Understanding Beta-Thalassemia with Focus on the Indian Subcontinent and the Middle East

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Abstract: Beta-thalassemia is one of the most prevalent autosomal disorders in the world. Mutations in the *HBB* gene underlie deficiencies in hemoglobin production, which can interfere with oxygen delivery resulting in wide range of disease severity. Although >535 mutations have been characterized in the *HBB* gene, beta-thalassemia is broadly classified into three groups, based on clinical severity: beta-thalassemia major, beta-thalassemia intermedia and beta-thalassemia minor. In this article we review: 1) the molecular and biochemical basis of beta-thalassemia; 2) clinical features; 3) the range of common molecular variants of beta-thalassemia in a subset of geographic regions within the Indian Subcontinent and the Middle East; 4) potential molecular diagnostics; and 5) current and future treatments. We suggest that efforts to more completely characterize the *HBB* mutation distribution in high-risk areas, such as the Indian Subcontinent and the Middle East, may lead to improved diagnosis with earlier and more effective intervention strategies.

INTRODUCTION

Beta-thalassemia is an important disorder that has attracted the attention of medical research towards the various paradigms of this multifaceted disease [6-12]. Beta-thalassemia is an autosomal hematological disorder that is the result of genetically deficient synthesis of the beta-globin chains of hemoglobin [13]. Beta-thalassemia is one of the most common single-gene disorders with >400,000 newborns affected per year worldwide [14]. Most frequently, this disorder is found in the malarial, tropical and sub-tropical regions of Mediterranean countries, the Middle East, Transcaucasus, Central Asia, the Indian Subcontinent (South Asia) and Southeast Asia [15] (Fig. **1**).

Hemoglobin (Hb) is responsible for oxygen delivery from the lungs to peripheral tissues [16]. Hb is a tetrameric iron-containing protein, composed of two alpha-globin and two beta-globin chains. The alpha-globin and beta-globin chains join to form Hb in developing erythrocytes, or red blood cells (RBCs), and remain together for the life of the RBC [17]. In the event of altered beta-globin chain structure or function, in which one or both copies fail to quantitatively or qualitatively produce normal beta-globin, the alpha-globin

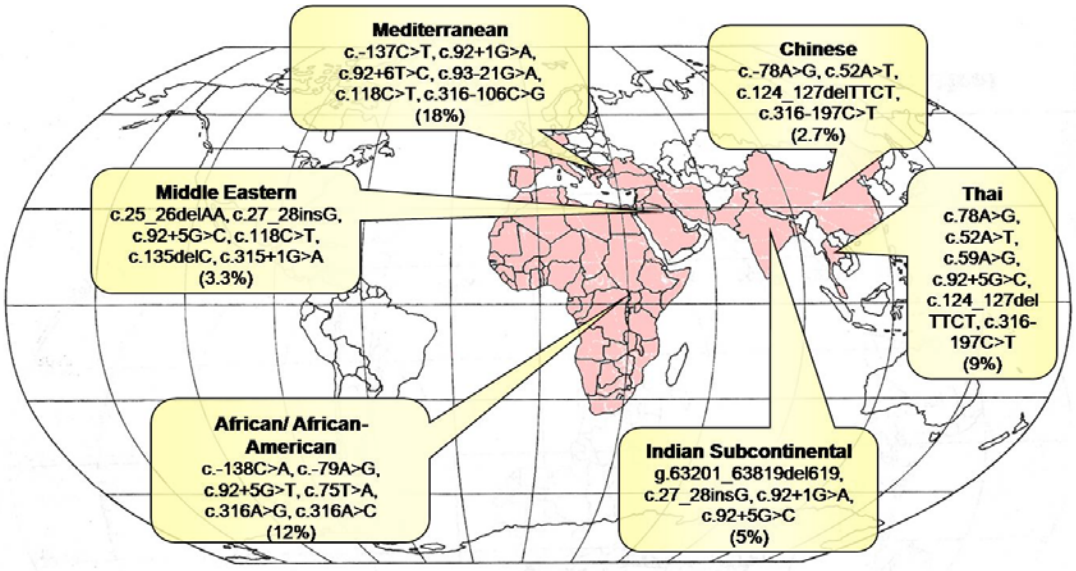

Fig. (1). Most common beta-thalassemia mutations in at-risk populations. Highlighted regions displaying the highest gene mutation frequencies of beta-thalassemia on a global scale. Mutations listed are those which are most common and represent 91-95% of affected individuals from these regions (or 75-80% of the population for individuals of African/African-American descent). Listed in brackets are the approximate upper ranges of beta-thalassemia carrier frequencies in each region [1-5].

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gene continues to produce quantitatively and qualitatively normal alpha-globin [16, 17]. The imbalance of the globin chains results in beta-thalassemia [16], with the precipitation of excess alpha-chains contributing to excessive destruction of RBCs, which begins a cascade that ends with significant morbidity and mortality [16].

THE BETA-GLOBIN LOCUS

The beta-globin chain is encoded by the *HBB* gene, which spans 1.6 kb on the short-arm of chromosome 11 (11p15.4; MIM: 141900) [18, 19]. The genomic sequence of *HBB* contains three exons, two intervening sequences (IVS1 and IVS2) and the 5' and 3' untranslated regions (UTRs) (Fig. **2a**). The *HBB* gene is regulated by a 5' promoter region that contains the classical TATA, CAAT and duplicated CACCC boxes (Fig. **2a**). Upstream of the beta-globin cluster is another regulatory element for *HBB*, namely the locus control region (βLCR). The expression of individual globin genes is governed by direct physical interaction between the βLCR and globin promoters, mediated by the binding of tissue-restricted and ubiquitous transcription factors [20]. The most important transcription factor for *HBB* is the erythroid Kruppel-like factor (EKLF), which binds the proximal CACCC box [18].

The beta-globin locus harbors, in addition to the *HBB* gene, the ε-globin (*HBE*) gene, the two γ-globin (*HBG*) genes (*HBG-G* and *HBG-A*), and the δ-globin (*HBD*) gene (Fig. **2b**) [21]. Most genes at this locus are expressed at specific time points in development. During embryonic development, the *HBE* gene is expressed along with the alpha-globin gene to form embryonic-hemoglobin or Hb-E [21]. Twelve weeks post-conception, the fetus primarily uses fetal hemoglobin (Hb-F) which is composed of two γ-globin and two alpha-globin chains [21]. Around the time of birth, γ-globin production decreases while beta-globin synthesis increases such that most individuals have only trace amounts of Hb-F detectable 7 or 8 months after birth [21, 22]. The combination of two alpha-globin genes and two beta-globin genes comprises a normal adult Hb, which becomes the predominant form within 18 to 24 weeks after birth [21, 22]. Less than 3% of adult hemoglobin is Hb type A2, which is composed of two δ-globin chains and two alpha-globin chains [22].

