal mRNA processing.[67] Because the normal donor site has to compete with this new site, the level of normally spliced β^E–mRNA is reduced, resulting in the clinical phenotype of a mild form of beta thalassemia. Single nucleotide substitution (point mutation) is the most common pattern of defect found in different types of hemoglobinopathies. Until recently more than 900 such point mutations have been detected that are known to cause hemoglobinopathy. This type of mutation causes the nucleotide sequence to remain "in frame" resulting in a single amino acid substitution that will not alter the overall size of the globin protein product. Therefore the defect detected in different hemoglobinopathies is a simple an amino acid substitution that changes the amino acid sequence affecting protein structure and function rather than quantity. This type of alterations in hemoglobin function include changes in oxygen binding affinity, molecular solubility and the way in which individual hemoglobin molecules interact with the red blood cells. There are two α-gene loci on each chromosome 16 and one β-gene locus on each chromosome of 11 in the human genome. Clinical severity depends on the presence of number of mutated gene. In human genome, there are four α-genes and two β-genes. Mutation of α-genes is expected to four levels of clinical manifestation, on the other hand a β-gene mutation is likely to present two types of severity. This principle correlates with both hemoglobinopathies and the thalassemias. But there are some documented mutations in either type of hemoglobin disorder that do not have any deleterious effect to the patient. Besides these innocent mutations, α-thalassemia and hemoglobinopathies usually correlates with the number of mutated alleles and clinical severity. In contrast, the statement regarding number of mutated alleles and clinical severity for α-thalassemia and hemoglobinopathies is not absolutely fit for β-thalassaemia because many of the mutations in β–thalassemia produce protein products in varying amount.[54] Thalassemia also results from different types of mutations that are diverse and broad and eventually affect the quantity of protein produced inside the red cells. More than 400 mutations have been identified as the etiology of β-thalassemia and these mutations are categorized into: deletions, promoter mutations, non-sense mutations, stop codon mutations and splice site mutations.[68, 69] As a result of such mutations; reduced β-globin chain is synthesized in varying amount. If very little or no protein product is produced it is called β^0-thalassemia, β^+-thalassemia is designated if mild to moderate amount of protein product is produced. Therefore, clinical presentation depends on the total number of β–globin genes affected and to the degree to which each individual gene produces the globin protein.[54] In β-thalassemia, mutation profile differs around the globe in different geographical regions and cultures. Therefore, mutational profiling is important for genetic counseling and prenatal diagnosis of beta thalassemia. By detecting specific mutation, disease severity can also be predicted.[70] Mutations in globin genes (α or β) reduces the synthesis of globin chain that lead to decreased or even absent erythropoiesis. In India, a meta-analysis comprising 8505 alleles among the Indian population has revealed five mutations [IVSI-5 (G>C), IVSI-1 (G>T), 619-bp del, Codon 41/42 (-TCTT) and Codon 8/9 (G) comprise about 90% of all beta-globin mutations.[71] Though Bangladesh is a hotspot of thalassemia carriers, mutational analysis

