Frequency of haemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency in Basra

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تواتر حدوث الثلاسيميا بيتا والهيموغلوبين المنجلي وعوز إنزيم نازعة هيدروجين فسفات -6- الغلوكوز في محافظة البصرة في العراق

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الخلاصة: قمنا بمُخارطة محافظة البصرة في جنوب العراق، (أي رسم خريطة تبيّن) الاعتلالات الهيموغلوبينيّة وعوز إنزيم نازعة هيدروجين الفسفات-6- غلوكوز فيها. ومن بين 1064 زوجاً ممن تتراوح أعمارهم بين 14-60 عاماً تم جسهم من مختبرات الصحة العمومية، كان 46 منهم حاملاً لخلّة الثلاسيميا-بيتا و69 منهم حاملاً لخلّة الخلية المنجلية، و2 منهم حاملَيْن لخلّة الهيموغلوبين D و2 منهم حاملَيْن لخلة الهيموغلوبين وواحد منهم حاملاً لهيموغلوبين جنيني مستمر. فيما تشكل الحالات التي تحمل اضطرابات كبرى للغلوبين بيتا 11.48٪، وتم كشف عوز إنزيم نازعة هيدروجين فسفات-6-غلوكوز في 133 شخص (12.5٪). فيما كان عشرة أزواج (0.94٪) معرضين لخطر أن يكون لديهم أطفال مصابون بمرض الخلية المنجلية أو الثلاسيميا الكبيرة بيتا. إن حالات الإصابات هذه تُعَدُّ من المشكلات الصحية الحقيقية وتوجب وضع خطة معالجة وتثقيف صحي عمومي للتشخيص الباكر والمعالجة الباكرة.

ABSTRACT Basra, southern Iraq, was mapped for haemoglobinopathies and glucose-6-phosphate dehydrogenase (G6PD) deficiency. Of 1064 couples aged 14–60 years recruited from the Public Health Laboratory, 49 had β-thalassaemia trait, 69 had sickle-cell trait, 2 had haemoglobin D trait, 2 had haemoglobin C trait and 1 had high persistent fetal haemoglobin. Carriers of major β-globin disorders comprised 11.48%. G6PD deficiency was detected in 133 individuals (12.5%). Only 10 couples (0.94%) were at risk of having children affected with either sickle-cell disease or β-thalassaemia major. These defects constitute a real health problem and necessitate a management plan and public health education for early diagnosis and therapy.

Fréquence des hémoglobinopathies et de l'anémie hémolytique enzymoprive dans le Gouvernorat de Bassora (Iraq)

RESUME Une cartographie de Bassora (sud de l'Iraq) a été établie pour les hémoglobinopathies et l'anémie hémolytique enzymoprive (G-6-PD). Sur les 1064 couples âgés de 14 à 60 ans recrutés dans le Laboratoire de santé publique, 49 avaient un trait β-thalassémique, 69 avaient un trait drépanocytaire, 2 avaient un trait d'hémoglobine D, 2 avaient un trait d'hémoglobine C et 1 présentait une persistance de l'hémoglobine fœtale élevée. Les porteurs d'anomalies majeures de la structure de la globine-β représentaient 11,48 %. Une anémie hémolytique enzymoprive (G-6-PD) a été détectée chez 133 individus (12,5 %). Seuls 10 couples (0,94 %) avaient un risque d'avoir des enfants atteints d'une drépanocytose ou d'une β-thalassémie majeure. Ces anomalies constituent un problème de santé réel et nécessitent un plan de prise en charge et une action d'éducation de santé publique pour le diagnostic précoce et le traitement.

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Introduction

Haemoglobinopathies, i.e. disorders of haemoglobin, are the most common single gene disorders worldwide [*1,2*]. Haemoglobinopathies can be divided into two main groups: the structural variants such as Hb S and more than 600 other variants, and thalassaemias, which are characterized by the abnormal expression of the genes for normal globin chains [*1,3*]. Sickle-cell haemoglobin, β-thalassaemia and glucose-6-phosphate dehydrogenase (G6PD) deficiency are the most frequent of the abnormal genes that affect red cell stability and integrity [*3,4*]. The haemoglobinopathies are a serious problem in many developing countries and as infant mortality rates fall with progress in controlling malnutrition and infections, these genetic diseases will represent an increasing challenge [*5*].