VARIANTS OF BETA-THALASSEMIA

There are >535 *HBB* gene mutations [21, 23] with a subset of ~40 mutations responsible for the majority of beta-thalassemia cases globally, as defined by population studies [15]. Moreover, frequencies of beta-thalassemia are very high in malarial, tropical and sub-tropical regions such as the Indian Subcontinent and the Middle East, a fact hypothesized to be the result of a thalassemia heterozygote advantage against severe forms of malaria [24].

Different classes of *HBB* mutations underlie beta-thalassemia, in descending order of frequency: missense/nonsense [25, 26], splicing [27], regulatory [28], small or gross gene deletions [27, 29], including the common deletion of the terminal portion of *HBB* [30], gene insertions [31], small insertion-deletions [32], and complex rearrangements [33]. In rare instances, the causative defect is due to a deletion of the βLCR [18], mutations in another gene within [34] or outside [16] the beta-globin locus. Beta-thalassemia rarely arises due to the complete loss of the *HBB* gene [34].

In the past, a descriptive, non-molecular ontology for beta-thalassemia deduced the existence of two disease-related alleles, B^0 and B^+. The B^0 allele was considered to produce non-functional beta-globin protein [16, 21]. Subse-

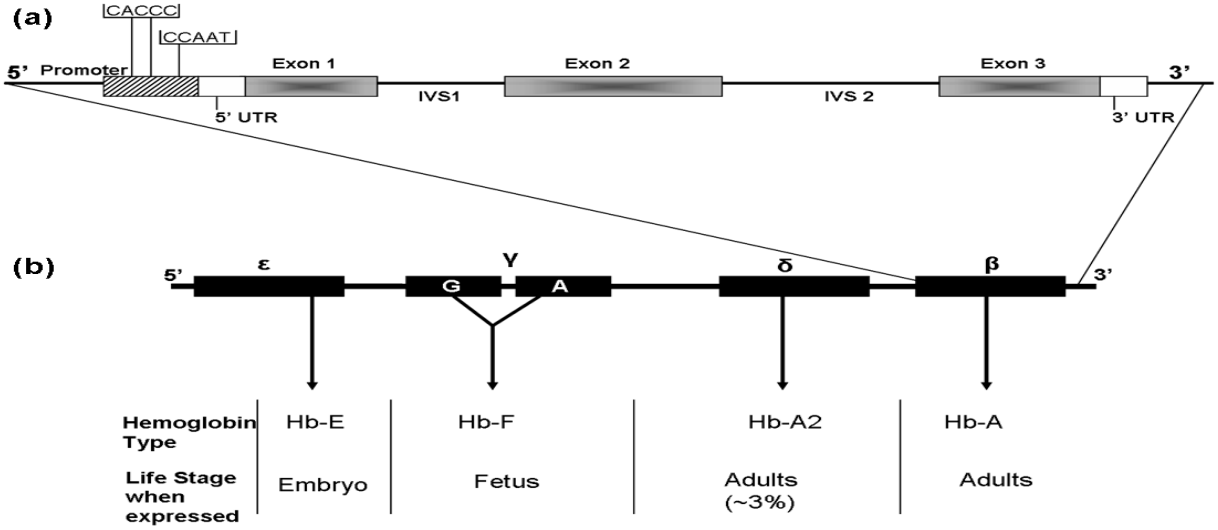

Fig. (2). Schematic representation of beta-globin locus. (a) Schematic structure of the beta-globin gene, with 3 exons (grey boxes) and the two intervening sequences (IVS). The gene is flanked by the 3' and 5' UTR regions (white boxes). The promoter region upstream of the 5' UTR (stripped box) contains the following regulatory sequences: TATA, CAAT, and duplicated CACCC boxes. (Adapted from Thein 2005); **(b)** Schematic structure of the beta-globin locus that resides on chromosome 11p15.4. From 5' the first gene, *HBE* (ε), is expressed at the embryonic stage to produce embryonic-hemoglobin (Hb-E). The next two genes, *HBG-G* (γ-G) and *HBG-A* (γ-A), are expressed during fetal development (12 weeks post-conception) to form fetal-hemoglobin (Hb-F). After 7 or 8 months after birth, adult-hemoglobin (Hb-A) is produced due to initiation of expression of the *HBB* (β) gene. 3% of adult-hemoglobin is adult-hemoglobin-type 2 (Hb-A2), composed of the δ-globin chain.

quent molecular characterization showed that the absent beta-globin chain from the putative "B^0 allele" was actually due to a variety of nonsense, frameshift, or sometimes splicing mutations [34]. In contrast, the B^+ allele was a considered to be mutation that resulted in reduced quantity of beta-globin protein [16, 21]. Subsequent molecular characterization showed that reduced beta-globin production related to the "B^+ allele" resulted from mutations in the *HBB* promoter region, polyadenylation signal, 5' or 3' untranslated region, or splicing sites [34].

Moreover, three classes of beta-thalassemia have long been recognized clinically: beta-thalassemia major, intermedia and minor. The three thalassemia types are differentiated clinically by the degree of anemia, with thalassemia major and minor having the most and least severe anemia, respectively. Subsequent molecular characterization has shown some genotype-phenotype correlation, with clinical severity roughly related to the mutation type (Fig. **3**) [34].

Thalassemia Major

Thalassemia major, also known as Cooley's anemia and Mediterranean anemia, is the most severe form of beta-thalassemia, since both mutations of both *HBB* alleles results in severely impaired beta-globin chain production [21, 35]. Three of the general allele combinations are responsible for this thalassemia phenotype — B^0/B^0, B^0/B^+, and sometimes B^+/B^+ (Fig. **3a**) [34].

In thalassemia major, the excess unpaired alpha-globin chains aggregate to form inclusion bodies [18]. These chain inclusion bodies damage RBC membranes, leading to intravascular hemolysis [18]. In addition, there is damage and premature destruction of RBC precursors, causing ineffective erythropoiesis [18]. Anemia is severe and oxygen transport is compromised [35]. In some patients, death would result without chronic blood transfusions. Other clinical manifestations include listless, fatigue, dyspnea, poor appetite, hepatosplenomegaly, heart failure and bone deformation and delayed puberty [16, 21, 35]. Laboratory abnormalities include microcystic anemia with abnormally shaped RBCs and abnormal Hb electrophoresis. Thalassemia major presents within the first two years of life and, with treatment affected individuals can live five decades or more [35].

