Percentage of HbS among cases of sickle-cell trait in Basra, Iraq

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النسبة المئوية للهيموغلوبين المنجلي بين حالات الخلة المنجلية في البصرة بالعراق

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خلاصة: تم تحديد نسبة الهيموغلوبين المنجلي بين 170 شخصاً يحملون الخلة المنجلية. فوجد أن 75 شخصاً (44.1%) كان لديهم هيموغلوبين منجلي يزيد عن 38%، بينما كان هناك 54 شخصاً (31.8%) لديهم نسبة من الهيموغلوبين المنجلي تتراوح بين 31% و38%. وكان هناك 41 شخصاً (24.1%) لديهم نسبة من الهيموغلوبين المنجلي تقل عن 31%. ووجد ارتباط إيجابي بين النسبة المئوية للهيموغلوبين المنجلي وبين تركيز الهيموغلوبين وحجم الخلايا المكدسة والمستوى الوسطي لهيموغلوبين الكريات والحجم الوسطي للكريات والنسبة المئوية للخلايا المستهدفة. وتبيَّن من تحليل التحوُّف (الانحدار) المتعدد أن قياس تركيز الهيموغلوبين والمستوى الوسطي لهيموغلوبين الكريات والنسبة المئوية للخلايا المستهدفة، يمكن أن تستعمل من أجل التنبؤ بالنسبة المئوية للهيموغلوبين المنجلي.

ABSTRACT The proportion of sickle haemoglobin (HbS) was determined in 170 sickle-cell-trait individuals; 75 (44.1%) individuals had HbS% > 38%, 54 (31.8%) had HbS% between 31% and 38% and 41 (24.1%) had HbS% < 31%. There was positive correlation between HbS% and haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin, mean corpuscular volume and target cell percentage. Multiple regression analysis indicated that measurement of haemoglobin concentration, mean corpuscular haemoglobin and target cell percentage could be used to predict the HbS%.

Pourcentage d'hémoglobine S dans les cas de trait drépanocytaire à Bassora (Iraq)
La proportion d'hémoglobine S a été déterminée chez 170 individus porteurs du trait drépanocytaire; 75 personnes (44,1%) avaient un pourcentage d'hémoglobine S > 38%, 54 (31,8%) avaient un pourcentage d'hémoglobine S se situant entre 31% et 38% et 41 (24,1%) avaient un pourcentage d'hémoglobine S < 31%. Il y avait une corrélation positive entre le pourcentage d'hémoglobine S et la concentration en hémoglobine, l'hématocrite, la teneur globulaire moyenne en hémoglobine, le volume globulaire moyen et le pourcentage d'hématies en cible. L'analyse de régression multiple a indiqué que la mesure de la concentration en hémoglobine, de la teneur globulaire moyenne en hémoglobine et du pourcentage d'hématies en cible pouvait être utilisée pour prédire le pourcentage d'hémoglobine S.

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Introduction

Sickle-cell trait (HbAS) is the heterozygous state of the sickle-cell gene. Consequently, every red cell in sickle-cell-trait individuals contains both normal haemoglobin (HbA) and sickle haemoglobin (HbS). Variable proportions of HbS (20%–45%) have been reported [*1–7*] with a mean value of 38 ± 5% [*3,6,8*].

A trimodal distribution of the amounts of HbS has been demonstrated in population surveys in various areas of the world [*9–13*]. Neel and co-workers in 1951 postulated that the variation in the proportion of HbS in sickle-cell trait is genetic rather than environmental in origin [*2*]. Subsequent studies have shown that the proportion of HbS in heterozygotes is reduced by co-inheritance of one or more genes of α-thalassaemia [*1,2,7,9–11,14–17*].

There has been no published report of the HbS distribution in our local population with sickle-cell trait. Thus, the aim of our study was to provide information about the proportion of HbS in Basra province and the possible use of haematological parameters to predict this proportion.

Materials and methods

Venous blood was collected in EDTA vacutainers from 301 patients of both sexes (age range: 1–70 years) attending family care centres and hospital outpatient clinics in Basra governorate. From these, 170 people with sickle-cell trait were selected according to the results of electrophoresis and sickling test.

Standard laboratory procedures [*18*] were used to assess haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), reticulocyte count and blood film for estimation of target cell percentage.

Haemoglobin electrophoresis was carried out on cellulose acetate strips (Shandon, United Kingdom), barbiton buffer (pH 8.6) and 250 volts for 75 minutes in a Gelman horizontal cell [*19*]. The strips were stained with Ponceau S 3% in trichloracetic acid and cleared with 5% acetic acid. HbA and HbS were quantified by scanning densitometry (Elvi 160).

Results

Data obtained from the 170 sickle-cell-trait individuals (according to HbS percentage) are shown in Table 1. The participants were subdivided into three groups: group A included 41 individuals (24.1%) with HbS% < 31%; group B included 54 individuals (31.8%) with Hbs% between 31% and 38% and group C included 75 individuals (44.1%) with HbS% > 38%.

