

Diagnosis of Beta-Thalassaemia Carriers in the Sultanate of Oman

*Shahina Daar¹, David Gravell²

تشخيص حاملي البيتا ثالاسيميا (β -Thalassaemia) في سلطنة عمان

الملخص: المقدمة: امراض خضاب الدم (الهيموجلوبين) من الاسباب الرئيسية للمراضة في سلطنة عمان. وقد تم تشجيع تقصي (غريلة) ما قبل الزواج لتقليل ولادة الأطفال المصابين بهذه الأمراض. أن التعرف على حاملي البيتا ثالاسيميا هو مطلب جوهري لأي برنامج للتقصي. على أية حال. مستوى خضاب الدم ($Hb A_2$) والمستخدم لكشف حاملي البيتا ثالاسيميا. يمكن أن يتأثر بعوامل أخرى كعوز الحديد. ووجود الفا ثالاسيميا (α thalassaemia) وكذلك في حالة طفرة في ال (دي ان أي) (DNA). **الهدف:** أجريت هذه الدراسة لمعرفة فيما إذا كان مستوى خضاب الدم $Hb A_2$ وسيلة مناسبة للتعرف على حاملي البيتا ثالاسيميا في عمان. **الطريقة:** تم قياس $Hb A_2$ بواسطة جهاز تحليل خاص عالي الفعالية (HPLC) ل 160 حامل مؤكد للبيتا ثالاسيميا. تم كشف صفات البيتا ثالاسيميا في 158 شخصا كان لديهم مستوى $Hb A_2$ أعلى من 3.5. واثنان فقط كان لديهم أقل من ذلك واكتشف انهما مصابين بعوز الحديد. بعد العلاج. ارتفع مستوى $Hb A_2$ الى أعلى من 3.5. الخلاصة: في غياب عوز الحديد يكون $Hb A_2$ مؤشرا دقيقا لوجود صفة البيتا ثالاسيميا في سلطنة عمان.

ABSTRACT Background: Haemoglobinopathies are a major cause of morbidity in the Sultanate of Oman and premarital screening is being encouraged in order to reduce the number of affected births. The identification of β -thalassaemia carrier status is an essential prerequisite of any screening programme. However, the level of Haemoglobin ($Hb A_2$), which is used to detect β -thalassaemia carriers, can be affected by other factors including iron deficiency, concurrent α thalassaemia and the type of DNA mutation present. **Objectives:** The following study was undertaken to ascertain if the $Hb A_2$ level is an appropriate tool for the identification of β -thalassaemia carriers in the Omani population. **Method:** $Hb A_2$ was measured by high performance liquid chromatography (HPLC) in 160 obligate carriers of β -thalassaemia. 158 subjects had $Hb A_2$ levels above 3.5% indicating β -thalassaemia trait. Two subjects had slightly lower levels and were found to be iron deficient. After therapy both these subjects' $Hb A_2$ levels increased to above 3.5%. **Conclusion:** In the absence of iron deficiency, $Hb A_2$ is an accurate marker for the presence of β -thalassaemia trait in the Sultanate of Oman.

Key words: $Hb A_2$, β -thalassaemia trait, Sultanate of Oman

IN THE NORMAL POPULATION HAEMOGLOBIN A_2 ($\alpha_2\delta_2$) is physiologically unimportant as it is present in very small quantities (<3% of total haemoglobin). However, the carrier state for β -thalassaemia is characterised by an increased level of $Hb A_2$ above 3.5%, low mean cell volume (MCV) and low mean cell haemoglobin (MCH).¹ Exceptions to this are a group of heterozygotes that may have normal $Hb A_2$ levels; these include the 'silent' thalassaemias and coinheritance of δ -thalassaemia.²

The mechanism underlying the increase in $Hb A_2$ appears to be through increased $\alpha\delta$ -dimer formation.

In a normal red cell, α chains will preferentially combine with β chains rather than δ chains which are more positively charged; however, in the presence of a reduced quantity of β chains and a relative excess of α chains as found in β -thalassaemia, more $Hb A_2$ will be formed.³

Beta-thalassaemia heterozygotes are usually clinically asymptomatic. Although there is a decrease in β globin gene output, there is very little ineffective erythropoiesis, with the proteolytic mechanisms within the red cell having the capacity to deal with this degree of chain imbalance. Thus, in presence of the normal

¹ Department of Haematology, Sultan Qaboos University, College of Medicine and Health Sciences, P.O.Box 35, Al-Khod, Muscat 123, Sultanate of Oman, ² Department of Haematology, Sultan Qaboos University Hospital, P.O.Box 35, Al-Khod, Muscat 123, Sultanate of Oman.