Thalassemia Intermedia

Patients with beta-thalassemia intermedia have mild to moderate anemia and in most cases do not require blood transfusions [36]. This condition is milder than thalassemia major due to inheritance of a *HBB* mutation associated with reduced beta-globin chain production (Fig. **3b**) [18]. The deduced genotype is most commonly B^+/B^+ [34]. The clinical phenotype of thalassemia intermedia is roughly intermediate between thalassemia major and minor [37]. Common clinical features include splenic enlargement due to entrapment of damaged RBCs, with risk of iron overload due in part to increased intestinal absorption [16, 21, 36]. Although thalassemia intermedia can be associated with poor growth and bone abnormalities, it presents later in life and rarely affects longevity [16, 36]. Patients require regular monitoring because the clinical severity varies widely between patients and within a patient over time, with possible deterioration to the thalassemia major phenotype.

Several rare *HBB* variants phenotypically manifest thalassemia intermedia. For instance, individuals with dominantly inherited beta-thalassemia or inclusion body beta-thalassemia clinically exhibit thalassemia intermedia [38, 39]. These patients have moderate anemia and splenomegaly. More than 30 dominantly inherited beta-thalassemia cases have been described, resulting from a spectrum of molecular lesions ranging from missense mutations to nonsense muta-

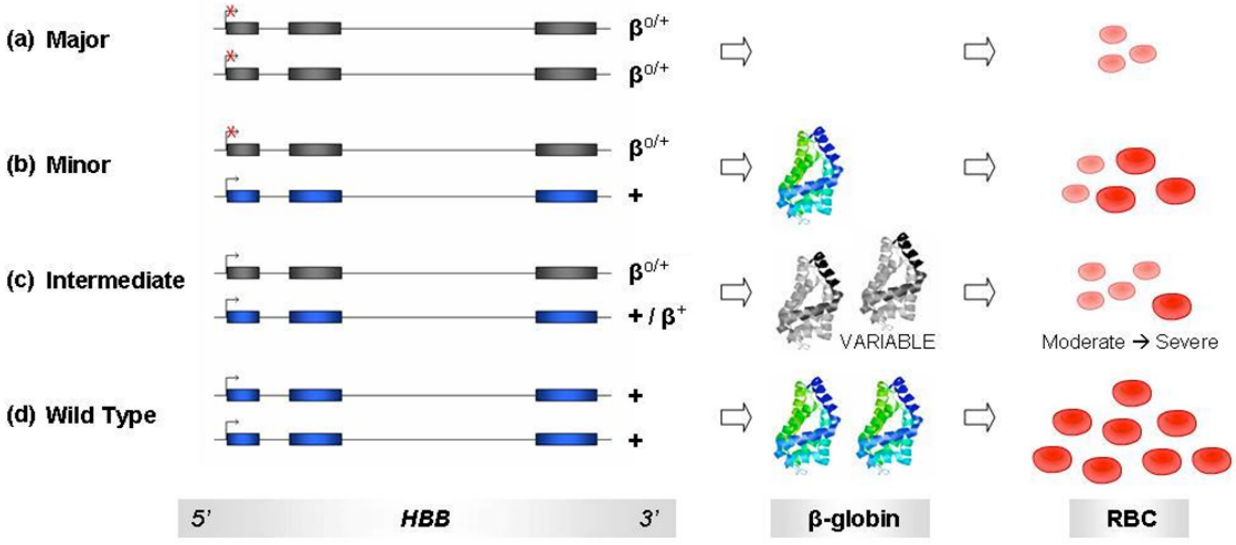

Fig. (3). Schematic representation of inherited beta-globin variants and related beta-chain and red blood cell (RBC) phenotype. The *HBB* variants are represented in grey exons while the wild type alleles are represented in blue exons. Production of beta-globin from a single/double wild type alleles are represented by one/two colored schematic of the beta-globin protein respectively. Grey colored beta-globin diagrams refer to below-normal synthesis levels of the protein, created by mutant *HBB* variants. Bright red-colored RBCs represent normal cell phenotype, while pink colored ones represent microcytic, hypochromic cells characteristic of beta-thalassemia phenotype. Relative number of RBC reflects relative levels of anemia amongst the three classes of beta-thalassemia and in comparison to the wild type RBC pool.

tions [40]. A rare variant form called "silent beta-thalassemia" results from a mild imbalance of globin chain synthesis due to reduced beta-globin synthesis, leading to thalassemia intermedia. Silent beta-thalassemia mutations are found mainly in the regulatory regions, *HBB* promoter and 5' and 3' UTRs. The most common silent mutation is the nt -101C>T (c.-151C>T) transition in *HBB* [41], which underlies most thalassemia intermedia cases in the Mediterranean region [42]. Thalassemia intermedia has also been observed in patients with a retro-transposition insertion of a L1 family transposable element in the *HBB* gene. This ~7 kb DNA insertion in IVS2 expresses ~15% of the full length beta-globin chain [43]. In addition, *trans*-acting genetic determinants, independent of the beta-globin locus, can cause the thalassemia intermedia phenotype [44]. For instance, mutations in the *XPD/ERCC2* gene, which is causative for trichothiodystrophy (MIM 601675) [19], have also been associated with the beta-thalassemia phenotype [45], as demonstrated by reduced levels of beta-globin synthesis and mRNA levels. The XPD protein coded by this gene is a subunit of the TF11H transcription factor, involved in basal transcription and DNA repair [45].

Thalassemia Minor

Thalassemia minor is most common form of beta-thalassemia, and is also known as the 'thalassemia trait', in which affected individuals are asymptomatic (Fig. **3c**) [18, 21]. These subjects are typically heterozygous for beta-thalassemia since they carry one normal *HBB* allele and one thalassemia allele - either B^0 or B^+ [34]. Asymptomatic patients are usually detected through routine hematologic testing, but in retrospect some newly diagnosed patients are observed to have mild anemia and small RBCs [16]. The primary caution for individuals with thalassemia minor is a potential risk of having children affected with more serious thalassemia if their partner is also a carrier of thalassemia minor [34].

GENETIC SUSCEPTIBILITY UNDERLYING BETA-THALASSEMIA COMPLICATIONS

Complications in older patients with beta-thalassemia appear to develop in part as a result of certain genetic susceptibilities [13]. For instance, hyperbilirubinemia and cholelithiasis are common complications among beta-thalassemia patients, since bilirubin is the breakdown product of RBCs and can be a component of certain gallstone types. There appears to be an increased incidence of these complications in patients who carry an insertion polymorphism in the promoter region of the uridine diphosphate-glucoronyltransferase IA (*UGT1A*) gene [13]. Also, common polymorphisms of the vitamin D receptor (*VDR*) and collagen type alpha1 (*COL1A1)* genes [13] can modulate the severity of progressive osteoporosis and osteopenia in beta-thalassemia. In addition, decreased frequency of the *APOE* (apolipoprotein E) E4 allele has been shown to be a risk factor for development of ventricular failure in beta-thalassemia [13].