of these patients are largely unknown. A recent study (n=256) in Bangladesh regarding the mutational profile of beta thalassemia showed the five most common mutations: IVSI-5 (G>C), Codon 41/42 (-TCTT), Codon 8/9 (G), Codon 15 (G>A) and Codon 30 (G>C). However, the most common mutation in Bangladeshi beta thalassemia patient is IVSI-5 (39.1%).[79] A small study comprising 16 samples revealed IVSI-5 (G>C) of about 56.3%.[72] However, the mutation profile (five common mutation) of West Bengal is almost similar to that of Bangladesh, IVSI-5 being the commonest form (71.4%) followed by Codon 30 (G>C) and Codon 15 (G>A).[77] Hemoglobin E-beta thalassemia constitutes about 50% of all severe beta thalassemia in the world.[73] This inherited double/compound heterozygous disorder ranks highest in India, Bangladesh, and throughout Southeast Asia, especially in Thailand, Laos and Cambodia, as in these countries it is common to inherit alleles for both hemoglobin E and beta-thalassemia gene. In these regions, hemoglobin E-beta thalassemia has been emerging as a major rigorous public health problem. In Thailand, approximately 3,000 children are born annually and in China, the carrier rate of gene for β-thalassemia and for hemoglobin E is four percent and about thousands of patients are affected.[74, 75] The basic pathophysiology of Hemoglobin E-beta thalassemia is that the affected individual inherit a β-thalassemia allele from one parent and structural variant hemoglobin E from the other. Factors affecting the pathophysiology of this compound hemoglobin E-beta thalassemia are: reduced β chain synthesis causing globin chain imbalance, ineffective erythropoiesis, apoptosis, oxidative damage and shortened red cell survival.[76, 77] Hemoglobin E is mildly instable but this instability plays very minor role in the pathophysiology of hemoglobin E-beta thalassemia except during intercurrent febrile illnesses during which such instability may cause escalated hemolysis.[78] The clinical presentation of patients with Hemoglobin E-beta thalassemia varies widely from a mild and asymptomatic anemia to life-threatening disorder requiring blood transfusion from infancy.[79] The unstable phenotypic behaviour and natural history of hemoglobin E-beta thalassemia varies with age. Over the first ten years of life, the clinical features are variable and changing in terms of severity of anemia and erythroid hyperplasia. A significant number of adult patients with hemoglobin E-beta thalassemia, the disease becomes more stable and require no blood transfusion with good quality of life and activities of daily living. However, a few data also describes that a few adult patients with hemoglobin E-beta thalassemia develops deteriorating anemia with the passage of time.[77] Attempts were made to classify hemoglobin E-beta thalassemia on the basis of clinical severity, commonly occurring genetic as well as environmental factors and categorized the patients as "severe" and "mild" disease groups with three other groups in between the two. A study conducted on Hemoglobin E-beta thalassemia by Premawardhene et al. which includes 109 patients, age ranging from one to 51 year was evaluated and classified into five group grading from very mild to very severely affected patients.[79] Group I: includes those patients who needs slightest blood transfusion and have normal growth, sexual maturity and quality of life as exceeding 5 on the 10-point scale. Group 2 patients are similar to group I except longer history of blood transfusion. Group 3 constitutes those patients who had previous history of splenectomy with an improvement in quality of life. Group 4 includes those patients who do not take blood transfusion though it is needed and have retarded growth, delayed sexual maturation and marginal self-reported quality of life (less than 5 on the 10-scale). Group 5 makes up those patients who have similar features like that of group 4 and needs regular blood transfusion. The genetic factors which influence the clinical severity of hemoglobin E-beta thalassemia are: type of beta-thalassemia mutation co-inherited with hemoglobin E, the co-inheritance of α-thalassemia and a type of polymorphism associated with increased synthesis of fetal hemoglobin. It was observed that patients who co-inherit a mild β-thalassemia with hemoglobin E have mild disease on one hand, on the other hand who co-inherit severe β^{+}or β^{0}thalassemia alleles might have very severe disease.[70] In 90 Thai patients whose genetic mutation for Hemoglobin E-beta thalassemia was found similar and had steady-state hemoglobin concentrations ranging from 4.2 to 12.6 g/dL.[75] Patients with Hemoglobin E-beta thalassemia having milder clinical symptoms co-inherited modifying mutation other than mild beta thalassemia allele, including XmnI polymorphism, α^{+}thalassemia or hemoglobin H-Constant spring which was found in 36 patients in a study.[71] Another study was conducted on 2000 Thai population with hemoglobin E-beta thalassemia, it was found that mild presentation of these patients was related to the interplay between hemoglobin E and the mutation at nucleotide -28 in the ATA box of β globin gene. In brief, it can be considered that β -thalassemia allele in trans to Hemoglobin E is not responsible for the broad spectrum of phenotypic presentation of hemoglobin E-beta thalassemia and obviously other genetic factors must be considered.[80]

In this patient, presence of moderate to severe anemia, generalized weakness and splenomegaly favors the diagnosis of compound heterozygous hemoglobin disorder, especially hemoglobin E-beta thalassemia as it is more common in this area. But presence of long standing fever, significant weight loss and anorexia are the points against the diagnosis of hemoglobin E-beta thalassemia.

