Hemoglobinopathies in the United Arab Emirates

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Introduction

The United Arab Emirates (UAE) is a federation of seven emirates situated on the Eastern Arabian Peninsula bordering Oman, Saudi Arabia and Qatar. In the north lie Iran and the Arabian Gulf. The population of the UAE is diverse and, like the other Gulf countries, is made up of immigrants from the Middle East, India, Pakistan, Iran and Europe. In the last two decades, the population of the UAE swelled significantly from 600,000 in 1985 to over 2.5 million in 1995 and boasts slightly over 4 million people today, of which only 20% are indigenous nationals. Approximately 80% of the UAE population is made up of expatriates, the majority of whom are Indians, Pakistanis, Iranians and Arab nationals. The term "expatriate" is used throughout the text to represent the above ethnic groups. The sharp increase in the UAE population is attributable to the rapid growth in economy, trade and tourism. The population spurt is mainly caused by foreign labor, which migrated to the UAE to work on the numerous ambitious development projects.

The birth rate in the UAE is estimated at 2% and infant mortality rate <1%. Live births are estimated at 60,000 per annum. The fertility rate is 4.04 births per national woman. The age structure in the UAE is a significant consideration as it reflects a bearing on the population growth; the age groups are represented as follows; 25 - 29 age group is the largest (500,000), followed by 30 - 34 (460,000) and 35 - 39 (430,000). Those in the 10 - 19 age group (510,000) comprise of 15% of the entire population. Those under the age of 50 account for 95% of the total population. In the 20 - 49 age group, males account for nearly three times the number of females.

Genetic Disorders in the UAE

Autosomal recessive disorders are common in the UAE. Hemoglobinopathies are one of the most common disorders among the UAE nationals. Other diseases include congenital abnormalities, cancers, metabolic disorders, chromosomal aberrations and mental retardation. Monogenic diseases such as cystic fibrosis, fragile-X and G6PD also exist at appreciable levels. During the last two years alone, the author's laboratory has carried out mutational identification and characterization of more than 50 cases of cystic Fibrosis, predominantly among the UAE nationals.

β-Thalassemia

β-Thalassemia (β-thal) is one of the most common single gene disorders affecting almost all the countries in the Mediterranean Basin, the Middle East, South East Asia, Far East, Australasia, the Americas and Africa. It is characterized by the deficiency or absence of β-globin chain production. More than 200 different mutations have so far been reported that result in β- thalassemia (Huisman *et al.,* 1997).

β-Thal constitutes a major public health problem in the UAE. During 1989-2004, more than 850 patients have been registered at the Dubai Genetics and Thalassemia Center. The exact number of thalassemics in the UAE is unknown although DNA-based studies from the author's laboratory project this number to be much higher than previously estimated especially when the other emirates are taken into account. Previous surveys have shown that the UAE exhibits one of the highest carrier frequencies of β-thal in the Gulf region. DNA studies performed on over 400 consecutive UAE National newborns and nearly 2000 adult college students and 800 randomly selected nationals, demonstrated that the frequency of β-globin gene defect including the β-thal, β^s gene and abnormal hemoglobins is estimated at 8.5% (Baysal, 2001), one of the highest in the Gulf Region.

Molecular studies were carried out on all β-thal patients registered at the Genetics and Thalassemia Center using the latest available techniques. α-Globin genes were routinely investigated according to Baysal and Huisman (1994) as the latter exist at a very high frequency (El-Kalla and Baysal, 1998b). Laboratory analyses included iso-electric focusing (IEF), quantitation of Hb types by column chromatography, PCR, restriction enzyme analysis (REA), β-strip hybridization, allele specific oligonucleotide (ASO) hybridization as well as manual and automated DNA sequencing.

The mutation analysis among the UAE national and expatriate β-thal patients clearly demonstrates that the UAE is arguably the most heterogenous β-thal population in the world with 50 different β-thal mutations reported to date. It is important to note that the majority of the β-thal mutations in the UAE are very severe. Except for the most common allele (which is β^+ thal), all other mutations are of severe β^0-thal type. The high degree of consanguinity, especially between the first cousin marriages, resulted in significant number of homozygotes who are on regular blood transfusion and chelation therapy. The high level of endogamy originates from centuries old socio-cultural and religious traditions in the Arab societies. Similar observations were made in the expatriate patient population most of whom are Muslims (Bener *et al.,* 1996; Al-Gazali *et al.*, 1997). Our existing data depict that 68% of the UAE nationals (212 out of 313 patients) were characterized as homozygous β-thal thus corroborating consanguinity. This was more than twice the number of compound heterozygotes.