PREVALENCE OF BETA-THALASSEMIA IN THE INDIAN SUBCONTINENT (SOUTH ASIA)

Within the Indian Subcontinent (or South Asia), which includes the countries of Pakistan, Sri Lanka and India, there are ~45 million carriers (carrier rate of ~1:20) of beta-thalassemia [46]. There are four common beta-thalassemia mutational 'hotspots' in the *HBB* gene found in this population: g.63201_63819del619 (619bp deletion), c.27_28insG, c.92+1G>A and c.92+5G>C [18] (Fig. **1**).

In beta-thalassemia patients from Pakistan, there was a high consanguinity rate (81%) [47, 48]. The polymorphism at c.92+5G>C, in the *HBB* gene, was the most common mutation (37.0%), followed by c.27_28insG and 619bp deletion with allele frequencies of 21.0% and 12.0%, respectively [48] (Table **1**).

In Sri Lanka, the two most prevalent *HBB* mutations were c.92+5G>C and c.92+1G>A with allele frequencies of 56.0% and 27.0%, respectively [48] (Table **1**).

In Indian patients, five mutations accounted for 92% of the beta-thalassemia alleles (Table **1**). The most common mutation was c.92+5G>C, as in Pakistan and Sri Lanka. The other four mutations were c.92+1G>T, 619bp deletion, c.27_28insG, and at c.124_127delTTCT. Regional differences in prevalence across north, east and south India, has also been demonstrated [48]. Within the Northern states, including Punjab, the mutations and allele frequencies are quite similar to those of Pakistan where the two most com-

Table 1. Frequency (in percentage) of common β-globin (*HBB*) gene mutation in Indian Subcontinent countries. All mutation detected were homozygous

Mutation	Pakistan	Sri Lanka	North India	East India	South India
c.27_28insG*	21	N/A[+]	N/A[+]	N/A[+]	N/A[+]
c.92+1G>A*	N/A[+]	27	38.5	N/A[+]	N/A[+]
c.92+5G>C*	37	56	43.5	48.8	N/A[+]
c.92+5G>T	N/A[+]	N/A[+]	N/A[+]	N/A[+]	74
c.124_127delTTCT	N/A[+]	N/A[+]	N/A[+]	N/A[+]	N/A[+]
619bp deletion[‡]	12	N/A[+]	6.8	N/A[+]	N/A[+]
[Ref]	[47]	[48]	[47, 48]	[49]	[50]

[+]N/A—data not available; [‡]619bp deletion: g.63201_63819del619.
*mutational hot spots shared with the Middle East.

mon mutations were c.92+5G>C (43.5%) and c.27_28insG (38.5%) [47]. The similarity in mutation frequencies in these regions reflects their geographical proximity. In addition, 6.6% of confirmed cases in northern India harbored the *HBB* c.124_127delTTCT mutation [47]. Within the Eastern region of India, including the state of West Bengal, the frequency of *HBB* c.92+5G>C was 48.8% [49]. Interestingly, 31.9% of mutations involved the *HBE* gene, a pattern that was particular for the eastern region of India. In South India, including the province of Andhra Pradesh, the *HBB* c.92+5G>T mutation frequency was 74% [50]. Other important South Indian *HBB* mutations were not found among other common South Asian *HBB* mutations, indicating regional differences in the genetic architecture of beta-thalassemia. Such differences in *HBB* mutation may reflect a high prevalence of consanguinity and non-random mating that occurs within some rural South Indian communities [50].

Overall these studies in the Indian Subcontinent demonstrate the importance of collecting and replicating *HBB* mutation frequencies, since this is the first step in establishing a successful prenatal diagnosis program [48].

PREVALENCE OF BETA-THALASSEMIA THROUGHOUT THE MIDDLE EAST

There is a high prevalence of beta-thalassemia in countries of the Middle East including Iraq, Lebanon, Egypt and Morocco, with an average carrier rate of ~1:30. More than 90% of affected individuals in the Middle East have *HBB* mutations within one of several mutational 'hotspots', including: c.25_26delAA, c.27_28insG, c.92+5G>C, c.118C>T, c.135delC, and c.315+1G>A (Fig. **1**).

In Iraq, beta-thalassemia is an evident health problem, specifically in the Dohuk region located in the northern part of the country [51]. The Dohuk region lies midway between Iran, Turkey and Syria, countries also characterized by a relatively high frequency of beta-thalassemia. The disease prevalence in Dohuk is reinforced by the high rate of consanguineous marriages in the region, which were estimated at 25.3% [51]. Molecular testing of 104 (50 male, 54 female) registered beta-thalassemia patients from Dohuk detected 12 mutations. The eight most common mutations observed accounted for 81.7% of the thalassemia alleles [51]. The mutations in descending order of frequency were: c.315+1G>A, c.135delC, c.17_18delCT, c.92+1G>A, c.92+6T>C, c.118C>T, c.27_28insG, and c.92+5G>C (Table **2**). The remaining four infrequent mutations were: c.25_26delAA, c.68_74delAAGTTGG, c.93G>C and c.93-21G>A. Further studies are required to provide a more thorough and accurate representation of common beta-thalassemia mutations in other Iraqi subpopulations. The frequencies of observed beta-thalassemia mutations in neighboring countries suggested mutational flow from Iraq; for instance, the *HBB* c.135delC mutation was much more frequent (12.5%) in Dohuk than in the neighboring regions of Syria, Turkey and Iran (0-2.6%), suggesting it originated in Dohuk.

In Lebanon, beta-thalassemia is the most common genetic disorder [52]. In a recent study of 255 patients from Lebanon, 6 *HBB* mutations were found in >85% of beta-thalassemia patients [52]. These mutations in descending order of prevalence, were: c.93-21G>A, c.92+1G>A, c.92+6T>C, c.90C>T, c.315+1G>A, and c.17_18delCT [52] (Table **2**). The Lebanese population also has a religious gra-

Table 2. Allele frequency (in percentage) of common β-globin (*HBB*) gene mutation in Middle Eastern countries

Mutation	Iraq	Lebanon	Egypt	Morocco
c.-79A>G	N/A[+]	N/A[+]	N/A[+]	7
c.17_18delCT	10.6	5	N/A[+]	N/A[+]
c.20delA	N/A[+]	N/A[+]	N/A[+]	10
c.25_26delAA	N/A[+]	N/A[+]	N/A[+]	15.5
c.27_28insG*	7.7	N/A[+]	N/A[+]	N/A[+]
c.90C>T	N/A[+]	9.6	N/A[+]	N/A[+]
c.92+1G>A*	8.7	15	19	13
c.92+5G>C*	6.7	N/A[+]	N/A[+]	N/A[+]
c.92+6T>C	8.7	14.4	36.3	14
c.93+21G>A	N/A[+]	34.2	25.8	N/A[+]
c.118C>T	8.7	N/A[+]	N/A[+]	15.5
c.135delC	12.5	N/A[+]	N/A[+]	N/A[+]
c.315+1G>A	18.3	8.6	N/A[+]	N/A[+]
c.316-106C>G	N/A[+]	N/A[+]	6.4	N/A[+]
[Ref]	[51]	[52]	[53-56]	[57, 58]

[+]N/A—data not available.
*mutational hot spots shared with the Indian Subcontinent.

dient of *HBB* mutational diversity. Sunni Muslims had the highest beta-thalassemia carrier rate and also the greatest mutational heterogeneity, with 16 *HBB* different mutations. Shiite Muslims had 13 *HBB* mutations, while Maronites had 7 different *HBB* mutations, while other religious factions including Orthodox, Druze, Greek Catholic and Latin, had progressively less mutational diversity. *HBB* locus haplotype analysis showed that the observed genetic diversity originated from both new mutational events and gene flow from population migration [52].