Hemoglobin E with visceral leishmaniasis

Visceral leishmaniasis, also known as kala-azar, is the most threatened form of leishmaniasis, which if not properly diagnosed and treated carries high mortality.[81] Leishmaniasis is a parasitic disease caused by the species Leishmania. Three distinct clinically recognized leishmaniasis: Visceral leishmaniasis, cutaneous leishmaniasis and mucocutaneous leishmaniasis.[82] In 1824, a few Western doctors first described the kala-azar in Jessore district of Bangladesh where it was initially thought to be a form of malaria. Assam gave the name Assam fever.[83] The most commonly used indigenous name "kala-azar" is derived from kala means black in Sanskriti and azar means fever in Persian and Hindustani because patients with this disease become dark on the skin of the extremities and abdomen.[84] In 1901 William Leishman, a Scottish doctor, observed the parasite in spleen smears of a soldier who died of the disease in Dumdum, Calcutta, India, hence also the name dumdum fever. Irish physician Charles Donovan also worked independently and they published their discovery simultaneously, that's why the species was named for both of them – *Leishmania donovani*.[85] Initially the species were thought to be trypanosomes but in 1903 Captain Donovan delineated them as being new. Major Ross ascertained the link between these organism and kala-azar and he was the first scientist who was successful in culturing the flagellated form of *L. donovani* and named them "*Leishman donovani*". In 1907, White Patton described that amastigote form could be found in the spleen, bone marrow, lymph nodes and occasionally peripheral blood whereas promastigote form occurred in the intestine of the sand fly fed upon kala-azar patient.[86] Leishmaniasis occurs in 88 countries throughout the world in tropical and temperate regions. Approximately 350 million populations are at risk and 10 million people are affected from this disease worldwide.[84] Death toll from leishmaniasis is the second most common among parasitic diseases after malaria. Five hundred thousand new cases of visceral leishmaniasis and more than 50,000 death occurs annually.[87] Six countries in the world, namely Bangladesh, India, Nepal, Sudan, Ethiopia and Brazil are the mostly affected state where more than 90% cases of the world are occurring. Factors influencing the increased incidence of leishmaniasis includes: migration, lack of control measures and HIV-visceral leishmaniasis co-infection.[88] An estimated 300,000 cases per year and 60% of the global burden of VL occurs in Bangladesh, India and Nepal. In these three countries, the disease is prevailing in 109 districts (45 in Bangladesh, 52 in India and 12 in Nepal). In India, visceral leishmaniasis is gradually increasing in number but there is fluctuating of the disease in Bangladesh and Nepal.[89] Kala-azar was found to occur more along the courses of Ganges and Brahmaputra rivers.[90] In Bangladesh, most affected districts in the early 1919s are: Tangail, Rajshahi, Jessore, Mymensingh and Noakhali. In early 1980s, the affected districts are: Sirajgonj, Pabna, Mymensingh, Rajshahi and Tangail. A total of 73,467 kala-azar cases were reported from 1994 to 2004 by Directorate General of Health Services, Government of Bangladesh. From 1994 to 1996, the highest number of cases was reported in Pabna district but after 1996, the incidence increases in Mymensingh superseding the Pabna district.[91, 92] The incubation period ranges from 3 to 8 months. Patients with visceral leishmaniasis usually present with fever, weight loss, hepatosplenomegaly (usually spleen much larger than the liver), lymphadenopathy, pancytopenia and hypergammaglobulinemia. Pigmentation (blackening) of the skin is also an important finding. However, in some cases it may be asymptomatic and self-resolving but usually runs a chronic course and may be fatal or even with treatment. Because of severe immunosuppression, patients usually develop life-threatening bacterial infection and may lead to death. In some patients, there is atypical presentation involving the lungs, pleura, oral mucosa, larynx, esophagus, stomach, small intestine, skin and bone marrow.[93, 94]

In this case, the patient presents with long standing fever, significant weight loss, moderate to severe anemia and splenomegaly as well as hailing from endemic zone of kala-azar, all favors the diagnosis of visceral leishmaniasis.