Genes for G6PD deficiency and sickle-cell disease were first reported in Iraq in 1963 and 1964 by Taj El-Deen et al. and Baker and Al-Qausi respectively [*6,7*]. More recently, a number of studies in Basra found a high prevalence of G6PD deficiency genes, ranging from 13.1% to 14.1% [*8,9*]. Most of these studies, however, were conducted in areas of Basra where the sickle-cell gene was thought to be present in high frequency, e.g. the Abu al-Khasib district where the frequency of Hb S ranged from 14.86% to 16% [*10,11*]. It has been shown that, in addition to sickle-cell genes, β-thalassaemia genes also occur frequently. A previous study in Baghdad reported a prevalence rate of 4.4% for β-thalassaemia gene [*12*]. The exact gene frequencies of Hb S, β-thalassaemia and G6PD deficiency in different areas of Basra are still unknown. This study, therefore, investigated previously unscreened areas and mapped Basra for haemoglobinopathies and G6PD deficiency.

Methods

This study was carried out from 1 August 2000 until the end of January 2001. The subjects were couples attending the Primary Health Care Department, Public Health Laboratory for routine premarital investigations. Their ages ranged from 14 to 60 years. This laboratory is the only laboratory in Basra offering premarital investigations, which include blood group and rhesus group, human immunodeficiency virus (HIV) and VDRL (Venereal Disease Research Laboratory slide test) screening, and thus, it receives individuals from all areas of Basra. The average number of attendees investigated by the laboratory is 60–70/day, i.e. 30–35 couples. The sample sizes for the different regions of Basra were determined according to the last census (1997) before starting the study. Accordingly, a representative number of subjects from each region was calculated and the number of respondents were recorded (Table 1).

A questionnaire was used for each individual and each person's name, age, sex, residence, consanguinity of parents and family history of blood diseases (haemolytic anaemia) were recorded. A total of 1112 individuals (556 couples) attending the medical laboratory during the study period were included. All subjects were of Iraqi nationality and lived in different areas of Basra. They were randomly selected, i.e. 30 samples were chosen 3 times per week and 1 out of 2 couples were randomly selected.

A 2 mL blood sample was collected from each subject by venepucture in EDTA

Table 1 **Numbers of individuals surveyed in the study according to their residence**

Residence	Expected no.	No. of respondents	Response rate (%)
City Centre	530	526	99.2
al-Qurnah	117	114	97.4
al-Madina	87	114	131.0
al-Zubair	175	154	88.0
Abu al-Khasib	101	112	110.9
Shatt al-Arab	45	44	97.8
Total	1055	1064	100.8

anticoagulated tube and delivered immediately to the laboratory where investigations were carried out within 24 hours. Haematological and red cell indices were estimated by an automated cell counter (Coulter Counter, MS9).

The activity of G6PD was determined by fluorescent spot test as described by Beutler et al. [*13*]. Fluorescence is produced due to reduction of NADP to NADPH. This reaction is catalysed by the enzyme G6PD and is coupled with the oxidation of glucose-6-phosphate to 6-phosphogluconate. Moderate enzyme activity, i.e. 20%–60% of normal residual activity, was determined if the spot showed weak fluorescence after 15 minutes. Severe deficiency referred to spots with no fluorescence after 30 minutes with enzyme activity less than 20%. This was the result of the presence in the reagent mixture of reduced glutathione, which reacted with small amounts of NADPH formed during the reaction [*14*].

Haemoglobin typing was performed quantitatively by an automated ion exchange high performance liquid chromatography system using β-thalassaemia short programme on the Bio-Rad Variant instrument (Bio-Rad Laboratories, Belgium). β-thalassaemia trait was identified by the characteristic elevation of Hb A_2 (>3.8%). Hb S, Hb C, Hb D and others were detected according to their specific peak area that was calculated after elution with the buffer solution.

The gene frequency for each haemoglobin disorder and G6PD deficiency was estimated by applying the Hardy–Weinburg equilibrium as follows:

$$(p + q)^2 = p^2 + 2pq + q^2 = 1$$

where frequency of trait in males = frequency of trait gene, q;
frequency of male normals = frequency of normal gene, $1 - q = p$;
frequency of female heterozygote, $2q(1 - q) = 2qp$;
frequency of female abnormal homozygotes, q^2;
and frequency of female normal homozygotes, $(1 - q)^2 = p^2$.

The affected birth rate was estimated from the carrier frequency and Hardy–Weinberg equation as above. Statistical analysis was done using the Fisher exact test.

