Original Research

The Prevalence of alpha-Thalassemia in Anemic US Veterans without Nutritional Deficiency or Abnormal Hemoglobin HPLC Pattern

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Alpha-thalassemia is one of the most common hemoglobin genetic abnormalities. The primary defect is the reduced or absent production of the alpha globin chains, which underlines 4 clinical conditions: 1) alpha^{+}-thalassemia (loss of one alpha globin), 2) alpha^{0}-thalassemia (loss of 2 alpha globins), 3) HbH disease (loss of 3 alpha globins), and 4) Hb Bart hydrops fetalis syndrome (loss of all alpha globins). Among the anemic US veteran patients, one subpopulation defies extensive workup and remains etiology unknown (normal status of iron, VitB12, and Folate, normal hemoglobin HPLC pattern, etc.). Here we present the data of alpha-thalassemia DNA analysis in this subpopulation. 156 patients were analyzed of the alpha-globin gene locus by multiplex ligation-dependent probe amplification (LabCorp, RTP, NC). The prevalence of alpha^{0}-thalassemia was 5%, alpha^{+}-thalassemia 26%, and negative 69%. We believe alpha-thalassemia DNA analysis might be a useful test choice in mild anemic patients with uncertain etiology.
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Key Words: *alpha-thalassemia, alpha-globin DNA analysis, multiplex ligation-dependent probe amplification*

INTRODUCTION

Alpha-thalassemia is one of the most common hemoglobin genetic abnormalities.[1] The primary defect is the reduced or absent production of the alpha globin chains, which results in 4 conditions: 1) alpha^{+}-thalassemia (loss of one alpha globin), 2) alpha^{0}-thalassemia (loss of 2 alpha globins), 3) HbH disease (loss of 3 alpha globins), and 4) Hb Bart hydrops fetalis syndrome (loss of all alpha globins). Clinically, conditions 1 and 2 are categorized as carrier state, while 3 and 4 as clinically related symptomatic forms. The carrier state is aslo divided into silent carrier and alpha thalassemia trait. The silent carrier is mostly resulted from a single alpha globin gene deletion (- alpha/alpha alpha), which, is characterized in the newborns with a very mild increase of Hb Bart (1-2%), a tetramer of gamma chains, but in adults with a complete silence state or associated with a moderate microcytosis and hypochromia with normal HbA2 and F. Alpha thalassemia trait refers to patients with 2 residual functional alpha genes, either in cis (- -/alpha alpha) or in trans (- alpha/- alpha), charactering a moderate increase (5-6%) of Hb Bart in the newborns and mild anemia with normal HbA2 and F in adults. HbH disease is usually manifested with pronounced microcytic hypochromic hemolytic anemia. Hb Bart hydrops fetalis syndrome is almost always lethal.[2-6]

Among the anemic US veteran patients, one subpopulation defies extensive workup and remains etiology unknown (normal status of iron, VitB12, and Folate, normal hemoglobin HPLC pattern, etc.). Here we present the studies of alpha-thalassemia DNA analysis in this subpopulation.

METHODS

156 veteran patients with anemia of unknown etiology who were submitted for alpha-thalassemia DNA analysis for the past 6 years were selected in this study. All patients were qualified for "unknown etiology" with: 1) normal iron studies, 2) normal Vitamin B12/folate level, and 3) with normal hemoglobin HPLC pattern. The DNA analysis tests were performed at Labcorp, RTP, NC, 27709. Briefly, multiplex ligation-dependent probe amplification (MLPA) was applied to the analysis of the alpha-globin gene locus (HBA1/HBA2 OMIM 141800 & 141850, 16pter-16p13.3). The mutations analyzed were alpha3.7, alpha4.2, Southeast Asian type (--SEA), Filipino type (--FIL), Thailand type (--THAI), and Mediterranean type (--MED).[7-10]

All patients' following data were collected and studied also. 1) Complete CBC, including RBC, HGB, HCT, MCV, MCH,

