SCIENTIFIC PAPER

Glucose-6-phosphate-dehydrogenase deficiency and haematopoietic stem cell transplantation in Chinese patients

WY Au 區永仁
JC So 蘇志釗
SK Ma 馬紹鈞
Albert KW Lie 李國維

Deficiency in glucose-6-phosphate dehydrogenase (G6PD), an X-linked recessive red cell enzymopathy, is endemic in Southern Chinese. Universal screening of newborn is done in Hong Kong, Taiwan and Singapore, among other places. In Hong Kong, 4.8% of males are affected and seven common G6PD alleles account for over 99% of all defects. Male hemizygotes suffer from severe deficiency, while female heterozygotes may also be affected. Deficiency of G6PD may affect haematopoietic stem cell transplantation (HSCT) recipients and donors, before and after HSCT. Female patients with clonal erythropoiesis (eg myelodysplasia/myeloproliferative diseases) will have the male population incidence of G6PD. Quantitative enzyme level screening is prudent for donors and recipients, and should be repeated after engraftment. Cotrimoxazole prophylaxis should be avoided in known male and female carriers, including those with low-normal G6PD enzyme levels. Our experience suggested that G6PD-deficient marrow, stem cell and cord blood donor units have no engraftment problems. Post-engraftment G6PD levels correlate with those in donors. An acquired change in G6PD status may serve as a surrogate marker for engraftment. For female heterozygote donors with normal G6PD levels, skewing of lyonized X-chromosome ratio during engraftment may result in over-expression of the deficient allele. This can result in unexpected significant G6PD deficiency. Hence, a repeat G6PD screening at stable engraftment is recommended, especially before commencement of oxidative medications.

Introduction

Deficiency in glucose-6-phosphate dehydrogenase (G6PD) is the commonest enzymopathy in the world. This is due to putative selective advantage against malaria falciparum infection. In Hong Kong, 4.47% of newborn males and 0.27% of newborn females are affected.[1] Universal screening is performed at birth locally, as well as in Singapore, Taiwan, Southern China, Malaysia, etc. Apart from a higher incidence of neonatal jaundice, deficient individuals are asymptomatic until they are exposed to oxidative stress, which can trigger off life-threatening intravascular haemolysis.[2]

Key words
Glucosephosphate dehydrogenase deficiency; Hematopoietic stem cell transplantation

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Department of Medicine, Queen Mary Hospital, Pokfulam Road, Hong Kong
WY Au, AKW Lie
Department of Pathology, Queen Mary Hospital, Pokfulam Road, Hong Kong
JC So
Department of Pathology, Hong Kong Sanatorium Hospital, Happy Valley, Hong Kong
SK Ma

Correspondence to: Dr WY Au
Tel: 852 28554792
Fax: 852 28726896
E-mail: auwing@hotmail.com

Mutations and enzyme levels in Chinese population

While over 400 G6PD variants had been biochemically characterised, only 129 were sequenced, and some biochemical variants were subsequently found to be genetically identical.[2,3] The genetic variants showed marked ethnic bias in frequencies. The three common Chinese G6PD variants—G6PD Canton (nt 1376 G→T), Kaiping (nt 1388 G→A), and Gaohe (nt 95 A→G)—are all Class II to III variants associated with mild-to-moderate clinical severity.[2,3]

Screening can be done by a quick and inexpensive fluorescent test. The substrate glucose-6-phosphate (G6P) is oxidised to 6-phosphogluconate by G6PD and nicotinamide-adenine dinucleotide phosphate (NADP) is reduced to NADPH. Since 6-phosphogluconate dehydrogenase is present, further reduction of NADP occurs. Although some NADPH is removed by oxidised glutathione, the excess NADPH shows up as fluorescence. For more elaborate results and to confirm dubious cases, G6PD assay can be performed. This is based on the spectrophotometric rate of NADP reduction during incubation with G6P. The activity is calculated from the rate of increase in absorbance due to NADPH generation, the millimolar extinction coefficient of NADPH, the haemoglobin (Hb) and the dilution factor (normal range, 6.35-10.33 IU/gHb).