Dr. Rahman's Diagnosis

Hemoglobin E disease with visceral leishmaniasis

Discussion

Dr. Rahman: After analyzing the patient's history, clinical signs and symptoms, results of few initial investigations, the most likely diagnosis might be hemoglobin E disease with visceral leishmaniasis. This patient's history revealed that he got admitted initially with moderate to severe anemia, fever, significant weight loss and splenomegaly and concerned physician on this finding advised to do hemoglobin-electrophoresis which unveiled the diagnosis of hemoglobin E disease. The physician and the patient got satisfied after getting one diagnosis and discharged the patient after transfusing several units of packed red cell and tablet folic acid. With this treatment, though his generalized weakness reduced but again after four months he reported to the hospital with the same symptoms. Therefore, the concerned physician thought about the persistence of second pathology in this patient. Re-evaluating the all clinical and physical findings, the concerned physician advised for more other laboratory investigations keeping in

mind the diagnosis of kala-azar. These investigations include bone marrow examination and rk39 test and sent the patient to AFIP for these two investigations. Bone marrow examination revealed numerous *L. Donovan* (LD) bodies both intra- and extracellularly as well as rk39 was found positive (Figure 1 and Figure 2). Therefore, the ultimate diagnosis was hemoglobin E disease with visceral leishmaniasis.

Dr. Durdana Mahin (Histopathology Resident): Why do not you consider the diagnosis as splenic lymphoma with villous lymphocytes (SLVL)?

Dr. Monir (Hematology Resident): Yes, it might be a one differential diagnosis. But initial complete blood count and blood film examination showed bicytopenia (suggestive of hereditary hemolytic anemia + leucopenia). Thereafter Hb-electrophoresis revealed hemoglobin E disease. And subsequently bone marrow examination and rk39 test confirms the diagnosis of visceral leishmaniasis. Patient with splenic lymphoma with villous lymphocytes usually present with symptoms of anemia and discomfort in the left upper quadrant due to splenomegaly. These features were present in this patient. Splenic lymphoma with villous lymphocytes patient is usually associated with moderate lymphocytosis and morphologically splenic lymphoma with villous lymphocytes cells are slightly larger than small lymphocytes with condensed chromatin, inconspicuous nucleoli, moderate amount of cytoplasm and fine villous projections on the cell surface.[95] In this patient, there is leucopenia and lymphocytes do not have the features of splenic lymphoma with villous lymphocytes cell.

Dr. Sifat–E-Moin (Hematology Resident): Could it be a case of tuberculosis? Did you perform MT?

Dr. Islam (Hematology Resident): Significant weight loss and fever point to the diagnosis of TB. But absence of cough and presence of splenomegaly disfavor the diagnosis of TB. MT was not done as bone marrow study, rk39 and Hb-electrophoresis unveiled the diagnosis, so it was not required.

Dr. Mreenal Kanti Sarker (Chemical Pathology Resident): Gastric poly and esophageal varices were found on upper gastrointestinal tract endoscopy. Is there any relationship with the diagnosis?

Dr. Lubna Naznin (Chemical Pathologist): I think it is a separate entity. However, this patient might also have cirrhosis of liver. In one study in Bangladesh, the authors described that the patients with kala-azar may develop cirrhosis of liver due to cause-effect relationship.[96]

Dr. Omme Hani (Histopathology Resident): Is there any relationship with hemoglobin E disease and visceral leishmaniasis?

Dr. Rahman (Hematologist): Extensive search through internet and Google scholars, we did not find any relationship between these two diseases. A case report was published showing rare association of visceral leishmaniasis with Hodgkin lymphoma. This might be due to immunosuppression occurred in patients with Hodgkin lymphoma.[97]

Dr. S. M. Rashidul Kabir (Microbiology Resident): Why serum ferritin is very high in this patient?

Dr. Islam (Hematology Resident): As the patient has been suffering from hereditary hemolytic anemia, so there is continuous breakdown of red cells resulting the deposition of ferritin in different organs of the body and that's why serum ferritin level is found elevated. Besides this, in chronic disease, because of failure of utilization of iron by macrophages, serum ferritin may also be elevated.[11]

Dr. Samia Mahmood (Microbiology Resident): How will you manage this high serum ferritin level?

Dr. Sharfin Nahar (Hematology Resident): If a patient has an elevated ferritin level, the treatment options include: observation, magnetic resonance imaging, liver biopsy, empirical phlebotomy or voluntary blood donation. In this patient, initially observation is the first option for treating high serum ferritin as this high level might be due to multiple (10 units) blood transfusion and hemolysis due to hemoglobin E disease. After symptomatic treatment (tablet folic acid) of hemoglobin E disease and visceral leishmaniasis (injection stibogluconate), I will look for serum ferritin level. Still if it remains more than 1000 ng/mL, then I will think about iron chelation treatment.