Among Egyptians, beta-thalassemia is the most common cause of chronic hemolytic anemia and represents a major health concern. A recent study of 95 Egyptian beta-thalassemic cases illustrated that the three most common *HBB* mutations observed were at c.92+6 (36.3%), c.93-21 (25.8%), and c.92+1 (19.0%) [53], similar to the findings in other studies carried out on Egyptians [54-56] (Table **2**). Moreover, the seven most frequent alleles in this study accounted for 84.2% of the observed thalassemic alleles in Egypt.

Finally, in a study of beta-thalassemia in Morocco, six *HBB* mutations were seen in >75% of patients [57, 58]. These were in descending order of frequency: c.118C>T, c.25_26delAA, c.92+6T>C, c.92+1G>A, c.20delA, and c.-79A>G (Table **2**). Regional predominance was observed in the Gharb and West regions for the c.92+6T>C mutation. The distribution of mutations was suggested to correlate with historical migration patterns of Berbers, Phoenicians, Carthaginians, Romans, Arabs, and Vandals, Byzantines and Sub-Saharan Africans [57].

Studies in the Middle East and Indian Subcontinent reveal an overlap in mutational hotspots for beta-thalassemia (Tables **1** and **2**). Such overlap may be due to genetic relatedness and intermarriages between these two regions. More importantly, it may be indicative of starting points for development of effective molecular therapy methods for both geographical regions.

MOLECULAR DIAGNOSTIC TESTING

While beta-thalassemia is observed in most global populations, each population or subpopulation has its own unique spectrum of beta-thalassemia mutations [48]. In both the Indian Subcontinent and the Middle East, beta-thalassemia is a major public health problem [46, 48]. It has been estimated that 10% of the world's beta-thalassemia major infants are born either in the Indian Subcontinent or the Middle East [5, 46]. In fact, there are ~12,300 total beta-thalassemia births per year in Indian Subcontinent and the Middle East. The cost for blood transfusions for beta-thalassemia major has been projected at ~3200 USD per child per year [46] while the lifetime healthcare costs of caring for a person born with thalassaemia major has been projected at 284,154 USD [59]. Since this exceeds the economic reach of most families in the Indian Subcontinent and Middle East, genetic counseling and prenatal diagnosis are an important component of multi-pronged strategy to reduce the burden of beta-thalassemia in these jurisdictions [46, 60]. Thus, it is important to collect, replicate and corroborate the frequencies of specific *HBB* mutations in these jurisdictions, especially when the presence of a few common mutations explains a large proportion of cases, enabling relatively cost-effective molecular diagnosis of carriers and prenatal diagnosis [48].

Unknown *HBB* mutations must be discovered using screening methods such as direct sequencing of genomic DNA, single strand conformation polymorphism analysis or denaturing gradient gel electrophoresis [61]. However, since these techniques require specialized skill and reagents, they are expensive [61]. They are presently not ideal for economically-unstable countries within Indian Subcontinent and the Middle East. Therefore, the strategy of developing dedicated allele-specific methods to find the most prevalent geographically-relevant mutations causing beta-thalassemia based on screening for a small number of known mutations that underlie a majority of cases is a more realistic goal [47].

Current technologies for clinical detection of specific mutant alleles include: 1) **A**mplification **R**efractory **M**utation **S**ystem-**P**olymerase **C**hain **R**eaction (ARMS-PCR) and 2) **R**everse **D**ot-**B**lot (RDB) analysis, among many others. ARMS-PCR amplifies both wild-type and mutant alleles, together with a control fragment, in a single tube reaction [62]. Two allele-specific (inner) primers and two non–allele-specific (outer) primers amplify both the wild-type and the mutant amplicons, resulting in products of different lengths that can be easily identified using agarose-gel electrophoresis [62]. In contrast, RDB involves amplification and biotin-labeling of the DNA sequence of interest, followed by hybridization of the amplified products to oligonucleotide probes immobilized on a membrane [63]. Indeed in many studies, fetal DNA from chorionic villus sampling was screened tests including ARMS-PCR method and by RDB analysis [47-50]. Once fetuses were diagnosed, the option of pregnancy termination can be offered, within the boundaries of consideration given to appropriate cultural and ethical sensitivities.

CURRENT TREATMENTS FOR BETA-THALASSEMIA

The outlook for patients with beta-thalassemia has improved steadily during the last two decades due to developments in treatment [11, 64]. In addition, newer treatments currently under investigation will have a great impact in the next few years and ease the clinical burden of this disorder [22]. However, as a result, some treatments have been associated with an increase in the development of iatrogenic complications.

Blood Transfusion Therapy and its Complications

The commonest form of life-long treatment for individuals with beta-thalassemia major is regular blood transfusions in order to maintain a Hb blood concentration >90 g/L and to compensate for ineffective erythropoiesis [16, 65]. Moreover for beta-thalassemia major, patients are unable to grow and develop at infancy and may die if untreated by regular transfusions [66]. However, regular and recurring blood transfusions can be complicated by high rates of blood-borne infection; for instance ~70% of transfused children in some developing countries may acquire hepatitis C and even hepatitis B [13, 24]. Aggressive treatment approaches for hepatitis are being used to avoid eventual liver cirrhosis and hepatocellular carcinoma [35].

Iron overload is an important complication due to the iron present in the transfused blood as well as excessive iron absorption [67]. Iron overload - acquired hemochromatosis - produces reactive oxygen species that damage the heart (cardiomyopathy), liver (fibrosis and cirrhosis), nervous system, can lead to diabetes mellitus, hypothyroidism, hyperparathyroidism as well as adrenal and pituitary insufficiencies [21]. Susceptibility to specific endocrinopathies should be monitored and treated as they develop. Iron overload also plays a pivotal role in cochlear siderosis and is the cause of various neurological disorders [18]. Pituitary iron overload, which results in hypogonadotrophic hypogonadism, may lead to low fertility [22].

