Hemoglobin H disease in Muscat, Oman - A 5 year study

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Abstract

Objective: Published data indicate that Alpha thalassemia trait is prevalent in 45% of population of Sultanate of Oman. Recent unpublished data suggest that this prevalence is higher than 45%. Yet clinical suspicion or investigations into α-thalassemias are lacking. Moreover, Hemoglobin H disease is considered rare in Oman. We decided, therefore to look for Hemoglobin H disease and characterize the clinico–hematopathological features of the disease.

Methods: Patient demographics, clinical details and detailed hematology parametry of Hemoglobin H disease cases, diagnosed by Department of Laboratory over a period of 5 years between February 2002 and January 2007 in patients presenting at Al-Nahdha Hospital and Genetic counseling unit in Muscat were compiled from hospital and laboratory records and analyzed.

Results: Twenty cases of Hemoglobin H disease in Omanis were diagnosed mainly during the second decade. 60% belonged to Al-Balushi tribe. 40% of cases presented with body pains. 35% presented with nonspecific symptoms. 50% of cases were erroneously labeled as Iron deficiency anemia. Microcytic erythrocytosis, high Red Cell Distribution Width, numerous misshapen Red Blood Cells, pseudothrombocytosis, low A2 and normal Ferritin were important diagnostic clues. Hemoglobin H inclusions in special reticulocyte smears and Hemoglobin H on HPLC or Electrophoresis were diagnostic.

Conclusion: Hemoglobin H disease is common in Oman. The need to do HPLC, G6PD activity and Ferritin studies in all cases of anemia in Oman to avoid missing diagnosis of Hemoglobin H disease is stressed. This study is intended to create awareness about Hemoglobin H disease in order to diagnose early, treat rightly, counsel correctly and pave the path for prevention of α-thalassemia disease in Oman.

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Introduction

Alpha thalassemias are hemolytic anaemias due to reduced synthesis of the α globin chain. They consist of Hydrops foetalis, Hemoglobin H disease and Alpha thalassemia trait. Hydrops foetalis is a severe form of α-thalassemia due the loss of all 4 α genes.

Hemoglobin H disease is a moderate form of alpha thalassaemia due to the loss of three α genes. Though most of the losses of α genes in Hemoglobin H disease are of deletional variety, pure non deletional forms and a mix of deletional & mutational type of Hemoglobin H disease are also evident.[1] Thus molecular studies are needed to know the genotypes.

Alpha thalassemia trait is a mild form of alpha thalassemia due to the loss of two or one α genes. The frequency of erythrocyte genetic defects in Omanis for α thalassemia trait is as high as 0.45.[2] Yet clinical or laboratory work up into α-thalassemia is lacking. This prompted our study that led to diagnosis of 20 cases of Hemoglobin H disease over a period of 5 years.

Methods

69000 Complete Blood Count (CBC) tests were sent to Al Nahdha hospital laboratory over a period of 5 years from 2002 to 2007. We diagnosed Hemoglobin H disease in 20 patients (0.03%). Patient demographics, symptoms, signs and clinical diagnosis of all 20 patients were documented. The CBC parametry of 20 cases done in an automated hematology analyzer (Cell Dyn 1700) included Hemoglobin, Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Red Blood Cell (RBC) count, Red Cell Distribution Width (RDW) and Platelet count. A Leishman stained peripheral blood smear was performed and examined under oil immersion lens of the microscope.

Sickle solubility test, Enzyme colorimetric assay for qualitative determination of G6PD activity, Reticulocyte count and a modified reticulocyte preparation (Hemoglobin H preparation) were also performed.

The remaining blood in EDTA vacutainer was referred for High Performance Liquid Chromatography (HPLC) to detect abnormal hemoglobin by Biorad 10 at Royal Hospital, Muscat. Hemoglobin H quantitation was done after running in Cellulose Acetate Electrophoresis at pH 8.6 at Sultan Qaboos University Hospital in Muscat. Ferritin estimation was done in our cases by the Electrochemiluminescence immunoassay on the Roche Elecsys 2010 analyzer.

Results

Al-Nahdha Laboratory diagnosed 20 cases of Hemoglobin H disease, over a period of 5 years from February 2002 to January 2007. All the affected were Omanis. The youngest patient was 4 years old while the oldest was 48 years old. Ten patients were male and ten female.

60% (12 patients) of our cases belonged to Al-Balushi tribe. One patient each belonged to Al-Zadjali, Al-Siyabi, Al-Mamari, Al-Buraimi, Al-Ahsani and Al-Mahrooqi tribes. The tribal information was not available in 2 patients.

40% (8 cases) of our patients complained of aches and pains namely headache, toothache, throat pain, chest pain, body ache and pain in the lower limbs respectively. Four cases had come for Genetic Blood Screening, 3 of which cough and weakness and one each had come for eye check up and recurrent Jaundice.

On physical examination, in one patient there was a suspicion of icterus and in another patient, icterus was definite and spleen was just palpable. The clinical diagnosis in 50% (10 patients) of cases was Iron deficiency anaemia. Four persons had voluntarily come for Genetic blood disease screening. Two cases each were diagnosed as Dental caries and acute upper respiratory infection. One case that came for eye check up was diagnosed as Keratoconus. Only one case was clinically diagnosed as G6PD deficiency hemolytic anemia.