The most common mutation in the UAE is the IVS-I-5 (G→C), a type of β^+ thal that exists in very high frequencies in the Indian subcontinent and among populations neighboring India. All the remaining 10 most common mutations were β^0-thal with very severe phenotype. The spectrum of β-thal mutations in the UAE also represents an extensive admixture of genes from the Mediterranean, Arabian and Indian framework. Among the first 11 most common mutations, five; [Cd39 (C→T), IVS-II-1 (G→A), Cd5 (-CT), IVS-I-1 (G→A), Cd30 (G→C)] exist at significantly high levels in the Mediterranean countries; three [IVS-I-5 (G→C), Cd 8/9 (+G), Hb D] are prevalent in India; and the others [-25 bp del, Cd 39 (C→T), IVS-II-I (G→A)] occur commonly in Mediterranean countries, Iran and the Eastern Arabian peninsula.

In contrast to the other countries where only a small spectrum of β-thal alleles occurs; UAE appears to have a significant heterogeneity even among the indigenous population. It is very likely that the IVS-I-5 (G→C) allele was introduced to the Arabian Peninsula by gene migration from Baluchistan, a region spanning southern Iran, Afghanistan, and Pakistan. Many of the UAE national families are thought to have their roots in surrounding countries such as Iran and Baluchistan, as evidenced from the common mutations and haplotype studies.

It is well documented that the IVS-I-5 (G→C) mutation was generally found in the Chinese, Asian-Indians and peoples of Baluchistan and Pakistan but not among the Middle East Arabs (Quaife *et al.,* 1994). The propensity of this allele in the Arabian Peninsula can be attributed to the population migration from the Indian subcontinent; its low frequency in Kuwait and high frequency in the UAE and Oman favors the speculation that the gene was introduced into the Arabian Peninsula across the Straits of Hormuz. This navigational route still constitutes a major trade link between the Indian subcontinent and the Gulf States.

Abnormal hemoglobins, namely HbS, HbD, Hb O-Arab, Hb Knossos, are also important in the epidemiology of hemoglobinopathies. In the UAE, abnormal hemoglobins, particularly HbS, contribute significantly to the genetic diversity of hemoglobinopathies. The β^S gene is a major genetic factor in a group of patients with co-inherited Sickle/β-thal (S/β-thal). Molecular studies demonstrate that β^S gene is indeed the second most common β-globin gene defect in the UAE and exists predominantly on the Arab-Indian haplotype (El-Kalla and Baysal, 1998a). Furthermore, HbE is present particularly in the expatriate community from South East Asia and Bangladesh.

The diversity of hemoglobinopathies in the UAE is most certainly caused by the admixture of genes between different groups in and around the Gulf region, Indian subcontinent, Middle Eastern countries and Africa. In recent times, this was exacerbated by the influx of many other nationalities into the UAE. The gene flow and heterogeneity of β-thal mutations represent complex anthropological influences from the East Mediterranean, Asia, India, sub Sahara and East Africa corroborating that the diversity of β-thal mutations reflects historical events and gene migration in the region.The β-thal distribution, heterogeneity of mutations, homozygous births due to consanguinity, founder effect compounded with cultural traditions and beliefs are some of the important factors that determine the propensity of hemoglobinopathies in the UAE. Public awareness programs, education and preventive measures have recently been implemented and seem to be showing positive effect.

The data reported here implicate the importance of the need for a comprehensive thalassemia control program and requirement for an effective and efficient system for patient management, population screening, genetic counseling and prenatal diagnosis.

α-Thalassemia and HbH Disease

α-Thalassemia (α-thal) is generally caused by the deletion of one (-α/αα) or both (-α/-α or --/αα) functional α-globin genes leading to α-thal-2 (-α/αα) and α-thal-1 (--/αα) conditions, respectively. Individuals who inherit two or three functional α-genes (-α/αα; -α/-α; --/αα) have α-thal trait with a mild hypochromic, microcytic anemia. Those who inherit a single α-gene (--/-α) have HbH (ß4) disease, a moderately severe hemolytic anemia with a variable clinical course. HbH Disease is the most severe form of the α-thal syndromes compatible with life. Hb Bart's Hydrops Fetalis syndromes arise from total absence of four α-globin genes (--/--) and such condition is incompatible with life. The majority of α-thal and Hb H cases in the Gulf Region are caused by point mutations characterized by relatively severe phenotype.

HbH disease is a moderately severe hemolytic anemia with microcytosis, hypochromia, low HbA_2 and HbF levels, and varying quantities of HbH (β_4; 2-30%). Most of the HbH syndromes were thought to be caused by the deletion or inactivation of three of the four α-globin genes. However, in the last decade, numerous reports have been published demonstrating an increasing number of non-deletional (α^T) α-thal as the molecular basis for many of the HbH syndromes, particularly from the Middle East (Adekile *et al.*, 1994; Baysal, 2001). For decades, hematological evaluation and gene mapping techniques have been used to identify these anomalies at the molecular level. More recently, novel techniques such as PCR have been devised which enable the molecular characterization of such patients rapidly, easily and accurately (Baysal and Huisman, 1994).