In order to minimize iron overload and its complications, iron chelation is provided as an adjunct to regular blood transfusion. Chelators inertly and tightly bind iron ions and remove excess iron. Deferoxamine (Desferal®), an iron chelator, is administered parenterally [67]. Chelator treatment is expensive because of the dosage, equipment and skill required [64]. To decrease expense and dosage requirement while increasing effectiveness, a combination of chelators should be employed, such as deferoxamine together with such orally-active chelators as deferiprone or deferasirox (ICL670) [22]. However, secondary complications have arisen from deferoxamine therapy. For instance deferoxamine predisposes patients to *Yersinia* septicemia, which is problematic in regions with endemic *Yersinia* such as the Middle East or Indian Subcontinent [24]. In addition, side effects of deferoxamine include ocular and auditory toxicity, growth retardation, and occasionally, renal impairment and interstitial pneumonitis [18]. Thus, patients with beta-thalassemia who receive regular blood transfusions together with deferoxamine should be monitored. Magnetic resonance imaging (using the T_2^* relaxometry method) can be utilized for non-invasive, safe and accurate assessment of affected tissues, such as cardiac tissue, since iron overload causes deposition in vulnerable areas such as ventricular walls and epicardial layer, potentially leading to serious arrhythmias or congestive heart failure [22, 68].

Depressed Immunity

Beta-thalassemia patients, having survived severe anemia due to early management and diagnosis often have depressed immunity [22]. As a result, bacterial and fungal infections contribute to high mortality in beta-thalassemia patients [24]. Major microbial infections, including *Klebsiella* (mainly in Asia), *Escherichia coli* and *Streptococcus pneumonia*, have been observed [24]. Recently, in Asia, a fungus-like infection called pythosis from *Pythium insidiosum* has been observed in patients with beta-thalassemia [24]. Treatment has been difficult because anti-fungal drugs and anti-fungal vaccines are often ineffective. Moreover, many of these infections are endemic in the Indian Subcontinent and the Middle East and pose a risk to immune-suppressed beta-thalassemia patients.

Other Common Therapies

Adults with beta-thalassemia are susceptible to thrombosis, the most serious clinical outcome of which is pulmonary embolism and pulmonary hypertension [7, 69]. A daily low-dose of aspirin prescribed prophylactically can reduce this risk [6, 16]. Other forms of treatment for thalassemia major and severe cases of thalassemia intermedia include folate supplements [18] and splenectomy to decrease transfusion requirements [35]. Severe infections, such as pneumococcal meningitis and pneumonia, may possibly complicate splenectomy [24, 70]. As a result patients would benefit from prophylactic antibiotics and vaccinations.

The only treatment that can cure thalassemia major is allogeneic bone marrow (stem cell) transplantation. Since it was developed by the Pesaro group in 1981 there have been more than a 1000 patients with transplantations with a thalassemia-free survival rate of 68% [71]. Thus far, allogeneic bone marrow transplantation seems to provide a definitive cure of this disease. Due to the high risks involved, careful decision-making is necessary when considering such transplants. When transplantation is the treatment of choice, it is pursued only for patients with human leukocyte antigen-matched donors [72].

FUTURE THERAPIES

A novel approach that provides an alternative to high-risk donor transplantation for severe beta-thalassemic patients is the transfer of normal human beta-globin gene in autologous hematopoietic stem cells [73]. Another approach to improve the clinical status of beta-thalassemia patients aims to increase the synthesis of Hb-F, an alternative source of Hb. This treatment option is especially used in cases of beta-thalassemia intermedia and can help alleviate anemia [74]. Production of Hb-F was noted to be reactivated during the recovery from marrow suppression after treatment of hematologic malignancies with cytotoxic drugs. Thus, it was postulated that the hypomethylating agent 5-azacytidine, which switches off expression of adult to fetal Hb form in adult baboons, and butyrate may alter the pattern of erythropoeisis in humans with beta-thalassemia and increase the expression of γ-chain genes [64]. Although the mechanism is unknown, hydroxyurea treatment has been observed to increase Hb levels, reduce brain masses and leg ulcers [65]. Also, studies in mouse models are being pursued for gene correction in hematopoietic stem cells using lentiviral vectors [64].

But while advances in therapy continue to be made, management of primary and secondary complications has had a major impact on the quantity and quality of life in patients with beta-thalassemia. Further investigations as well as individual-based therapies may allow for more efficient treatment methodologies in the future.

CONCLUSION

In conclusion, beta-thalassemia is highly prevalent and is a major public health problem in the malaria endemic areas of the Indian Subcontinent and Middle East. Cross-sectional surveys indicate that in many regions of the Indian Subcontinent and Middle East, only a few prevalent *HBB* mutations underlie the majority of patients with beta-thalassemia. This suggests that relatively cost-effective dedicated carrier screening methods could be implemented in these areas. However, imminent application of molecular screening in the Indian Subcontinent and Middle East is complicated by regional differences and the fact that sizable minorities of patients with beta-thalassemia in some areas do not result from previously known *HBB* mutations. Although early diagnosis and treatment measures are available, they can be

expensive and some, such as blood transfusion and treatment with iron-chelators, can have potentially serious complications. Thus, prenatal diagnosis or other preventative approaches may be the most important strategy to control the clinical problems arising from beta-thalassemia. Along with this, millions of abortions are performed annually due to molecular diagnostic testing that raise many ethical and economical challenges, for which time and financial assistance should be invested to effectively handle this issue.

AUTHORS' CONTRIBUTIONS

All authors participated in literature review and manuscript preparation. All authors approved the final version of the manuscript.

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REFERENCES

[1] Kham SK, Quah TC, Loong AM, *et al.* A molecular epidemiologic study of thalassemia using newborns' cord blood in a multiracial Asian population in Singapore: results and recommendations for a population screening program. J Pediatr Hematol Oncol 2004; 26 (12): 817-9.

[2] Wong P, Thanormrat P, Srithipayawan S, *et al.* Risk of a couple having a child with severe thalassemia syndrome, prevalence in lower northern Thailand. Southeast Asian J Tropical Med Public Health 2006; 37 (2): 366-9.

[3] Livingstone FB. Frequencies of Hemoglobin Variants: Thalassemia, the Glucose-6-Phosphate Dehydrogenase Variants, and Ovalocytosis in Human Populations. Oxford, UK: Oxford University Press 1985.

[4] Weatherall DJ. Pharmacological treatment of monogenic disease. Pharmacogenomics J 2003; 3(5): 264-6.

[5] Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bulletin of the World Health Organization 2001; 79(8): 704-12.

[6] Aessopos A, Kati M, Farmakis D. Heart disease in thalassemia intermedia: a review of the underlying pathophysiology. Haematologica 2007; 92(5): 658-65.

[7] Ataga KI, Cappellini MD, Rachmilewitz EA. Beta-thalassaemia and sickle cell anaemia as paradigms of hypercoagulability. Br J Haematol 2007; 139(1): 3-13.

[8] Barton JC. Chelation therapy for iron overload. Curr Gastroenterol Rep 2007; 9(1): 74-82.