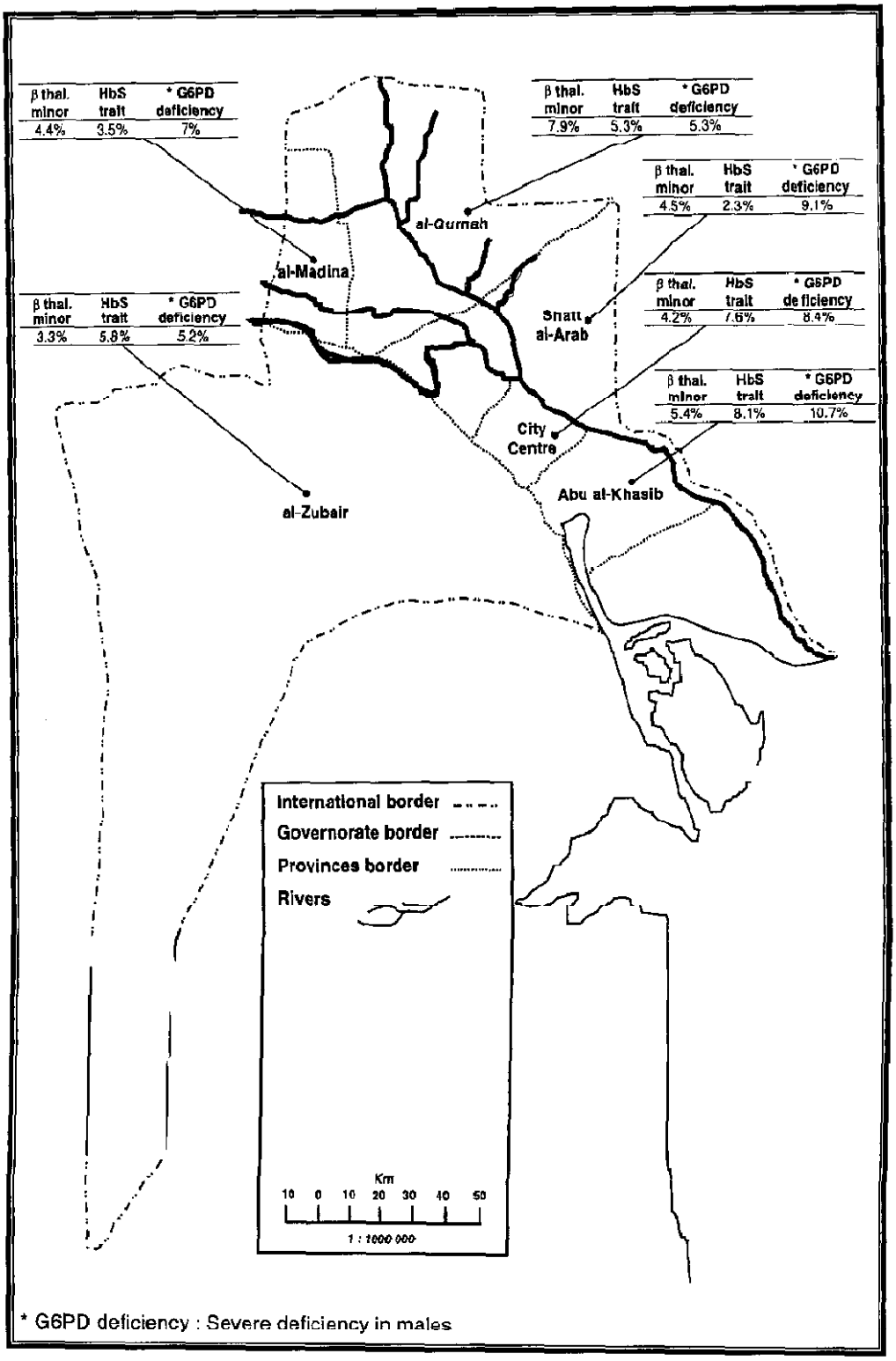

Figure 2 **Distribution of haemoglobinopathies and G6PD deficiency in different regions of Basra governorate**

Table 5 **Interaction of G6PD deficiency with different haemoglobinopathies**

Hb type	Total no. investigated	G6PD deficiency No.	%	*P*-value[a]
Hb A	941	107	11.4	
Hb S	69	15	21.7	$P = 0.018$
β-thalassaemia	49	11	22.4	$P = 0.026$
Others	5	0		

[a]*P-value was calculated using Fisher's exact test.*

the Centre, Abu al-Khasib, Shatt al-Arab, al-Zubair, al-Madina and al-Qurnah. A representative sample of the population from each region was investigated in this study in order to estimate, as close as possible, the true prevalence rate of the defective genes in that area.

The overall frequency of G6PD deficiency in males in the present study was 15.3%, which was comparable to that reported in previous studies in Basra [*10,11*]. The frequencies ranged from 11.4% in al-Madina to 16.1% in Abu al-Khasib districts. These frequencies were higher than those reported in other areas of Iraq such as Baghdad (6.3%) and Nineveh (9.5%) [*15*]. In this study, the majority of the individuals investigated were Iraqis from traditionally agricultural areas. Consanguineous marriage is common and malaria is endemic; these factors might explain the high frequency of G6PD in our study.

