Alpha-2-Globin Gene Polyadenylation (AATAAA → AATAAG) Mutation in Hemoglobin H Disease among Kuwaitis

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Abstract
Objectives: In the Arabian Gulf region, hemoglobin (Hb) H disease usually results from homozygosity or compound heterozygosity involving the α2-globin gene polyadenylation (poly A) signal (AATAAA → AATAAG) mutation (ααT). Here we document the clinical and hematological characteristics of children with Hb H disease being followed in Kuwait. Subjects and Methods: Twenty-four patients (0.5–12 years old, mean 4.7 ± 3.5 years) with persistent microcytic, hypochromic anemia (and normal iron status as well as normal Hb A2 levels) were referred to the pediatric hematology clinic for further investigations. They were all screened for the α-thalassemia (α-thal; –3.7 kb) deletion using a standard PCR method. They were also screened for the α2-globin gene α7α allele and the 5nt deletion (–α5nt) in the first intervening sequence, which are common α-thal alleles in this population. They were followed up for periods ranging from 2 to 8 years. Results: Of the 24 patients, 4 (16.7%) also had sickle cell trait (Hb-AS), while 7 (29.2%) were glucose-6-phosphate dehydrogenase deficient. Only 1 patient had significant hepatosplenomegaly and 1 developed gallstones. While none was on chronic transfusion therapy, 8 (33.3%) had been transfused at least once and, in 3 instances, this was secondary to parvovirus B19 +ve aplastic crisis. The α-globin genotype was successfully determined in almost all patients. The results showed that 17 (70.8%) patients were homozygous for the poly A mutation (αααT), 6 (25.0%) were compound heterozygotes for this and the αα-thal (–3.7 kb) deletion (–α/αTα) and 1 (4.2%) was undetermined. There were no significant differences in the phenotypes of the 2 genotypes and their hematological features were identical. Conclusions: Hb H disease involving the poly A mutation is a mild thal intermedia phenotype among Kuwaitis. There are no serious complications and there is no need for regular blood transfusion.

Introduction

Normal individuals have two linked α-globin genes on chromosome 16, with the genotype αα/αα. The two most common determinants of α-thalassemia, α-thal-2 and α-thal-1, result from the loss of one (−α/) or both (−/−) α-globin genes, respectively. The former is associated with α+, while the latter is associated with α0-thal trait. Compound heterozygosity of α0-thal and α+α+ alleles (−/−/α) results in inactivation or loss of 3 α-globin genes,
with significant α/β-globin chain imbalance causing the clinical syndrome called hemoglobin (Hb) H disease. Less common α-thal determinants are nondeletional lesions in which a mutation on one or the other α-globin gene (α2α/α) reduces the synthetic production of the affected gene. Homozygotes (α2α/α2α) or compound heterozygotes (α/α2α, α2α/α) are also associated with the Hb H phenotype of varying severity [1–4].

The clinical phenotype of Hb H disease is quite heterogeneous, but is usually one of that intermedia with mild to moderate microcytic, hypochromic anemia, usually not requiring regular blood transfusion. The excess mild to moderate microcytic, hypochromic anemia, usually not requiring regular blood transfusion. The excess.

α-Thal trait is quite prevalent in the Arabian Peninsula with the frequency of α-thal-2 allele being as high as 40–60% in some areas [7–9]. While the α2-thal alleles are uncommon, the nondeletional, α-globin polyadenylation (poly A; AATAAA→AATAAG) allele has been described in Eastern Saudi Arabia, United Arab Emirates and Kuwait [3, 4, 10–12]. There have been mainly anecdotal reports of Hb H disease among Gulf Arabs [8–10], and the present communication is an account of cases that are being followed in the pediatric hematology clinic in Kuwait. It documents their clinical presentation, laboratory findings and molecular characterization. In addition, it looks at the influence (if any) of coexistent sickle cell trait and glucose-6-phosphate dehydrogenase (G6PD) deficiency on the phenotype.

Subjects and Methods

A total of 24 patients were studied, 14 males and 10 females aged between 6 months and 12 years with a mean age of 4.7 ± 3.6 years. Eleven (45.8%) had weight and height of ≥50th centile. Their Hb ranged from 7.4 to 10.6 g/dl (9.0 ± 0.8), mean cell volume from 48.1 to 66.6 fl (56.7 ± 4.0) and mean cell hemoglobin from 15.7 to 21.2 pg (18.4 ± 1.4). All patients had inclusion bodies in their peripheral blood smears.

The patients were being followed in the pediatric hematology clinics of the Amiri and Mubarak Hospitals in Kuwait. Both are part of the teaching hospitals’ complex of the Kuwait University Faculty of Medicine. The diagnosis of Hb H disease was based on the finding of microcytic, hypochromic anemia in a child who did not have iron deficiency and whose Hb electrophoresis was not consistent with the β-thal syndrome. In addition, they all showed Hb H inclusion bodies in more than 80% of the peripheral red blood cells following cresyl blue staining. Family screening was carried out and in all cases both parents had varying degrees of microcytic and hypochromic anemia.