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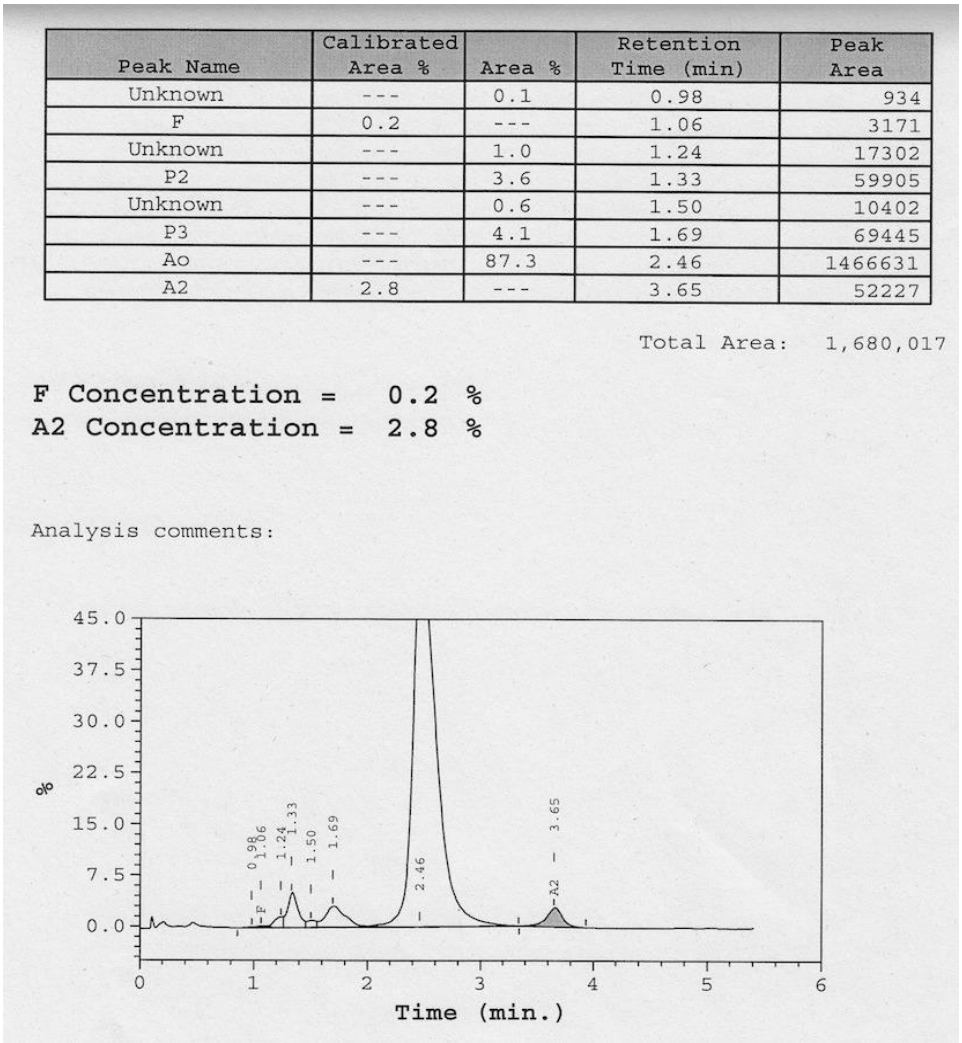
MCHC, RDW, WBC, and platelet, 2) iron studies, including serum iron, TIBC, iron saturation, and ferritin, 3) serum level of Vitamin B12 and folate, and 4) hemoglobin HPLC pattern.

RESULTS

All patients are male, with an average age of 70.6 Y, Hb of 12.65 g/dL, RBC of 4.41 M/uL, and MCV of 87.10 fL. Regarding patients' race, 95/156 (61%) are white (including Hispanic white), 33/156 (21%) are black, 28/156 (18%) are others (Asian, Hispanic nonwhite, etc.) The prevalence of alpha^0-thalassemia was 5% (8/156), alpha$^+$-thalassemia 26% (40/156), and negativity 69% (108/156). alpha^0-thalassemia patients had lowest Hb and MCV, and highest RBC number (Table 1). As the precondition for the patient selection, all iron studies, level of Vitamin B12 and folate, and hemoglobin HPLC patterns **(Figure 1)** are within normal limits.

Table 1. Complete CBC, Iron status, hemoglobin HPLC pattern in anemic US veterans with or without alpha-thalassemia.

	alpha^0-thalassemia	alpha$^+$-thalassemia	negative	Reference Range
Patients (total 78)	8/156 (5%)	40/156 (26%)	108/156 (69%)	
Age (year)	62.5	55.6	76.7	
Hemoglobin (g/dL)	12.4	13.1	12.5	14-17.5
HCT (%)	38.3	39.5	37.4	41.5-50.4
RBC (M/uL)	5.25	4.88	4.17	4.5-5.9
MCV (fL)	73.3	82.1	90.0	80-96
Iron status	normal	normal	normal	
Vitamin B12	normal	normal	normal	
Folate	normal	normal	normal	
Hemoglobin HPLC pattern	normal	normal	normal	



Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.98	934
F	0.2	---	1.06	3171
Unknown	---	1.0	1.24	17302
P2	---	3.6	1.33	59905
Unknown	---	0.6	1.50	10402
P3	---	4.1	1.69	69445
Ao	---	87.3	2.46	1466631
A2	2.8	---	3.65	52227

Figure 1. A representative HPLC analysis of hemoglobin from one of the patients. All fractions are within normal limits.