Dr. Hasan Mahmud Shihab (Histopathology Resident): Why the serum lactate dehydrogenase level is high in this patient?

Dr. Golam Rabbani (Hematology Resident): Lactate dehydrogenase is often used as a marker of tissue breakdown as lactate dehydrogenase is abundant in red cells and can function as a marker of hemolysis. Hemoglobin E disease, which is present in this patient, is a hereditary hemolytic anemia where red cell lysis leads to high lactate dehydrogenase level in serum.

Dr. Dilder Alam (Clinical Pathology Resident): Are there any other investigations for the diagnosis of visceral leishmaniasis?

Dr. Mehedi Hasan Robin (Clinical Pathology Resident): Splenic puncture is another direct evidence of diagnosing visceral leishmaniasis. Some other investigations support the diagnosis of visceral leishmaniasis: DAT (direct agglutination test), indirect hemagglutination test, indirect fluorescence antibody test, aldehyde test, Chopra antimony test which are now-a-days rarely used.

Dr. Arif Ahmed Khan (Microbiologist): Which form of *L. donovani* is found in spleen, bone marrow and peripheral blood?

Dr. Wasim Selimul Haque (Histopathologist): Amastigote form of *L. donovani*. It is an intracellular, non-motile form and devoid of external flagella and found in the mononuclear phagocyte and circulatory system of humans. Amastigote form is oval in shape and measures 3–6 µm in length and 1–3 µm in breadth. The kinetoplast and basal body lie towards the anterior end.

Dr. Mostare Khondoker (Histopathology Resident): Can we get the amastigote form in peripheral blood?

Dr. Imana Shahreen (Microbiology Resident): Yes, we can detect the amastigote form of *L. donovani* in the buffy coat. The buffy coat is the fraction of an anticoagulated blood sample that contains most of the white blood cells and platelets following density gradient centrifugation. After centrifugation, the volume is about 1% of the total blood volume centrifuged. Buffy coat is used to extract DNA from the blood of mammals. Quantitative buffy coat is a laboratory test to detect infection with malaria, leishmania or other blood parasites. The blood is taken in a quantitative buffy coat capillary tube which is coated with acridine orange (a fluorescent dye) and centrifuged; the fluorescing parasites can then be observed under ultraviolet light at the interface between red blood cells and buffy coat. The test is more sensitive than the conventional thick smear and in >90% of cases the species of parasite can be detected. Leishmania amastigotes are found by buffy coat microscopy in 93.5% of those positive by buffy coat PCR and 92.4% of those positive for leishmania amastigotes by spleen smear microscopy.[98]

Dr. Md Husenuzzaman Chowdhury (Biochemistry Resident): How can we find promastigote form of leishmania?

Dr. Samia (Microbiology Resident): By culture in NNN (Novy, MacNeal, Nicolle) medium, this is a common and world known medium for the isolation of the genus leishmania. Some other media have also been used which can be categorized into: Semisolid, biphasic and liquid. Other in vitro culture media includes RPMI 1640, Evans and Schneider. Culture of this organism for 4 weeks give rise to motile, extracellular form called promastigote.[99]

Dr. Tonima Talukder (Histopathology Resident): In detecting the amastigote form of *L. donovani*, among bone marrow and splenic smear which one is superior?

Dr. Rahman (Hematologist): Splenic smear microscopy is superior to bone marrow microscopy in detecting the *L. donovani* bodies. Bone marrow is a safer but less sensitive method in the diagnosis of visceral leishmaniasis as compared to splenic aspiration. Bone marrow microscopy in the diagnosis of visceral leishmaniasis has a lower sensitivity of approximately 60–85%.[99] Splenic aspirate, though consociated with danger of calamitous hemorrhage from a soft and enlarged spleen in inexperienced hands, is one of the most valuable methods for diagnosis of kala-azar with a sensitivity exceeding 95%.[100]

Final Diagnosis

Hemoglobin E disease with visceral leishmaniasis

Conflict of Interest

Authors declare no conflict of interest

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