

Red cell glucose-6-phosphate dehydrogenase phenotypes in Iraq

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الأنماط الظاهرية لإنزيم نازعة هيدروجين الغلوكوز - 6 - فسفات في الكريات الحمراء في العراق

فربال حنمي، نصير العلوي، ميسون رسام، غسان الشماخ وعامر الهاشمي

المخالصة: جاربنا في هذه الدراسة تبيّن خصائص الضروب المختلفة من إنزيم نازعة هيدروجين الغلوكوز - 6 - فسفات من الناحية الكيميائية الحيوية في الأفراد العراقيين. وقد تم اختيار عشوائي لـ 758 من الذكور العراقيين الأصحاء الذين تتراوح أعمارهم بين 18 و 60 سنة، وكان 46 منهم (6.1%) مصابين بعوز إنزيم نازعة هيدروجين الغلوكوز - 6 - فسفات. ورغم أن النمط الظاهري السائد لدى غير المصابين بعوز هذا الإنزيم كان من النمط B في 92.6% من الحالات فإن النمط A من عوز هذا الإنزيم وُجد في الأشكال المتعددة بمقدار (1.3%). وبين المصابين بعوز هذا الإنزيم كان 31 حالة منهم تظهر عليهم الخصائص كاملة، و 17 حالة كانت ملاحظتها تتماشى مع الضرب السائد في منطقة البحر المتوسط، و 12 حالة لها خصائص عرفت بأنها ليست من الضرب السائد في منطقة البحر المتوسط فيما تم تصنيف الخائين المتبقيين من عوز الإنزيم على أنهما من الضرب السليبي الـ A. ورغم أن الضرب الكثير التكرار من عوز الإنزيم هو الضرب السائد في منطقة البحر المتوسط فإن وجود ضرب إيجالي A وآخر سيلي A بين العراقيين يتماشى مع التقارير التي وردت من الجزيرة العربية، كما أن وجود عدد كبير من الضروب غير السائدة في منطقة البحر المتوسط لم يكن متوقفاً وقد يعود إلى التكوين المتغاير في سكان العراق.

ABSTRACT We attempted to characterize biochemically glucose-6-phosphate dehydrogenase (G6PD) variants in Iraqi individuals. Thus 758 healthy Iraqi males aged 18–60 years were randomly selected and 46 (6.1%) were G6PD deficient. Although the predominant non-deficient G6PD phenotype was G6PD B (92.6%), G6PD A+ was found in polymorphic frequency (1.3%). In the deficient group, 31 cases were fully characterized, including 17 cases with features consistent with G6PD Mediterranean variant, while 12 had other biochemical features and were labelled as non-Mediterranean variant. The remaining two deficient cases were characterized as G6PD A– variant. The presence of a significant number of non-Mediterranean variant was unexpected and may be related to the more heterogeneous background of the Iraqi people.

Les phénotypes de la glucose-6-phosphate-déshydrogénase érythrocytaire en Iraq

RESUME Nous avons essayé de procéder à une caractérisation biochimique des variantes de la glucose-6-phosphate-déshydrogénase (G-6-PD) chez des individus irakiens. Sept cent cinquante-huit (758) hommes irakiens en bonne santé, âgés entre 18 et 60 ans, ont donc été choisis au hasard et 46 (6,1 %) présentaient un déficit en G-6-PD. Si le phénotype non déficient prédominant de la G-6-PD était la G-6-PD B (92,6%), la G-6-PD A+ a été trouvée à fréquence polymorphique (1,3 %). Dans le groupe déficient, 31 cas étaient complètement caractérisés, dont 17 cas ayant des caractéristiques correspondant à la variante méditerranéenne de la G-6-PD, alors que 12 cas avaient d'autres caractéristiques biochimiques et étaient classés sous la variante non méditerranéenne. Les deux cas déficients restants étaient caractérisés comme variante G-6-PD A–. Tandis que la fréquence élevée de la variante méditerranéenne déficiente de la G-6-PD et la présence de G-6-PD A+ et A– chez les irakiens sont comparables aux rapports provenant de la péninsule Arabique, la présence d'un nombre important de la variante non méditerranéenne était inattendue et peut être liée au contexte plus hétérogène de la population irakienne.

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Introduction

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common red cell enzymopathy, with around 400 biochemically characterized variants [1]. The Middle East is believed to have the world's highest overall frequency of this genetic defect, and this, in addition to the region's unique geographical and historical features, makes it especially important and desirable to characterize G6PD variants in the region [2]. Iraq lies almost at the centre of the Middle East and is well known to have a relatively high frequency of G6PD deficiency [3,4], however there are no published studies addressing the question of G6PD variants in Iraq, and it is the aim of this study to address this issue through studying a random sample of Iraqi adult males.