DISCUSSION

Alpha-thalassemia is one of the most common hemoglobin genetic abnormalities, which, is caused most frequently by deletions and less commonly by nondeletional defects. In normal genotype, alpha globin genes are duplicated into alpha1 and alpha2 (this makes total of 4 alpha genes in 2 copies of chromosomes) and localized in the telomeric region of chromosome 16 (16p 13.3). The level of transcription ability of these duplicated alpha genes is different, with the alpha2 gene produces 2-3 times more mRNA than alpha1 gene. Consequently, the amount of hemoglobin present in alpha1 or alpha2 deletional mutation could generate different phenotype: most likely silent in alpha1 deletion, might showing mild anemia in alpha2 deletion.[1]

The most common deletions are the -alpha$^{3.7}$ and -alpha$^{4.2}$ deletions. Both of these deletions are caused by chromosome recombination resulting in a 3.7kb deletion or a 4.2kb deletion, and both of the deleted segments contain only one alpha gene, resulting in alpha^{+} alpha-thalassemia, with a silent phenotype or an alpha-thalassemia trait, depending on the degree of alpha globin chain deficiency relative to beta-globin production. Simultaneous deletion of 2 copies of them causes alpha^{0} alpha-thalassemia, showing an alpha-thalassemia trait.[11,12]

The test method used in this study is multiplex ligation dependent probe amplification (MLPA), which detects copy number changes by targeting 24 different sequences in the alpha-globin gene region, and will, in principle, detect all genomic deletions and duplications involving this locus, including alpha,$^{3.7}$ alpha,$^{4.2}$ Southeast Asian type (--SEA), Filipino type (--FIL), Thailand type (--THAI), and Mediterranean type (--MED), as well as Constant Spring point mutation (LabCorp test manual).

Among the anemic veterans, the majorities are attributable to nutritional deficiency (iron deficiency, Vitamin B/folate deficiency, etc.), chronic illness (anemia of chronic diseases), or chronic attrition due to malignancies, etc. However, a small population of anemic veterans defies route lab tests by showing normal iron study, normal Vitamin B and folate levels, and a within normal limits hemoglobin HPLC pattern. The latter eliminates most of the hemoglobinopathic diseases that would show more or less abnormal patterns on HPLC.[13] Furthermore, veterans went through physical examination and laboratory screen when they enlisted. Therefore, anybody with symptomatic hemoglobinopathy or anemia of other etiologies is excluded from enlisting. However, those silent alpha-thalassemia carriers obviously can pass the enlisting screen, by showing negativity for anemia or a low normal range of hemoglobin level.

Our study shows 31% of this subgroup of anemic veterans harbors alpha-globin gene defects, as 26% with alpha^{+}-thalassemia and 5% alpha^{0}-thalassemia. Of note, among the detected defects, 89% are alpha$^{3.7}$ deletion, 11% are alpha$^{4.2}$ deletion. No other type of defects is identified.

The anemia in those 5% alpha^{0}-thalassemia patients are likely caused by their alpha-globin genetic defects. These patients have slightly lower hemoglobin level (12.4 g/dL) and show microcytic RBCs (MCV 73.3 fL) comparing with the alpha^{+}-thalassemia patients in this study (Hb 13.1 g/dL, MCV 82.1 fL) or in that published in literature.[13] How these veterans slipped through enlist lab testing is unknown.

It's a surprise that up to 26% of the study subjects are of alpha^{+}-thalassemia by revealing one alpha-globin genetic defect, mostly alpha$^{3.7}$ deletion. Loss of one alpha-globin gene supposedly results in a silent phenotype or a thalassemia trait with mild anemia. Since we only select anemic veterans as research subjects, literally all silent carriers are excluded from this study, which makes it impossible to know the frequency of silent carriers in US veterans. Furthermore, our research subjects are all showing normal hemoglobin pattern in HPLC study, normal iron status, and normal blood level of Vitamin B and folate, we are confident that patients' anemia is directly related to the single alpha-globin defect.

The rest 69% anemic veterans are free of alpha-globin defects in this study. Of note, the applied test method in this study did not detect other point mutation in the target region than indicated in materials and methods section, nor did it detect mutations in the alpha-globin gene regulatory region-the multispecies conserved sequences (MCS) located about 40 kb upstream.[1] In addition, other potential factors, such as low erythropoietin level, early stage of myelodysplasia deterring normal erythropoiesis, etc., should all be in the differential diagnosis list.

CONCLUSIONS

Total 31% of the patients harbor one or two alpha gene deletion, which might be attributable to their mild anemia symptom. Alpha-thalassemia DNA analysis might be a useful test choice in mild anemic patients with uncertain etiology.

CONFLICT OF INTEREST

The authors disclaim no conflict of interests.

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