Hemoglobin of our patients ranged from 8.2 g/dL to 11.5 g/dL(Normal range: 9.5 – 13.5 in children; 11.5 to 16 in adult females; 13 to 18 in adult males). Mean Cell Volume (MCV) ranged from 45.4 fL to 57.1 fL(Normal range: 78 – 92). The Mean Cell Hemoglobin (MCH) ranged from 15.6 pg to 20.9 pg (Normal range: 27 – 31). The RBC count ranged from 4.9 M/μL to 6.4 M/μL (Normal range: 4 – 5.2 in children; 3.8 – 5.8 in adult females; 4.5 – 6.5 in adult males) . In our cases RDW ranged from 21.1% to 34.7% (Normal range: 11 – 14).The peripheral blood smear examination showed numerous small misshapen red cells in all our cases (Figure 1). The reticulocyte count of all our cases was increased and ranged from 4.1% to 12% (Normal range: 0.2 – 2).

The Platelet count on automated counters ranged from 266 K/μL to 1233 K/μL (Normal range: 150 – 400). On Hemoglobin H preparation, all were positive for Hb H Inclusion. Sickling solubility test was negative in all our cases. 18 cases had normal G6PD activity, while one had partial and another had nil G6PD activity.

HPLC and Hemoglobin electrophoresis revealed that Hb A ranged from 80.5% to 96% with a mean value of 91.2%; Hb F from 0.1% to 1.2% with a mean value of 0.5% and Hb A_2 from 0.9% to 1.9% with a mean value of 1.5%. Hb H percentage ranged from 2.2% to 18%.

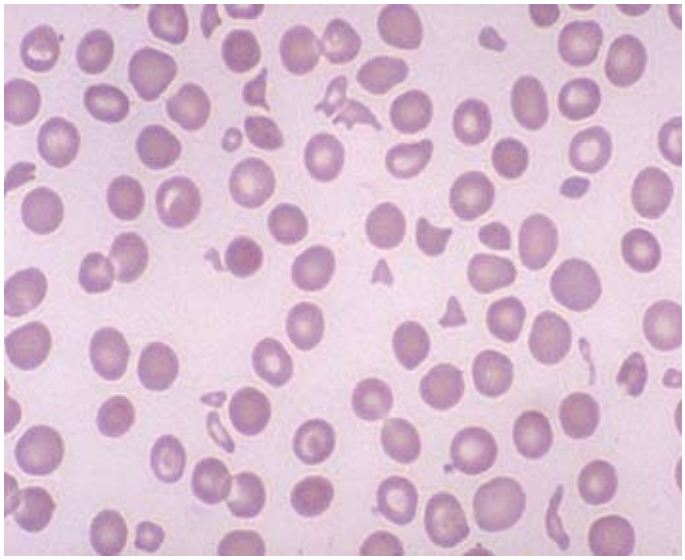

Figure 1: Smear shows many misshapen red blood cells

Ferritin values in our cases were normal with a mean value 87.6 μg/L (Normal range: 7 – 282 in females; 18 – 323 in males).

Discussion

Age distribution revealed that 4 patients belonged to the first decade, 12 to the second decade, 3 to the third decade and one in the fifth decade.

The ethnic origin of population of Oman is mainly Arab but over the centuries considerable mixing has taken place with East African and East Asian Subcontinents. Defining tribal variation can identify high and low risk group and assist in efficient targeting of health resources.[3]

It is worthwhile to remember that splenomegaly is observed in Hemoglobin H disease.[4] Except for one, none of the cases clinically entertained a diagnosis of hemolytic anaemia.

Hemoglobin of our patients ranged from 8.2 g/dL to 11.5 g/dL. The mean was 9.6 g/dL, indicating mild anemia. This is in concurrence with the classical presentation of Hemoglobin H disease cases where the anemia is mild to moderate in severity.[5]

In mild anemia, usually the MCV and MCH are mildly reduced. But the disproportionately low MCV (Microcytosis) and MCH compared to mildly low hemoglobin and normal RBC count is the first clue pointing towards alpha or beta thalassemias.[6]

Red cell Distribution Width (RDW) indicates variation in size of red cells. Greater the variation, greater would be the RDW. In our cases RDW ranged from 21.1% to 34.7%. This disproportionately high RDW compared to mildly decreased Hemoglobin value is the second clue towards the diagnosis.[7] The peripheral blood smear

examination showing numerous small misshapen red cells in all our cases was the third clue towards the diagnosis. Apart from misshapen red cells, polychromasia and Target cells were seen.

Another fascinating clue was the Platelet count. The Platelet count on automated counters ranged from 266 K/μL to 1233 K/μL and the mean was 734.7 K/μL. But the puzzling feature was the platelet count was either normal or very slightly increased on peripheral blood smear in all our cases. We also found that, patients with very high platelet counts of 1223 K/μL and 1136 K/μL had correspondingly high RDW of 34.7% and 29.5% respectively. The reason for this falsely high platelet count was due to the presence of many very small misshapen red cells which were falsely counted as platelets by automated analysers. This was the penultimate clue towards the diagnosis.