Methods

A total of 758 Iraqi men, aged 18–60 years, comprising 568 blood donors (national blood bank in Baghdad), 121 medical students (University of Baghdad) and 69 hospital personnel (Medical City Teaching Hospital, Baghdad) were selected at random for inclusion in this study. The majority of those included were permanent residents of Baghdad (75.7%), and of the surrounding central provinces (18.6%). While the majority were Arabs (703 subjects), there were some Kurds, Turkomans and other minorities (34, 18 and 3 subjects respectively). All the participants had a short medical history taken, including special emphasis on history of fava bean consumption, any history of haemolytic episodes following such consumption, whether they were ever diagnosed as having favism or G6PD deficiency, and if

they had any family history of this condition. All those included were screened for G6PD deficiency using the methaemoglobin reduction test [5]. In addition, regardless of the result, a full blood count, film, reticulocyte count and electrophoretic characterization of the G6PD enzyme on cellulose acetate membrane in tris-EDTA borate buffer, pH 8.6, (using a modification of the method of Sparks et al. [6]) were performed for all participants. A random sample of 25 of the men who were not G6PD-deficient, who had B electrophoretic enzyme mobility on electrophoresis, were taken as normal controls. Quantitation of enzyme activity was performed using a Sigma G6PD kit on all G6PD-deficient cases, on the controls mentioned above and on participants who were not G6PD-deficient but who displayed altered mobility. Further biochemical characterization using the methods and criteria set by WHO [7,8] on partially purified enzyme were performed on all control cases, 31 of the 46 deficient cases, and on all the non-deficient cases with altered mobility. The kinetic studies performed included Km for G6P, use of the analogues 2-deoxy G6P, galactose 6 phosphate, and deamino NADP.

Statistical analysis included the *t*-test and chi-squared test.

Results

Frequency of G6PD deficiency

Of 758 Iraqi male participants screened for G6PD by the methaemoglobin reduction test, 46 were found to be deficient (overall relative frequency of 6.1%). In those currently resident in Baghdad, which included the bulk of the cases, the frequency of G6PD deficiency was found to be 6.3%. Table 1 shows the frequency of G6PD deficiency in relation to the ethnic origin of

Table 1 Frequency of G6PD deficiency by ethnic group in 758 Iraqi adult males

Ethnic group	Total cases	% G6PD deficient
Arab	703	6.0
Kurd	34	8.8
Turkoman	18	5.6
Other	3	-

the participants. There was no significant association between the age distribution and the frequency of G6PD deficiency.

Non-deficient G6PD variants

Electrophoresis on cellulose acetate revealed B mobility in 702 and A mobility in 10 non-deficient individuals. Further characterization of a random 25 individuals with B mobility (the control group) and all

Table 2 Summary of biochemical characteristics of G6PD variants studied

Variants	No.	Electrophoretic mobility (%)	Activity (U/g Hb)	Km G6P (mm)	2-deoxy G6P use ^a	Galactose 6P use ^a	dNADP use ^b
Non-deficient							
B	25	100	(7.3 ± 2.01)	(58.3 ± 13.43)	(4.4 ± 2.15)	(3.4 ± 1.54)	(62.2 ± 7.79)
A	10	110	(7.1 ± 1.53)	(59.1 ± 9.93)	(4.8 ± 1.42)	(3.7 ± 1.7)	(65.6 ± 6.58)
Deficient							
Mediterranean	17	100	(0.4 ± 0.28)	(19.4 ± 7.1)	(35 ± 15.91)	(32.5 ± 15.0)	(213.6 ± 48.31)
Non-Mediterranean	12						
1		100	0.6	32.8	42	33.6	168.4
2		100	0.7	59.7	18.2	22.8	171.4
3		100	0	47.7	26.5	32	212
4		100	0	83.4	87.5	50	233
5		100	0.1	70.2	28.5	28.5	171.4
6		100	0.1	61.3	22.7	18.1	222
7		100	0.2	35.8	50	27.4	245
8		100	0.7	42.3	20.8	22.3	208.9
9		100	0.2	37.7	21	26.3	336.8
10		100	0.3	32.1	41.3	24	201.3
11		100	0.4	37.7	37.3	37.3	230
12		100	0.6	51.8	10.5	14	109
A-	2	110	1.1, 1.3	43, 82.5	8, 6	4.9, 6	51.9, 52

Figures in parenthesis refer to mean ± 1 standard deviation.

^aExpressed as a percentage of the rate of use of G6P.

^bExpressed as a percentage of the rate of use of NADP.

10 with A mobility was performed and showed features consistent with G6PD B normal phenotype and A phenotype respectively (Table 2).

Deficient G6PD variants

Of the 46 cases with deficient G6PD enzyme, 42 had B mobility, 2 had A mobility, while the remaining 2 were not visualized. Further characterization was done on 29 of the 42 cases with B mobility and on the two cases with A mobility.

Of the 29 G6PD B-, 17 had enzyme activity less than 10% and low Km for G6P with increased use of analogues as shown in Table 2, and were thus considered as G6PD Mediterranean. The remaining 12 cases had features different to one extent or another from G6PD Mediterranean (in particular all had normal Km), and were thus collectively labelled as non-Mediterranean variants, since they require further biochemical and/or molecular tests for proper characterization (Table 2). Both variants with A mobility were characterized as G6PD A- (a summary of results of deficient variants is in Table 3). Thus 92.6% of the Iraqis included in this study had a normal G6PD B phenotype, 1.3% a non-deficient G6PD A phenotype, 0.26%

had the G6PD A- variant, 0.26% were unidentified Class II variants, while 5.5% had Class II G6PD B- variants (and of those characterized, 58.6% were G6PD Mediterranean and the rest were non-Mediterranean variants).

