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ORIGINAL ARTICLE

Molecular Characterization of β-Thalassemia in Nineveh Province Illustrates the Relative Heterogeneity of Mutation Distributions in Northern Iraq

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Abstract Beta thalassemia is an important health problem in Nineveh province, a large province in Northwestern Iraq. No previous study of significance had focused on the spectrum of β-thalassemia mutations in this part of the country. A total of 94 unrelated β-thalassemia minor subjects from the latter province were recruited. Their carrier status was confirmed by full blood count, Hb A2 and F estimation. Thereafter their DNA was subjected to multiplex polymerase chain reaction and reverse hybridization to detect 20 β-thalassemia mutations. A total of eleven different β-thalassemia mutations were documented. The most frequent mutation was IVS-I-110 (G>A) documented in 34 %, followed by IVS-I-6 (T>C) in 9.6 %, IVS-I-5(G>C) in 8.5 %, codon 39 (C>T) and codon 44 (−C) in 7.4 % each, while IVS-I-1(G>A) and IVS-II-1(G>A) were encountered in 6.4 % each. Other mutations were less frequent including codon 8 (−AA), IVS-I-130 (G>C), codon 5 (−CT) and IVS-II-745(C>G). The current study revealed notable differences in the relative frequencies of several β-thalassemia mutations in Nineveh province as compared to other parts of Northern Iraq. Such an observation may be reflective of different ethnic backgrounds and varying historical population interactions. It is believed that these findings complement those of earlier studies on β-thalassemia mutations from the country, and are quite essential in the setting of a proposed national preventive program.

Keywords Beta thalassemia · Nineveh · Iraq · Molecular basis

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Introduction

Beta thalassemia is an inherited disorder of hemoglobin synthesis due to reduced (β^+) or absent (β^o) synthesis of the β-globin chains. More than 200 different mutations have been implicated, most being point mutations leading to defective expression of the β-globin gene [1, 2]. The distribution of these mutations varies in various parts of the world and in different ethnic groups, although generally a hand full of mutations constitute the bulk of these mutations in each particular population [1]. β-thalassemia is an important health problem in Iraq, with an estimated 15,000 registered patients with thalassemia major and intermedia, and an average of 4 % heterozygous carrier rate [3]. The problem is further aggravated by around 30 % consanguineous marriage rate [4]. Such a situation would make initiating a preventive program for this disorder a necessity. The determination of the mutations responsible for β-thalassemia in particular populations is an important prerequisite in establishing such a program in that population.

Nineveh Province is a large Northwestern Iraqi province, with Syria and Turkey to its West, and the three Northern Kurdish Iraqi provinces of Duhok, Erbil and Sulaimaniyah to its East (Fig. 1). It has an area of 37,323 km^2 and an estimated population of more than 2.5 million. Nineveh has been inhabited since 6000 BC and saw

Fig. 1 A map of Iraq showing the most frequent mutations in Nineveh province and other provinces in Northern Iraq, as well as surrounding countries

the rise of the Assyrian Empire, which was one of the early civilizations that flourished in ancient Mesopotamia. After the fall of Assyrian empire, this area exchanged hands between Median, Seleucid, Parthian, Persian and Islamic Empires, while it fell to the Mongols in the thirteenth century, and Turkmens in the sixteenth century and then and for the next four centuries it became part of Othman Empire, until the establishment of modern day Iraq in the early twentieth century [5]. Its population currently includes, in addition to a majority of Arabs, Kurdish and Turkmen ethnic groups. Previous studies have focused on β-thalassemia mutations in Northern and Northeastern Iraq where Kurds predominate, while none of significance have studied these mutations in this Northwestern province where other ethnic groups predominate [6–8]. Accordingly, this study was initiated to determine the spectrum of these mutations in this part of the country, in the setting of a proposed national preventive program for hemoglobinopathies.

Materials and Methods

A total of 94 unrelated β-thalassemia minor subjects were recruited for the purposes of this study. These subjects were parents of known patients with thalassemia major/intermedia, registered at the thalassemia centre in Nineveh province—Northwestern Iraq. The diagnosis of β-thalassemia minor was confirmed based on a combination of reduced MCV (<80 fL) and an increased Hb A2 (>3.5 %)

Table 1 The number and the relative proportion of different β-thalassemia mutations identified in the current study

Mutation	Phenotype	No. (%)	Origin
IVS-I-110 (G>A)	β^+	32 (34.0)	Mediterranean
IVS-I-6 (T>C)	β^+	9 (9.6)	Mediterranean
IVS-I- 5 (G>C)	β^+	8 (8.5)	Asian Indian
Codon 44 (−C)	β^o	7 (7.4)	Kurdish
Codon 39 (C>T)	β^o	7 (7.4)	Mediterranean
IVS-I-1 (G>A)	β^o	6 (6.4)	Mediterranean
IVS-II-1(G>A)	β^o	6 (6.4)	Mediterranean
Codon 8 (−AA)	β^o	5 (5.3)	Turkish
IVS-I-130 (G>C)	β^o	3 (3.2)	Turkish
Codon 5 (−CT)	β^o	3 (3.2)	Mediterranean
IVS-II-745 (C>G)	β+	2 (2.1)	Mediterranean
Uncharacterized		6 (6.4)	