Several studies were conducted in the author's laboratory in an attempt to elucidate the frequency of α-thal in the UAE. Cord blood samples were collected from 418 consecutive UAE national newborns. The PCR-based analysis of the α-globin gene status demonstrated that the incidence of α-thal, particularly the $-\alpha^{3.7}$ deletion, was extremely high (El-Kalla and Baysal, 1998b). The DNA-based newborn screening survey demonstrated that 49 % of the neonates had α-thal, one of the highest in the world. The distribution of mutations was as follows: αα/αα: 51%; $-\alpha^{3.7}/\alpha\alpha$: 34%; $-\alpha^{3.7}/-\alpha^{3.7}$: 11%; $-\alpha^{4.2}/\alpha\alpha$: 1.0% and one newborn was compound heterozygous for the $-\alpha^{3.7}/-\alpha^{4.2}$ genotype. The remaining 3% of the chromosomes were identified with the non-deletional type of α thal (α^T). Four different α^T alleles were identified; PolyA-1 [α^{PA-1}(AATAA**A**→AATAA**G**)], PolyA-2 [α^{PA}-2 (AAT**A**AA→AAT**G**AA)], Hb Constant Spring [HbCS ($\alpha^{CS}\alpha/\alpha^{CS}\alpha$) **T**AA→CAA] and pentanucleotide deletion [$\alpha^{-5nt\ del}$ (GA**GGTGA**GG→GAGG)].

Furthermore, the author's laboratory was able to define the genotype of 41 patients with HbH disease using direct PCR diagnosis, allele-specific oligonucleotide (ASO) hybridization, manual and automated DNA sequencing. Of these, 30 were UAE Nationals, 5 Omanis, 3 Sudanese, 2 Thai and 1 Pakistani. Nine UAE nationals and four Omani patients were homozygous for the poly A-1 mutation ($\alpha^{PA-1}\alpha/\alpha^{PA-1}\alpha$) characterized by the AATAA**A**→**G** substitution in the α_2 globin gene. Moreover, 11 UAE national patients were diagnosed with the $-\alpha^{3.7}/\alpha^{PA-1}$ α genotype. In a large UAE national family, two individuals were homozygous for the Hb Constant Spring ($\alpha^{CS}\alpha/\alpha^{CS}\alpha$) which affects the termination codon (α142 **T**AA→**C**AA) of the α_2 globin gene and three were compound heterozygous for the $-\alpha^{3.7}/\alpha^{CS}\alpha$ genotype. Interestingly, the HbCS accounted for 11.5% of the chromosomes. In addition, 9% of the α-thal chromosomes had the -5 nucleotide deletion ($\alpha^{-5nt\ del}$), at the splice junction between exon 1 and intron 1. Two UAE nationals were identified with a new α^T mutation; a frameshift caused by a single nucleotide deletion at codon 19 in exon 1 of the α_2 globin gene [Cd 19 (-G)]. Both patients were homozygous for this particular mutation. In addition, two sisters of Thai origin had the --SEA deletion and one boy from Oman was characterized with the $-\alpha^{3.7}$/--MED-I genotype. Both of these deletions were characterized through simple PCR-based approaches. A total of nine different α-thal genotypes were identified; 4 deletional and 5 non-deletional (α^T).

Of the 82 α-thal chromosomes, almost half (47.4%) were the Poly A-1 mutation; 13 of 41 patients were homozygous for the mutation, $\alpha^{PA-1}\alpha/\alpha^{PA-1}\alpha$, with mod-

erate clinical severity. The data presented here demonstrate a considerable heterogeneity of α-thal in the UAE. The genotype/phenotype correlation suggests that HbH disease in the UAE has, in general, a moderate presentation.

In conclusion, data obtained from DNA studies in the author's laboratory demonstrate an extremely high incidence of α-thal in the UAE, perhaps one of the highest in the world. The occurrence of α^T mutations, particularly in homozygous state implicates the degree of consanguinity as well as the clinical significance of HbH Disease in the community. The clinical data suggest that HbH Disease in the UAE has, in general, mild to moderate phenotypic expression and should be considered as a genuine public health issue. Finally, the molecular characterization of the patients with HbH disease may be clinically useful for the prediction of the clinical outcome, correct management and to provide appropriate genetic counseling to patients and families in the UAE.

Summary

The use of modern DNA techniques enabled us to characterize and identify 50 discrete β-thal mutations and 9 α-thal determinants in the UAE population. All of the β-thal mutations were severe β^+ or β^o types often resulting in transfusion dependent phenotype. Furthermore, a large number of α^T alleles in the α-thal carriers and in patients with HbH Disease accentuate the importance of HbH disease as a public health concern. The overall data presented here will be useful for better quality patient management, carrier screening, genetic counseling, and ultimately for the establishment of a comprehensive prenatal diagnosis program.