*To whom correspondence should be addressed. Email: asdoc@omantel.net.om

Table 1. Analysis of β -thalassaemia mutations in Omanis (234 alleles from 117 unrelated homozygous beta thalassaemia individuals)

Mutations	No. Alleles	% Total	Type
IVS 1-5 (G-C)	146	62.4	β^+
Codon 44 -C	26	11.1	β^0
Hb Dhofar ($\beta^{29} \beta^{58}$)	17	7.3	β^+
IVS 1-3 -25bp	14	6.0	β^0
619 del	8	3.4	β^0
IVS II -1 (G-A)	7	3.1	β^0
	218	93.3	β^0

The remaining 16 alleles were spread between 10 mutations found in very low frequency, namely, IVS 1-1 (G-A) (β^0), codon 39 (C-T) (β^0), codon 5 -CT (β^0), HbE (β^+), IVS1-110 (G-A) (β^0), codon 15 (G-A) (β^0), codon 30 (G-C) (β^0), codon 36/37 -T(β^0), Codon 37 (G-A) (β^0), IVS 1-128 (G-A) (β^+)

complement of α chains, most β -thalassaemia heterozygotes can be diagnosed by an increase in Hb A₂. However, the presence of coexistent α -thalassaemia could theoretically lower the Hb A₂ level. The Omani population is known to have a high prevalence of α -thalassaemia and a substantial number of the population are carriers of either Haemoglobin S or β -thalassaemia.^{4,5} Pre-marital screening and counselling are now encouraged. All blood samples at the Sultan Qaboos University Hospital (SQUH) that have a low MCV and MCH are routinely analysed by high performance liquid chromatography (HPLC). For these reasons, it is important to know the range of Hb A₂ among β -thalassaemia carriers in this population and, in particular, to observe if the presence of α -thalassaemia has lowered the levels Hb A₂ to overlap with the normal range. This study also compared Hb A₂ levels in β^0 and β^+ heterozygotes.

METHOD

DNA analysis (for beta globin gene mutations and α -thalassaemia) had previously been performed on 117 unrelated Omani patients with homozygous β thalassaemia being treated at SQUH in the Sultanate of Oman.⁶ For the present study, blood samples from 160 Omani obligate carriers (parents of known β -thalassaemia homozygotes) were collected into K EDTA and analysed by HPLC Biorad variant, β -thalassaemia 'short' programme. The samples included 28 parents heterozygous for Hb Dhofar ($\beta^{58} \text{ C-G}$, $\beta^{29} \text{ C-T}$) a known β -thalassaemia mutation, 86 from parents of patients

homozygous for either β^0 or β^+ mutations and 46 parents of patients who were compound heterozygotes for β^0/ β^+ . 40 of these samples had been previously analysed for the presence of α thalassaemia. Ten (25%) had no α -thalassaemia, 14 (35%) had a single gene deletion and 16 (40%) had two alpha genes deleted.⁷

RESULTS

158 of 160 samples from obligate β -thalassaemia carriers, including Hb Dhofar, had Hb A₂ levels above 3.5% (mean 4.8%, range 3.6%-6.7%) (Figure 1). The two exceptions had Hb A₂ levels of 3.4%; they were both found to be severely iron deficient and after six months of treatment with haematinics, the Hb A₂ levels had risen to 3.6% and 3.5% respectively.

Hb Dhofar elutes in the 'D' window, very close to Hb A₂ and raises the baseline of Hb A₂ resulting in a falsely low reading (Figure 2). In these cases an accurate value for Hb A₂ was obtained by elution after electrophoresis on cellulose acetate. The range of Hb A₂ in Hb Dhofar simple heterozygotes was 3.6%-5.5%, (mean $4.5\% \pm 0.47\%$).

Of the 160 samples, 69 were from known β^+ heterozygotes and 26 from known β^0 heterozygotes. Of the sixty-nine β^+ samples, all but two had an Hb A₂ level above 3.5% (range 3.6%-6.5%); the two exceptions were found to have iron deficiency. The mean level in this group was $4.9\% \pm 0.65\%$. The range of Hb A₂ in the twenty-two β^0 samples was 3.7% - 7.1% (mean $4.9\% \pm 0.62\%$).