Further analysis of the three groups showed that haematological values were lower in group A individuals (HbS < 31%) than group B (HbS = 31%–38%) and group C (HbS > 38%) (Table 2). The differences were statistically significant for A < B < C except in the values of RBC count and reticulocyte count ($P > 0.05$). There was a positive correlation between the mean cor-

Table 1 Distribution of sickle-cell-trait individuals according to HbS percentage

HbS percentage	No.	%
Group A (HbS% < 31%)	41	24.1
Group B (HbS% = 31%–38%)	54	31.8
Group C (HbS% > 38%)	75	44.1
Total	170	100

Table 2 **Haematological profiles in sickle-cell-trait individuals according the HbS percentage**

Haematological variable	Group A HbS% < 31% (*n* = 41)	Group B HbS% = 31%–38% (*n* = 54)	Group C HbS% > 38% (*n* = 75)
Haemoglobin (g/dL)	11.13 ± 1.02	11.94 ± 1.01	13.15 ± 1.39
Packed cell volume (%)	34.66 ± 3.03	36.93 ± 3.10	40.72 ± 4.18
Red blood count (/mm^3)	4.55 ± 0.60	4.35 ± 0.51	4.51 ± 0.51
Mean corpuscular volume (fL)	74.66 ± 5.26	85.37 ± 7.72	89.47 ± 7.99
Mean corpuscular haemoglobin (pg)	23.58 ± 1.73	27.29 ± 2.40	29.31 ± 2.44
Reticulocyte count (%)*	0.57 ± 0.56	0.55 ± 0.55	0.80 ± 1.18
Target cell (%)*	0.24 ± 0.34	1.12 ± 1.08	2.04 ± 2.22
HbS%	27.51 ± 1.80	35.81 ± 1.68	43.79 ± 2.92

**Statistically insignificant (P > 0.05)*
Values are expressed as mean ± standard deviation

puscular volume (MCR), mean corpuscular haemoglobin (MCH) and HbS level.

Univariate analysis of the different haematological parameters and HbS% revealed a moderate positive correlation between HbS% and the Hb concentration, PCV, MCV, MCH and target cell percentage ($r = 0.656$, $r = 0.797$ and $r = 0.511$ respectively) with a P-value of < 0.01.

Multiple regression analysis showed that the Hb concentration, MCH and target cell percentage are the best three predictors of HbS%. Therefore, HbS% can be estimated using the following equation:

$$\text{HbS\%} = 0.874\ \text{Hb} + 0.936\ \text{MCH} + 0.916\ \text{target cell percentage}$$

Discussion

The heterogeneous distribution of the proportion of HbS% in sickle-cell trait is related to genetic factors, which result in co-inheritance of one or more genes of α-thalassaemia [*1,5,7,9–11,13,15–17*]. The genotypes of the three groups of sickle-cell trait with low, medium and high amounts of HbS are $-\alpha/-\alpha$, $-\alpha/\alpha\alpha$ and $\alpha\alpha/\alpha\alpha$ respectively [*9*].

The mechanism for the depression of β^s biosynthesis in the presence of α-thalassaemia is unclear. Since this depression occurs in the peripheral blood reticulocytes, the influence of the thalassaemia gene must occur at the translational or post-translational level of protein synthesis [*1*]. At the translational level, it has been claimed that free alpha chains aid in the release of beta chains from polysomes [*6*], but it has been shown that in sickle-cell-trait individuals who also have deletion of alpha genes, there is a decrease in net synthesis of the absolute mean cell concentration of HbS, while there is no change in the absolute mean cell concentration of HbA [*9*]. So this mechanism is unlikely. The more likely mechanism occurs at the post-translational level [*5*] where the alpha chain has a greater affinity for binding with the β^A chain than with the β^S chain [*20*].

Therefore, when the amount of alpha chain available is reduced as a result of alpha gene deletion, one would expect that

such individuals will synthesize a greater amount of HbA than HbS.

We attempted to compare the haematological profile of all sickle-cell-trait individuals classified according to HbS% (Table 2). The Hb concentration, PCV, MCV and MCH were significantly lower in group A (HbS% < 31%) than group B (HbS% = 31%–38%) and group C (HbS% > 38%), the differences being statistically significant (A < B < C).

The observed relationship in these haematological parameters and the associated reduction in HbS% is compatible with the finding that α-thalassaemia caused the reduction of HbS in sickle-cell trait [*1,5,9–11,13–17*]. Thus, lowering HbS% is accompanied by parallel changes in MCV and MCH which are significantly reduced as a result of co-inheritance of α-thalassaemia [*5,16*]. On the other hand, the lower Hb value associated with decreased HbS% is most likely due to depression in HbS synthesis as a result of the alpha gene deletion [*5*]. Because the alpha chain has a higher affinity in binding with β^A than with β^S chain, the β^A chain will bind most of the alpha chain available and there will be a greater rapid breakdown of some of the newly synthesized β^S in individuals with sickle-cell trait with α-thalassaemia than those without α-thalassaemia [*21*].

The target cell percentage follows a similar pattern as the other haematological parameters, being lower in group A than groups B and C as shown in Table 2. The presence of target cells in peripheral blood film is characteristic of most haemoglobinopathies although not specific for them [*9*]. In haemoglobinopathies, the presence of target cells is related to the presence of abnormal haemoglobin, and in such instances their occurrence may be the initial indication that an abnormal haemoglobin is present, so it is expected that the number of the target cells will increase when the amount of the abnormal haemoglobin increases.

Regression analysis revealed that the three best predictors of HbS% in sickle-cell trait were: Hb concentration, MCH and target cell percentage. The Hb concentration and MCH have a positive function, indicating that higher HbS percentages are associated with higher Hb concentration and MCH values and vice versa. This is explained by the fact that the lower HbS percentages in sickle-cell trait are mostly caused by α-thalassaemia, which in turn also causes a decrease in Hb concentration and MCH. Similarly, the target cell percentage also has a positive effect as higher amounts of this abnormal haemoglobin are associated with an increase in the number of target cells.

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