Blood was drawn from all the patients by venepuncture; complete blood count was done with the Coulter electronic cell counter. A qualitative test for G6PD deficiency was used for screening the patients [13]. DNA was extracted by the method of Poncz et al. [14]. All the patients were screened for the α-thal-2 (–α3.7 kb) deletion with a PCR method [15]. The α-globin gene region was again amplified and dot blottting was done followed by allele-specific hybridization with probes specific for the poly A mutation [4, 11], AATAAA→AATAAG (α2α/α) and 5nt deletion (–α500) in the 5′ splice junction of the first intervening sequence [16]. These are the two nondeletional α-thal alleles prevalent in this region [10–12].

All the patients had detailed physical examination and were followed regularly in the clinic. The patients received daily folic acid supplement; otherwise, no other routine treatment was given. Their charts were reviewed for this report and details of any hospitalization were obtained. Particular attention was paid to frequency of blood transfusion and any complications, e.g. aplastic crisis.

Results

Of the 24 patients studied, 7 (29.2%) had G6PD deficiency, while 3 (12.5%) had Hb AS (heterozygous normal /sickle-Hb). Eight (33.3%) had been transfused at least once and none was on regular transfusion. Four (57.1%) of the G6PD-deficient patients had been transfused; the difference in frequency was not significant (χ2 = 1.0, p > 0.05). There were also no other significant differences in the hematological parameters among those with G6PD deficiency or Hb AS; this may be due to the small sample size.

Seventeen (70.8%) were homozygous for the poly A mutation (α2α/α2α) and 6 (25.0%) were compound heterozygous for the α-thal-2 deletion and the poly A mutation (–α3.7/α2α), while 1 (4.2%) was undetermined. There were no significant differences in the hematological parameters among the homozygotes and the compound heterozygotes.

The patients did not show any common complication, but 1 (4.2%), with genotype α2α/α2α, who was also G6PD deficient, had persistent hepatosplenomegaly, first noticed when he was about 6 months old and persisting at age 9 years. He was also transfused on 3 occasions in the first 2 years of life. Three (12.5%) patients developed aplastic crisis secondary to parvovirus B19 infection. Four (16.7%) patients had reactive thrombocytosis with platelet count ranging from 600 to >1,000 × 109/l lasting for varying lengths of time. None had evidence of concurrent iron deficiency. Only 1 (4.2%) patient had gallstones and required cholecystectomy.
Hb H disease is indeed common in Kuwaitis with ancestral origin in Eastern Saudi Arabia [17]. The patients are usually referred to the pediatric hematology clinic because of significant hypochromic, microcytic anemia not responding to iron supplements and with normal Hb A2 levels, ruling out β-thal trait. Almost all cases involve the α2-globin gene AATAAA→AATAAG mutation either as homozygotes or compound heterozygotes with the −α3.7 kb α-thal deletion.

The phenotype of Hb H disease among our patients is uniformly mild, with none of them requiring regular transfusions. Patients with chronic hemolytic anemia are prone to aplastic crisis following documented parvovirus B19 infection [18–20]. This was encountered in 3 of our patients; it was self-limiting in all cases, although they all required at least one blood transfusion. Four of the patients in this study showed evidence of transient reactive thrombocytosis. This appears to be nonspecific and accompanies the anemia of the disease.

Concomitant G6PD deficiency may be responsible for frequent hemolytic crisis in sickle cell disease. Its effect, if any, on the phenotype in Hb H disease has not been widely documented. Sanna et al. [21] reported higher G6PD enzyme levels in patients with Hb H disease and Öner et al. [22] did not see any effect of the enzyme deficiency on the hematological parameters of their patient. Similarly, we could not demonstrate any significant effects of G6PD deficiency on our patients and the frequency of blood transfusion was not significantly higher.

Matthay et al. [23] have suggested that sickle cell trait may modify Hb H disease because the relatively low Hb A level reduces the availability of β-chains to form Hb H. On the other hand, α-thal trait is known to ameliorate the course of sickle cell disease, because intracellular concentration of Hb S is low in patients with coexistent α-thal trait [24, 25]. It was therefore interesting that 3 of the Hb H patients in the present study had sickle cell trait. Two of them had Hb H inclusions in more than 80% of their peripheral erythrocytes and 1 had in about 40%. However, the baseline Hb level and other hematological parameters were not significantly different. We therefore cannot make a case for a modifying role for sickle cell trait in our Hb H patients.

No specific treatment is offered to our Hb H disease patients. Full family screening is carried out on all suspected cases to document complete cell count, iron status, Hb electrophoresis and α-globin genotype. Genetic counseling is carried out and the affected patients are started on regular folic acid supplementation. Patients who maintain their Hb at 10 g/dl and above are not followed in the clinic.

Conclusions

Hb H disease involving the poly A mutation is a mild thal phenotype among Kuwait patients. There are no serious complications and they do not require regular blood transfusion.

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References