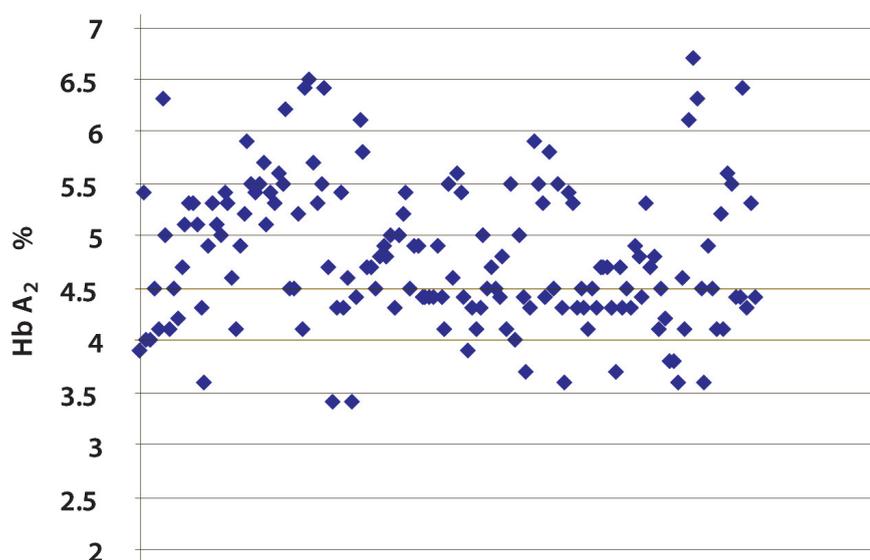


Figure 1. Hb A₂ in obligate β -thalassaemia carriers

DISCUSSION

A large number of β -thalassaemia mutations have been identified worldwide; some of these result in a total absence of beta globin chain production (β^0 mutations) while others cause a variable decrease in beta globin chain production (β^+ mutations). In the present study, there was no significant difference in Hb A₂ levels between the carriers of β^+ , β^0 or Hb Dhofar. This concurs with the separate findings of investigators who have found that despite the immense heterogeneity of β -thalassaemia mutations, the level of Hb A₂ in heterozygotes is remarkably similar. Huisman analysed data from six hundred β -thalassaemia heterozygotes and found Hb A₂ levels to be between 4.5% and 5.5% in persons with β^0 or severe β^+ thalassaemia and between 3.6% and 4.2% in those with milder mutations.² Altay and Gurgey obtained similar data from Turkish individuals.⁸ It is only when very mild β^+ alleles are present that the Hb A₂ level may drop. Other than the single allele of Hb E, (which poses no diagnostic difficulty), none of the mutations thus far identified in the Sultanate can be classified as mild Table 1.⁶ However, it should be noted that our studies have all been hospital based; there may be a number of milder beta thalassaemia mutations in the general population that have yet to be identified.

A complicating issue in the accurate identification of β -thalassaemia trait is that the coexistence of iron deficiency may suppress the level of Hb A₂ leading to misdiagnosis. The association of iron deficiency with

lowered Hb A₂ is not a constant finding. Wasi *et al*⁹ describe normal levels of Hb A₂ in β -thalassaemia trait individuals who were also iron deficient but Alperin *et al*¹⁰ found that the more severe the anaemia the lower the Hb A₂ level. In contrast, Madan found the mean Hb A₂ to be significantly higher in those patients with iron deficiency anaemia than in those without in a group of Indian β -thalassaemia heterozygotes.¹¹ Our results suggest that iron deficiency lowers the Hb A₂ level in some individuals.

The effect of concurrent α -thalassaemia on Hb A₂ is also a potential pitfall in the diagnosis of the β -thalassaemia carrier state, as in parts of the world where β -thalassaemia is found, α -thalassaemia is often present in high prevalence.¹²⁻¹⁴ Martinez and Menendez have shown that lower amounts of Hb A₂ may be formed when the supply of α chains is limited¹⁵ but a recent study in Singapore has shown that routine haematological tests can confirm β -thalassaemia carrier status in the simultaneous presence of α and β -thalassaemia.¹⁶ In addition, the results of a study in Sardinian children conducted by Galanello *et al* showed that when α -thalassaemia coexists with β -thalassaemia, although the mean cellular volume (MCV) and mean cellular haemoglobin (MCH) in heterozygote β -thalassaemics may fall within the normal range, the levels of Hb A₂ are always increased compared to the normal.¹⁷ Thus, in regions where both α and β -thalassaemia are prevalent the diagnosis of heterozygous β -thalassaemia should not be excluded on the basis of normal indices