[9] Efremov GD. Dominantly Inherited beta-Thalassemia. Hemoglobin 2007; 31(2): 193-207.

[10] Ghosh K, Ghosh K. Pathogenesis of anemia in malaria: a concise review. Parasitol Res 2007; 101(6): 1463-9.

[11] Quek L, Thein SL. Molecular therapies in beta-thalassaemia. Br J Haematol 2007; 136(3): 353-65.

[12] Theodorsson E, Birgens H, Hagve TA. Haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency in a Scandinavian perspective. Scandinavian J Clin Lab Invest 2007; 67(1): 3-10.

[13] Thein SL. Genetic modifiers of beta-thalassemia. Haematologica 2005; 90(5): 649-60.

[14] Angastiniotis M, Modell B. Global epidemiology of hemoglobin disorders. Ann N Y Acad Sci 1998 30; 850: 251-69.

[15] Flint J, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. Bailliere's Clin Haematol 1998; 11(1): 1-51.

[16] Bunn HF FB. Hemoglobin: Molecular, Genetic and Clinical Aspects W.B. Saunders Company 1984.

[17] Bridges K. How Do People Get Thalassemia? Information Center for Sickle Cell and Thalassemic Disorders 1998 [cited 16/11/2007; Available from: http: //sickle.bwh.harvard.edu/thal_inheritance.html.

[18] Cao A GR. Beta-Thalassemia. Gene Reviews 2005 [cited 16/11/2007; Available from: www.genetest.org.

[19] OMIM. Hemaglobin-Beta Locus; HBB. John Hopkins University 1986.

[20] Grosveld F, de Boer E, Dillon N, *et al*. The dynamics of globin gene expression and gene therapy vectors. Semin Hematol 1998 ; 35(2): 105-11.

[21] Rund D, Rachmilewitz E. Beta-thalassemia. New Eng J Med 2005; 353(11): 1135-46.

[22] Cohen AR, Galanello R, Pennell DJ, Cunningham MJ, Vichinsky E. Thalassemia. Hematol Education Prog Am Soc Hematol Am Soc Hematol 2004: 14-34.

[23] Patrinos GP, Giardine B, Riemer C, *et al.* Improvements in the HbVar database of human hemoglobin variants and thalassemia mutations for population and sequence variation studies. Nucleic acids Res 2004 1; 32(Database issue): D537-41.

[24] Vento S, Cainelli F, Cesario F. Infections and thalassaemia. The Lancet Infectious Diseases 2006; 6(4): 226-33.

[25] Waye g JS, EnB, Patterson M, Barr RD, Chui DH. De novo mutation of the beta-globin gene initiation codon (ATG-->AAG) in a Northern European boy. Am J Hematol 1997; 56(3): 179-82.

[26] Grignoli CR, Carvalho MH, Kimura EM, *et al*. beta0-thalassemia resulting from a novel mutation: beta66/u-->stop codon. Eur J Haematol 2000; 64(2): 137-8.

[27] Fisher CA, Premawardhena A, de Silva S, *et al.* The molecular basis for the thalassaemias in Sri Lanka. Br J Haematol 2003; 121(4): 662-71.

[28] De Angioletti M, Lacerra G, Sabato V, Carestia C. Beta+45 G --> C: a novel silent beta-thalassaemia mutation, the first in the Kozak sequence. Br J Haematol 2004 ; 124(2): 224-31.

[29] Shaji RV, Edison ES, Poonkuzhali B, Srivastava A, Chandy M. Rapid detection of beta-globin gene mutations and polymorphisms by temporal temperature gradient gel electrophoresis. Clin Chem 2003; 49(5): 777-81.

[30] Orkin SH, Old JM, Weatherall DJ, Nathan DG. Partial deletion of beta-globin gene DNA in certain patients with beta 0-thalassemia. Proc Natl Acad Sci USA 1979; 76(5): 2400-4.

[31] Flatz G, Wilke K, Syagailo YV, Eigel A, Horst J. Beta-thalassemia in the German population: mediterranean, Asian and novel mutations. Mutations in brief no.228. Online. Human Mutation. 1999; 13(3): 258.

[32] Agarwal S, Hattori Y, Agarwal SS. Identification of a novel frameshift beta-thalassemia mutation in an Asian Indian. Clin Genet 2000; 57(4): 311-2.

[33] Zertal-Zidani S, Ducrocq R, Sahbatou M, Satta D, Krishnamoorthy R. Foetal haemoglobin in normal healthy adults: relationship with polymorphic sequences cis to the beta globin gene. Eur J Hum Genet 2002; 10(5): 320-6.

[34] Thein SL. Genetic insights into the clinical diversity of beta thalassaemia. Br J Haematol 2004 ; 124(3): 264-74.

[35] Wonke B. Clinical management of beta-thalassemia major. Semin Hematol 2001; 38(4): 350-9.

[36] Taher A, Isma'eel H, Cappellini MD. Thalassemia intermedia: revisited. Blood cells, molecules & diseases. 2006; 37(1): 12-20.

[37] Phadke SR, Agarwal S. Phenotype score to grade the severity of thalassemia intermedia. Indian J Pediatrics 2003; 70(6): 477-81.

[38] Thein SL, Hesketh C, Taylor P, *et al.* Molecular basis for dominantly inherited inclusion body beta-thalassemia. Proc Natl Acad Sci USA 1990 ; 87(10): 3924-8.

[39] Stamatoyannopoulos G, Woodson R, Papayannopoulou T, Heywood D, Kurachi S. Inclusion-body beta-thalassemia trait. A form of beta thalassemia producing clinical manifestations in simple heterozygotes. New Eng J Med 1974 25; 290(17): 939-43.

[40] Thein SL. Is it dominantly inherited beta thalassaemia or just a beta-chain variant that is highly unstable? Br J Haematol 1999; 107(1): 12-21.

[41] Gonzalez-Redondo JM, Stoming TA, Kutlar A, *et al.* A C----T substitution at nt--101 in a conserved DNA sequence of the promotor region of the beta-globin gene is associated with "silent" beta-thalassemia. Blood 19891; 73(6): 1705-11.

[42] Maragoudaki E, Kanavakis E, Traeger-Synodinos J, *et al.* Molecular, haematological and clinical studies of the -101 C --> T substitution of the beta-globin gene promoter in 25 beta-thalassaemia intermedia patients and 45 heterozygotes. Br J Haematol 1999; 107(4): 699-706.

[43] Galanello R, Dessi E, Melis MA, *et al*. Molecular analysis of beta zero-thalassemia intermedia in Sardinia. Blood 1989 1; 74(2): 823-7.

[44] Giordano PC, Harteveld CL, Haak HL, *et al*. A case of non-beta-globin gene linked beta thalassaemia in a Dutch family with two additional alpha-gene defects: the common -alpha3.7 deletion and the rare IVS1-116 (A-->G) acceptor splice site mutation. Br J Haematol 1998 ; 103(2): 370-6.