[9]. The red cell indices were determined using a hematology analyzer (Beckman Coulter, Fullerton, CA, USA) and Hb A2 and F by high performance liquid chromatography (VARIANT^{TM}, Bio-Rad Laboratories, Hercules, CA, USA). Informed consent was obtained from all subjects, and the study was approved by the colleges of Medicine, universities of Duhok and Mousel, Iraq. All enrollees had their DNA extracted by a phenol–chloroform based method. The DNA was amplified in a multiplex reaction mixture, followed by hybridization to specific wild and mutant oligoprobes designed to detect 20 β-thalassemia mutations which are encountered in Mediterranean countries (β-Globin StripAssay MED® kit, Vienna Labordiagnostica GmbH, Vienna, Austria). The choice of this kit was based on previous studies from Iraq and surrounding countries [6–8, 10–13]. The β-thalassemia mutations that were screened for, included: −101 (C>T), −87 (C>G); −30 (T>A); codon 5 (−CT); codon 6 (−A); codon 8 (−AA); codon 8/9 (+G); codon 15 (TGG>TGA); codon 27 (G>T); IVS-I-1 (G>A); IVS-I-5(G>C); IVS-I-6 (T>C); IVS-I-110 (G>A); IVS-I-116(T>G); IVS-I-130 (G>C); codon 39 (C>T); codon 44 (−C); IVS-II-1 (G>A); IVS-II-745 (C>G) and IVS-II-848 (C>A). The amplification, hybridization and detection procedures were performed as recommended by the manufacturer.

Statistical analysis utilized the Mann–Whitney U test. p value <0.05 was considered significant.

Results

The enrolled thalassemia minor patients had a median age of 34 years (range 19–58 years) and included 35 males and 59 females. Seventy-one (75.5 %) were Arabs, 19 (20.2 %) were Turkmen, and 4 (4.3 %) were Kurds.

Table 2 The means ± standard deviations of the main hematological parameters in the eight main mutations in the current study

Mutation	No.	MCV (fL)	MCH (pg)	Hb A2 (%)	Hb F (%)
IVS-I-110 (G>A)[1]	32	63.85 ± 3.92	19.31 + 1.72	4.85 ± 0.71	1.1 ± 1.06
IVS-1-6 (T>C)[2]	9	68.79 ± 2.88	21.78 ± 1.28	4.53 ± 0.65	0.3 ± 0.42
IVS-1-5 (G>C)[3]	8	65.79 ± 5.85	20.26 ± 2.19	4.79 ± 0.58	1.38 ± 1.02
Codon 44 (−C)[4]	7	63.46 ± 2.81	20.64 ± 4.33	5.03 ± 0.47	1.64 ± 1.02
Codon 39 (C>T)[5]	7	62.8 ± 2.22	19.72 ± 0.95	4.68 ± 0.74	1.3 ± 1.04
IVS-1-1 (G>A)[6]	6	64.55 ± 7.09	20.20 ± 2.41	4.72 ± 0.67	1.23 + 0.81
IVS-II-1 (G>A)[7]	6	62.8 ± 1.94	18.64 ± 1.16	5.54 ± 0.23	1.54 ± 0.81
Codon 8 (−AA)[8]	5	63.58 ± 4.24	18.90 ± 1.68	5.42 ± 0.63	0.8 ± 1.03
Comparisons (*p* value if significant)		1 versus 2 (0.0014)	1 versus 2 (0.014)	1 versus 7 (0.009)	1 versus 2 (0.002)
		2 versus 4 (0.004)	2 versus 7 (0.0009)	2 versus 8 (0.045)	2 versus 3 (0.012)
		2 versus 5 (0.005)	2 versus 5 (0.003)	2 versus 7 (0.025)	2 versus 4 (0.005)
		2 versus 7 (0.006)	2 versus 8 (0.01)	5 versus 7 (0.036)	2 versus 5 (0.018)
		2 versus 8 (0.046)		6 versus 7 (0.011)	2 versus 7 (0.006)
					3 versus 6 (0.005)

The last row outlines the p value of significant differences between various carrier states. Non-listed comparisons are not significant ($p > 0.05$)

A total of eleven mutations were identified using StripAssay method (ViennaLab-Austria). Of the latter mutations, eight were the most frequent, seen in 85.1 % of the carriers screened. Of these eight mutations, the most frequent was IVS-I-110 (G>A), seen in 34.0 %, followed by IVS-I-6 (T>C), IVS-I-5(G>C), codon 39(C>T), codon 44(−C), IVS-I–I (G>A), IVS-II-1 (G>A) and codon 8 (−AA) (Table 1). Other mutations were less frequent, and included: IVS-I-130 (G>C), codon 5 (−CT) and IVS-II-745 (C>G).While only six subjects (6.4 %) remained uncharacterized by the employed procedures. Table 1 outlines the number and relative proportion of each of the characterized mutations.