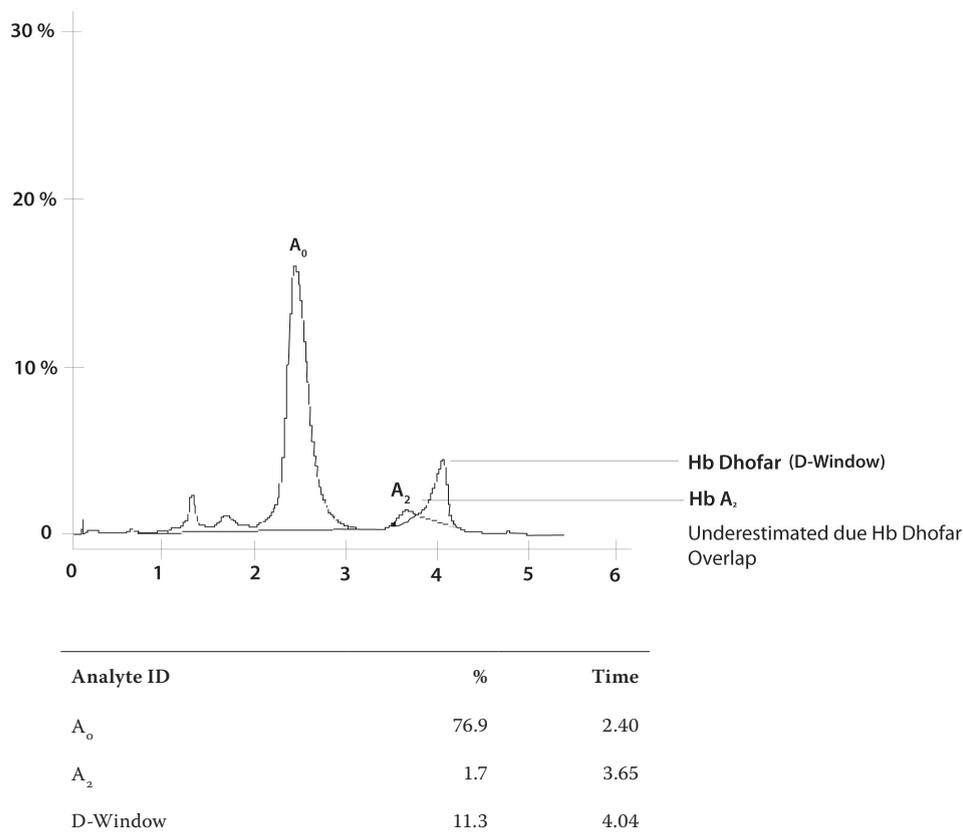


Figure 2. HPLC of Hb Dhofar trait

and Hb A₂ must always be evaluated.

Statistically, at least 75% of Omanis are expected to have either single gene or two-gene deletional α -thalassaemia.⁴ The same ratio was found in a study group of Omani thalassaemia major patients, with 36% having no α -thalassaemia, 32% having a single alpha gene deletion and 32% having two alpha genes deleted.⁷ A smaller study of 40 of the obligate carriers showed 10 (25%) to have no α -thalassaemia, 14 (35%) with a single gene deletion and 16 (40%) with two alpha gene deletion.

The results of the present study show that, other than the two carriers with iron deficiency, all the obligate carriers had Hb A₂ levels over the diagnostic range for β -thalassaemia trait.

CONCLUSION

For the purposes of genetic screening and premarital counselling it is reassuring to find that, despite the high prevalence of α -thalassaemia in the Omani population, the levels of Hb A₂ in β -thalassaemia carriers are above the normal range and that β -thalassaemia trait in Omanis can be diagnosed by the presence of Hb A₂

equal to or above 3.5% as long as there is no concomitant iron deficiency. In order to minimise false negatives, all individuals with Hb A₂ of 3.3%-3.4%, or who have anemia, should be screened for iron deficiency; if this is present, the Hb A₂ level should be repeated after adequate treatment with hematinics.

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