The gene for G6PD deficiency is transmitted as a sex-linked trait with severe enzyme deficiency occurring only in hemizygote males and homozygote females, while heterozygous females often have normal or only moderately lower enzyme level [*16*]. Our observed gene frequency for males was 15.3%, i.e. 82 of 536 were deficient (Table 3). All had severe G6PD deficiency. Comparable results have been found in different parts of Iraq [*8,17,18*]. Not all heterozygotes have intermediate activity; some may be normal and others may have low activity. The best estimate of gene frequency is from data in males, since enumeration of affected males will give the gene frequency [*16*]. The expected frequency of severe and intermediate G6PD deficiency in females was calculated from the 15.3% hemizygous. This means that for females 25.6% would be expected to be heterozygous and 2.3% homozygous. In our study, the observed combined frequency for heterozygotes was 3.6% and for homozygotes 6.1%, so under half of the expected heterozygotes were detected. These discrepancies are similar to the findings of other researchers and are attributable to the considerable overlap of heterozygote activities with normal range [*19*] and in part to random X-chromosome inactivation [*16*]. The effects of the high rate of consanguinity and other forms of intermarriage cannot be ruled out. Furthermore, the fluorescent spot test used in our study is known to have 100% reliability detecting G6PD activity in hemizygote males and homozygote females but not in heterozygote females [*13,14*]. This may explain the low number of heterozygote females detected (only 30%). Other researchers have made similar observations [*18*].

The results revealed diversity in gene frequencies of Hb S and β-thalassaemia in the different areas of Basra. Al-Zubair and Shatt al-Arab had the lowest frequencies for β-thalassaemia and Hb S respectively while Abu al-Khasib and al-Qurnah had the highest frequencies for Hb S and β-thalassaemia genes respectively. The overall carrier frequency of β-thalassaemia was 4.6% (gene frequency, 0.023) and was comparable to that reported in a previous study in Baghdad [*12*] but higher than the overall reported carrier rate in Iraq (3%) [*20*] and in neighbouring countries like Jordan (3.0%–3.5%) [*1,21,22*] and Lebanon (1.7%–3%) [*20–22*]. Previous studies in Basra have found that the frequency of Hb S genes ranges from 2.5% in the City Centre up to 16% in Abu al-Khasib district [*10,11*]. The increased frequency of Hb S reported in this study in the City Centre, along with the decreased frequency in Abu al-Khasib, can be attributed to migration of people towards the City Centre following the war.

The overall frequency of Hb S was 6.48% (0.032). We could not compare this frequency with those from other governorates of Iraq since no such data were available. This gene frequency was comparable to that reported in some neighbouring areas in Saudi Arabia [*23*] and Jordan [*24*].

An association between G6PD deficiency and each of Hb S and thalassaemia was found in our study that was similar to earlier studies done in our locality [*11*] that indicate that they interact even though their modes of inheritance are on separate genes. Similar findings have also been reported from countries neighbouring Iraq [*25,26*].

From our study, the number of homozygous births for haemoglobinopathies was estimated to be 1.57/1000 live births (≈2/1000) and the annual number of births of homozygotes was approximately 110 according to the records supplied by the health authorities in Basra.

Of the many inherited disorders of haemoglobin, only two, i.e. sickle-cell disease and thalassaemia, are a major drain on health resources. It has been estimated that the cost of treatment for one patient with β-thalassaemia major, depending on age and weight, ranges from US$ 2500 to US$ 10 500 annually [*20*]. As effective management of sickle-cell disease and thalassaemia major involving blood transfusions and iron chelating agents is too expensive for most developing countries, it is clear that population screening and disease prevention are a critical part of management. Therefore, we recommend that for the prospective control of the major β-globin disorders, we need heterozygote detection through premarital screening, which is also vital for the identification of couples at risk, and/or neonatal screening, genetic counselling and health education. This can be begun with the global education of all medical and paramedical staff, community education programmes through booklets, posters, television, video and newspapers, as well as the implementation of formal education into the school curriculum on the inherited anaemias.

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The Genomic Resource Centre

We would like to draw our readers attention to the WHO Genomic Resource Centre website. This resource base has been developed by the WHO Human Genetics Programme (HGN) to provide information and build awareness on human genomics, a new and rapidly developing science. The Genomic Resource Centre includes individual sections designed to cater for the needs of the major stakeholder groups in genomics, namely the public and the patients, the health professionals and the policy-makers. In addition, information is provided on the ethical, legal and social implications of genomics and the latest updates in genomic research. This centre is also instrumental in understanding the work of the WHO Human Genetics Programme as well to all related departments within WHO. The website can be accessed at: http://www.who.int/genomics/en/