[45] Viprakasit V, Gibbons RJ, Broughton BC, *et al.* Mutations in the general transcription factor TFIIH result in beta-thalassaemia in individuals with trichothiodystrophy. Human Mol Genet 2001; 10(24): 2797-802.

[46] Agarwal S, Gupta A, Gupta UR, Sarwai S, Phadke S, Agarwal SS. Prenatal diagnosis in beta-thalassemia: an Indian experience. Fetal Diagnosis And Therapy 2003; 18(5): 328-32.

[47] Baig SM, Azhar A, Hassan H, *et al.* Prenatal diagnosis of beta-thalassemia in Southern Punjab, Pakistan. Prenatal Diagnosis 2006; 26(10): 903-5.

[48] Old JM, Khan SN, Verma I, *et al.* A multi-center study in order to further define the molecular basis of beta-thalassemia in Thailand, Pakistan, Sri Lanka, Mauritius, Syria, and India, and to develop a simple molecular diagnostic strategy by amplification refractory mutation system-polymerase chain reaction. Hemoglobin 2001; 25(4): 397-407.

[49] Bandyopadhyay A, Bandyopadhyay S, Basak J, *et al*. Profile of beta-thalassemia in eastern India and its prenatal diagnosis. Prenatal Diagnosis 2004 15; 24(12): 992-6.

[50] Bashyam MD, Bashyam L, Savithri GR, Gopikrishna M, Sangal V, Devi AR. Molecular genetic analyses of beta-thalassemia in South India reveals rare mutations in the beta-globin gene. J Hum Genet 2004; 49(8): 408-13.

[51] Al-Allawi NA, Jubrael JM, Hughson M. Molecular characterization of beta-thalassemia in the Dohuk region of Iraq. Hemoglobin 2006; 30(4): 479-86.

[52] Makhoul NJ, Wells RS, Kaspar H, *et al*. Genetic heterogeneity of Beta thalassemia in Lebanon reflects historic and recent population migration. Ann Hum Genet 2005; 69(Pt 1): 55-66.

[53] El-Gawhary S, El-Shafie S, Niazi M, Aziz M, El-Beshlawy A. Study of beta-Thalassemia mutations using the polymerase chain reaction-amplification refractory mutation system and direct DNA sequencing techniques in a group of Egyptian Thalassemia patients. Hemoglobin 2007; 31(1): 63-9.

[54] Hussein IR, Temtamy SA, el-Beshlawy A, *et al.* Molecular characterization of beta-thalassemia in Egyptians. Hum Mutation 1993; 2(1): 48-52.

[55] Novelletto A, Hafez M, Deidda G, *et al.* Molecular characterization of beta-thalassemia mutations in Egypt. Hum Genet 1990; 85(3): 272-4.

[56] Omar A, Abdel Karim E, Gendy WE, Marzouk I, Wagdy M. Molecular basis of beta-thalassemia in Alexandria. The Egyptian J Immunol/ Egyptian Associat Immunol. 2005; 12(1): 15-24.

[57] Agouti I, Badens C, Abouyoub A, *et al.* Genotypic correlation between six common beta-thalassemia mutations and the XmnI polymorphism in the Moroccan population. Hemoglobin 2007; 31(2): 141-9.

[58] Lemsaddek W, Picanco I, Seuanes F, *et al.* The beta-thalassemia mutation/haplotype distribution in the moroccan population. Hemoglobin 2004 ; 28(1): 25-37.

[59] Ginsberg G, Tulchinsky T, Filon D, Goldfarb A, Abramov L, Rachmilevitz EA. Cost-benefit analysis of a national thalassaemia prevention programme in Israel. Journal of medical screening. 1998; 5(3): 120-6.

[60] Winichagoon P, Thitivichianlert A, Lebnak T, Piankijagum A, Fucharoen S. Screening for the carriers of thalassemias and abnormal hemoglobins at the community level. Southeast Asian J Tropical Med Public Health 2002; 33 Suppl 2: 145-50.

[61] Bhardwaj U, Zhang YH, Lorey F, McCabe LL, McCabe ER. Molecular genetic confirmatory testing from newborn screening samples for the common African-American, Asian Indian, Southeast Asian, and Chinese beta-thalassemia mutations. Am J Hematol 2005; 78(4): 249-55.

[62] Piccioli P, Serra M, Gismondi V, *et al.* Multiplex tetra-primer amplification refractory mutation system PCR to detect 6 common germline mutations of the MUTYH gene associated with polyposis and colorectal cancer. Clin Chem 2006; 52(4): 739-43.

[63] Steinlein LM, Crawford JT. Reverse dot blot assay (insertion site typing) for precise detection of sites of IS6110 insertion in the Mycobacterium tuberculosis genome. J Clin Microbiol 2001; 39(3): 871-8.

[64] Atweh GF, DeSimone J, Sauntharajah Y, *et al.* Hemoglobinopathies. Hematol/Education Prog Am Soc Hematol Am Soc Hematol 2003: 14-39.

[65] Morris CR, Singer ST, Walters MC. Clinical hemoglobinopathies: iron, lungs and new blood. Curr Opin Hematol 2006; 13(6): 407-18.

[66] Olivieri NF. The beta-thalassemias. New Eng J Med 1999; 341(2): 99-109.

[67] Bridges K. Chelators for Iron Overload. Information Center for Sickle Cell and Thalassemic Disorders 1999 [cited 16/11/2007; Available from: http: //sickle.bwh.harvard.edu/chelators.html.

[68] Argyropoulou MI, Astrakas L. MRI evaluation of tissue iron burden in patients with beta-thalassaemia major. Pediatr Radiol. 2007.

[69] Eldor A, Rachmilewitz EA. The hypercoagulable state in thalassemia. Blood 2002; 99(1): 36-43.

[70] Moshtaghi-Kashanian GR, Gholamhoseinian A, Hoseinimoghadam A, Rajabalian S. Splenectomy changes the pattern of cytokine production in beta-thalassemic patients. Cytokine 2006; 35(5-6): 253-7.

[71] Schrier SL, Angelucci E. New strategies in the treatment of the thalassemias. Ann Rev Med 2005; 56: 157-71.

[72] Rund D, Rachmilewitz E. Advances in the pathophysiology and treatment of thalassemia. Crit Rev Oncol/Hematol 1995; 20(3): 237-54.

[73] Sadelain M, Boulad F, Galanello R, *et al.* Therapeutic options for patients with severe beta-thalassemia: the need for globin gene therapy. Human Gene Therapy 2007; 18(1): 1-9.

[74] Olivieri NF. Reactivation of fetal hemoglobin in patients with beta-thalassemia. Semin Hematol 1996; 33(1): 24-42.

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