The main hematological parameters for the carriers of the eight most frequent mutations are outlined in Table 2. The latter table demonstrates an evident overlap in the distribution of these parameters among the carriers of various mutations. However, one notable observation was that carriers of IVS-I-6 (T>C) had the least reductions in the MCV and the MCH, as well as the least Hb A2 and F values. The significance of these differences, as well as other significant differences between various genotypes are outlined in Table 2.

Discussion

The most frequent mutation encountered in the enrolled patients was IVS-I-110 (G>A), a severe Mediterranean β^+ mutation, which is also the most frequent mutation in neighboring Turkey, Syria as well as Lebanon, Jordan and upper Egypt (Fig. 1) [10–12, 14–18]. Haplotype studies have revealed that this mutation has the highest haplotype diversity in Turkey, and there were suggestions that it may have originated there in Neolithic period, and thereafter it was spread to other parts of the Eastern Mediterranean and Eastern Europe through gene flow, where its frequency had increased by Malaria selection in these fertile agricultural lands [19]. Interestingly, the predominance of IVS1-110 mutation in Nineveh province (NW Iraq) is in contrast to its sporadic presence in neighboring Iraqi Kurdish provinces of Erbil and Duhok [7], but is consistent with that recently reported in Central Iraq [20]. In the latter study and similar to the current study, Arabs constitute the majority. Thus it appears that the reason for the latter observation is likely to be related to differences in ethnic backgrounds, and variable admixture and interactions with surrounding populations.

The second most frequent mutation was IVS-I-6 (T>C), and this is a mild Mediterranean β^+ mutation, first described in a Portuguese patient, with one of its highest frequencies worldwide among Palestinians [21, 22]. Furthermore, it is also the second most common mutation in Turkey, and is frequent in Lebanon, but less frequent in Jordan, Syria and central Iraq [10–12, 15–20].

The third most common mutation encountered in the current study was IVS-I-5 (G>C). This mutation is the most common mutation in the Indian subcontinent [23, 24], and its presence in relatively high frequency is also shared by other parts of Northern as well as central Iraq [6–8, 20]. The latter is most likely related to the fact that Nineveh and Mesopotamia in general, and at various periods of history, served as important trade routes between India and the Mediterranean [5].

One notable observation of the current study was that the Mediterranean IVS-II-1 (G>A) mutation was encountered

in a mere 6.4 %, in contrast to frequencies of 18.3–28.7 % in the three neighboring Kurdish provinces in Northern and northeastern Iraq, where it was the most frequently encountered mutation [6, 7]. This mutation has its highest frequency in Iran, where it may have originated, and decreases in frequency as we go west, and its frequencies in Turkey, Syria and Lebanon are comparable to some extent to our figures [10–13].

Codon 44 (−C) is another frequent mutation in the current study, it is a Kurdish mutation with a high frequency in the nearby Duhok province just to the north of Nineveh [8], but is quite sporadic in the two other Kurdish provinces to the East [6, 7]. It was suggested that this mutation may have originated in the Duhok province more than 2,000 years ago [8]. The presence of this Kurdish mutation in Nineveh is not unexpected, because of the inevitable and close interaction between the populations living in these two provinces throughout their mutual history.

The overlap in hematologic parameters among carriers of different β-thalassemia mutations as demonstrated by the current study has been also documented by previous investigators [25]. The most notable relevant observation, was the association of the mild β^{+}IVS-I-6 with lesser reduction of MCV and MCH, and lower Hb A2, and this is consistent with the bulk of the literature [18, 25].

The choice of the technique employed in the current study, which is multiplex polymerase chain reaction (PCR) with reverse hybridization using a commercial kit (β-Globin StripAssay MED® kit), proved to be quite justified, as it managed to pick up 93.6 % of all β-thal mutations. Thus such a technique may be employed in the settings of a future prenatal diagnostic program because of its ability to detect the bulk of mutations, leaving only a small proportion of patients requiring sequencing. Furthermore, the findings may be the basis for introducing more cost-effective molecular diagnostic techniques, like amplification refractory mutation system (ARMS), since it identified the most common mutations in this region [24, 26].

In conclusion, this study, which is the first such report from NW Iraq, has revealed that a relatively wide spectrum of β-thal mutations including those of Mediterranean, Asian-Indian, Turkish and Kurdish origins was implicated. The latter spectrum when compared to previous reports from other parts of Northern Iraq, clearly shows an evident heterogeneity in the mutations distribution, which is likely to be a reflection of ethnic heterogeneity and variable historical interactions even within a relatively restricted geographical area. Moreover, the findings provide the health authorities with one of the essential pre-requisites before embarking on the much needed national preventive program for thalassemia, which has long been overdue.

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Conflict of interest The authors have no conflict of interest to report.

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