Of the 46 G6PD deficient subjects only one had history of favism in early childhood, while six gave a family history of favism, but none had any history of haemolysis following infection or drugs, and all had consumed fava beans regularly with no ill effects, except for the patient with history of favism.

On evaluating the haematological parameters in G6PD deficient and non-deficient subjects, it was found that the deficient group had significantly lower haemoglobin ($P = 0.003$) and haematocrit ($P = 0.001$) and higher reticulocyte counts ($P = 0.0005$) than the non-deficient group.

Discussion

The overall frequency of G6PD deficiency of 6.3% for male adults resident in Baghdad, as determined in this study, is less than that reported previously by Amin-Zaki et al. and Al-Hamamy and Saeed [3,4] of 8.9%

Table 3 Biochemical characterization of the 46 individuals with G6PD deficiency identified in this study

WHO Class	Electrophoretic mobility	Total number	No.	Final designation
Class II	B	42	17	G6PD Mediterranean
			12	G6PD B- Non Mediterranean
			13	G6PD B- Not characterized
	Not visualized	2	2	Not characterized
Class III	A	2	2	G6PD A-

and 12.4% respectively. Furthermore, the frequency of G6PD deficiency in Arabs (in the current study) of 6% is less than the figures obtained by Amin Zaki et al. of 9.5% [3]. These differences are mostly related to the different contributions of various ethnic groups in the three studies, in the patient selection, and the sample size. It appears that our study, with random sampling and larger sample size, is probably more representative of Baghdad residents and of Arabs than the two former studies. However, the relatively small numbers of Kurds and Turkomans included in our study make any conclusions on the frequency of G6PD deficiency in these particular ethnic groups unreliable. The relatively high frequency of G6PD deficiency in Iraqis is not found in isolation from surrounding countries in the Middle East, and appears to be intermediate between the very high figures in some regions of Saudi Arabia and in Kurdish Jews of 65% and 58% respectively, and the lower figures in Jordan and Lebanon of 2% and 3% respectively [9].

Electrophoretic characterization of the G6PD enzyme revealed that, while G6PD B+ is the predominant enzyme, G6PD A+ (a non-deficient normal variant) exists in polymorphic frequency (1.3%). On the other hand, there was evident heterogeneity in the deficient variants, with more than half of the variants characterized based on enzyme activity, electrophoretic mobility, Km for G6P and analogue utilization showing features consistent with being Mediterranean variants. Such a finding is not unexpected since the Mediterranean variant is the most common deficient variant in Saudi Arabia [10, 11], United Arab Emirates [12] and Bahrain [13]. Molecular studies on 21 Middle Eastern individuals, including two Iraqis, revealed that all had mutation

563, in addition to the silent mutation 1311, which is identical to that characteristic of the Mediterranean mutation in European Mediterranean countries [9] and is different from that reported from the Indian sub-continent, where the silent 1311 mutation is not detected. This indicates a different origin of the Middle Eastern and European Mediterranean variants from that from India [14]. The possibility that the Mediterranean variant arose somewhere in the Mediterranean and then spread through the interaction between Greeks or southern Europeans with the Arabs and other ethnic groups in the Middle East [2], before and/or after the establishment of the Islamic empire, is a plausible explanation. However, the possibility that the latter variant arose independently in Arabia, and its frequency increased because of various selective forces, cannot be ruled out. One of the major selective forces in Iraq and the surrounding Middle Eastern countries is malaria, which was highly endemic throughout Iraq until the 1950s [15].

The presence of two cases with G6PD A- variant, coupled with the presence of polymorphic frequency of G6PD A+ variant is also seen in several Middle Eastern countries including Saudi Arabia [10], and is mainly due to the gene flow from Africa, mostly during the prosperous days of the Islamic empire, where Africans were brought in or immigrated and settled in various parts of the Islamic empire, including Iraq.

The presence of several deficient Class II variants, with B mobility but features different from the Mediterranean variant to one extent or another is interesting; some variants simulate G6PD Baghdad variant reported in Kurdish Jews [16], or El-Fayoum variant reported from Egypt [17], while others simulate Hamm or Dushanbe

variants reported from Germany and central Asia respectively [18]. Such a high frequency and relative heterogeneity of non-Mediterranean variants appears rather different from that seen in the Arabian peninsula, where the Mediterranean variant constitutes the large majority of cases. This is most likely due to the much wider ad-

mixture of the people of Mesopotamia throughout history with other peoples and civilizations.

It is our hope that the above findings will trigger the interest of fellow haematologists and biochemists to undertake further biochemical and molecular studies on G6PD variants in this part of the world.

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Note from the Editor

We would like to inform our readers that the next three Special Issues of the EMHJ will be on HIV/AIDS/STDs, Tropical and other Communicable Diseases, and Nutrition and are scheduled for 2002, 2003 and 2004 respectively.