Hemoglobin H preparation revealed numerous red cells with regular bluish dots resembling golf ball and these are nothing but Hemoglobin H inclusions and the presumptive diagnosis of Hemoglobin H disease was made. It is worthwhile to remember that G6PD patients with low red cell indices (MCV and MCH) should be screened for concurrent Hemoglobin H disease.[8]

HPLC and Hemoglobin electrophoresis revealed mean Hb H to be 6.8%. Low levels of Hemoglobin H are seen in black individuals and may be related to more effective proteolysis of excess β globin chains.[9] On Cellulose acetate electrophoresis at pH 8.6, Hemoglobin H is the fastest moving Hemoglobin. On HPLC it is the first peak that is observed. But the most significant fact is that Hemoglobin H is unstable and volatile and especially samples with low Hemoglobin H content may precipitate out during storage thereby leaving undetectable quantities in red blood cells. Thus if HPLC or Hemoglobin electrophoresis is delayed then one may not be able to detect Hemoglobin H. Hence it is very important to process the samples fresh and document the fact that Hemoglobin H inclusions were seen. A very helpful finding even when HPLC or Hemoglobin electrophoresis is normal is the low Hb A_2. The mean of our cases was 1.5%. The presence of low A_2 should make one extract and process a fresh sample for HPLC or Hemoglobin electrophoresis to avoid missing the diagnosis of Hemoglobin H disease.

Ferritin values in our cases were normal. Iron therapy erroneously given in 50% of our cases due to mistaken diagnosis of Iron deficiency anemia can lead over a period of time to iron overload.[10]

There are various associations and interactions of Hemoglobin H disease with other diseases. Splenomegaly, an unusual feature seen in some of the older patients of sickle cell disease in Oman could be due to co–inheritance of Hemoglobin H disease.[11] Co-inheritance of alpha thalassemias and homozygous β-thalassemias and consequent reduction in α-globin chain excess often results in a milder clinical and hematological phenotype.[12] The characterization could also be useful in genetic counselling.

Hemoglobin H inclusions in red cells are seen in some patients with premalignant conditions and acute myeloid or lymphoid leukemia and this is termed as acquired Hemoglobin H disease but none of our cases had any myelo or lymphoproliferative disorders.[13]

Hemoglobin H disease is neither rare nor a benign disorder as has been generally thought.[14] The major issue in Oman is that Hemoglobin H disease is erroneously diagnosed and treated as Iron deficiency anemia. Iron therapy in such patients is not only useless but could be harmful.

In view of the prevalence in 45% of population with alpha thalassemia trait and in 25% of the population with G6PD deficiency anemia, sickle cell trait and beta thalassemia trait the policy should be to do HPLC, G6PD activity and Ferritin in all cases of anemia in children in Oman before treatment to avoid missing such diagnosis. At Al-Nahdha Hospital Laboratory in Muscat, our Genetic blood screening unit do once a life time testing and mention G6PD, Sickling and HPLC data in a plastic laminated card and is given to the patient/person.

This significant carrier rate for Genetic blood diseases especially α-thalassemias in Oman warrants the need to do molecular studies.[15] Molecular testing for genetic blood disease including Hemoglobin H disease would provide the final answer to queries that come up even after HPLC or Hemoglobin electrophoresis testing. Non deletional Hemoglobin H disease tends to be more severe than deletional type.[10] Hence molecular studies can explain the varying clinical severity of different Hemoglobin H disease patients. It would play a great role in a definitive approach towards genetic counseling of the concerned couple and in identification of families who are at risk for having pregnancies affected with Hydrops foetalis or Hemoglobin H disease.[16]

Conclusion

Hemoglobin H disease in Omanis in our study of 20 cases from 2002 to 2007 was diagnosed by Al Nahdha Laboratory Department mainly during the second decade. Both sexes were equally affected. 60% of the cases belonged to Al Balushi tribe. 40% of cases complained of aches in various parts of body. None of the cases were clinically suspected as α-thalassemia / Hemoglobin H disease, despite a high prevalence of α-thalassemia trait in Oman.

Microcytic erythrocytosis, high RDW and numerous misshapen RBCs on blood smear, pseudothrombocytosis in automated counters were important clues towards diagnosis in our cases. Low HbA2 values and normal Ferritin values were additional clues towards the diagnosis. Hemoglobin H inclusions in special reticulocyte preparation was the qualitative diagnostic test and was confirmed by quantitation of Hemoglobin H by HPLC and Hb electrophoresis.

CBC, HPLC, G6PD activity and Ferritin tests need to be done in all cases of anaemia especially in children, before treatment, in Oman to detect Hemoglobin H disease. Even in diagnosed cases of Sickle Cell anaemia, Beta thalassemias and G6PD deficiency anaemias a co-inheritance of Hemoglobin H disease needs to be seriously considered. Finally, molecular studies in such cases will play a great role in a definitive approach towards Genetic Counselling. Let this study be the nidus for research in Hemoglobin H disease in Oman.

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