In our retrospective review of 181 G6PD-deficient cases among 5000 consecutive screening requests, only seven G6PD mutations were detected. Over 70% of cases are contributed by the three commonest variants: G6PD Canton, Kaiping, and Gaohe (Table 1). Such a genetic distribution is characteristic for Southern Chinese (Table 2).[3-5] All male hemizyotes have low enzyme activity (<1 IU/gHb except G6PD Chinese–4 and Chinese–

葡萄糖六磷酸鹽脫氫酶（G6PD）缺乏症與華人血幹細胞移植（HSCT)的關係

葡萄糖六磷酸鹽脫氫酶（G6PD）缺乏症為一性連遺傳的紅血球毛病。在華南、台灣及東南亞十分普遍，嬰孩多有G6PD的普查。香港男性中4.8%有G6PD缺乏，主要基因突變有7種。女性異合子（heterozygotes）亦可有G6PD酶缺乏。此症對血幹細胞移植（HSCT）的捐髓者和受髓者皆有影響，兩者在HSCT前後皆要注意G6PD酶素，作量化的評估。女血癌患者（如MPD、MDS、CML）的克隆性紅血球會有男性一般的G6PD率。男女癌者如G6PD不高，應避免服用甲氧苄氨／磺胺惡唑（cotrimoxazole）等藥。G6PD酶缺乏的骨髓，週邊及臍帶血幹細胞均可無礙生長，而移植後的G6PD度數大致和捐髓者相近，甚至可作成功移植佐証。但女性異合子在移植後由正常X染色體轉用G6PD缺乏X染色體，因而造成預計外的G6PD缺乏，所以所有移植後的病人都應再受G6PD測試，以策萬全。

5), as do female homozygotes (or compound heterozygotes). In female heterozygotes, the overall G6PD level is usually an (intermediate low-normal) average of normal and abnormal red cells. However, some heterozgyotes will randomly skew towards the deficient allele.[6] Our data showed that 95% of G6PD-deficient females are skewed heterozygotes. Similarly, 93 to 100% of G6PD-deficient newborn females are reported to be skewed heterozygotes.[4,7] Hence, skewed heterozygosity is the main cause of female G6PD deficiency.

Glucose-6-phosphate dehydrogenase deficiency in haemopoietic stem cell transplantation recipients

Deficiency for G6PD is routinely screened in

TABLE 1. The spectrum of glucose-6-phosphate dehydrogenase (G6PD) mutations in Hong Kong Chinese patients with clinical referral*

G6PD variant	Male subjects (n=139)			Female subjects† (n=40)		
	No.	Age (years)	G6PD activity (IU/gHb)	No.	Age (years)	G6PD activity (IU/gHb)
G6PD Canton (nt 1376 G→T)	40	37±4	0.43±0.09	16	43±5	3.19±0.82
G6PD Kaiping (nt 1388 G→A)	46	37±3	0.90±0.23	7	35±10	4.94±0.48
G6PD Gaohe (nt 95 A→G)	14	38±7	0.60±0.19	5	38±10	5.20±1.89
G6PD Viangchan (nt 871 G→A)	9	21±6	0.42±0.12	5	46±10	2.88±0.77
G6PD Chinese–4 (nt 392 G→T)	7	33±13	1.05±0.19	0	-	-
G6PD Union (nt 1360 C→T)	4	59±15	0.22±0.13	0	-	-
G6PD Chinese–5 (nt 1024 C→T)	2	42	1.95	0	-	-
Unknown	9	-	-	4	-	-
Poor DNA quality	8	-	-	3	-	-

* Age and G6PD activity are shown in mean±standard error

† In addition to 40 female heterozygotes, one female subject is homozygous for G6PD Canton, while another female subject is compound heterozygous for G6PD Canton and Chinese–4

TABLE 2. The prevalence of glucose-6-phosphate dehydrogenase (G6PD) variants characterised at the molecular level in Chinese populations

	Studies*			
	Chiu et al,[3] 1993	Huang et al,[4] 1996	Ainoon et al,[5] 1999	Present study
Patients	Adults (n=43)	Newborns (n=162)	Newborns (n=38)	Adults (n=179)
Sex (M/F)	43/0	112/50	38/0	139/40
G6PD Variant				
G6PD Canton (nt 1376 G→T)	12 (27.9%)	78 (48%)	19 (50%)	56 (31.3%)
G6PD Kaiping (nt 1388 G→A)	11 (25.6%)	27 (16.7%)	13 (34.2%)	53 (29.7%)
G6PD Gaohe (nt 95 A→G)	8 (18.6%)	9 (5.6%)	2 (5.3%)	19 (10.6%)
G6PD Chinese-3 (nt 493 A→G)	0	15 (9.3%)	0	0
G6PD Viangchan (nt 871 G→A)	0	1 (0.6%)	0	14 (7.8%)
G6PD Chinese-5 (nt 1024 C→T)	2 (4.6%)	10 (6.2%)	1 (2.6%)	2 (1.1%)
G6PD Chinese-4 (nt 392 G→T)	3 (7%)	2 (1.2%)	0	7 (3.9%)
G6PD Union (nt 1360 C→T)	0	1 (0.6%)	0	4 (2.2%)
G6PD Mahidol (nt 487 G→A)	0	2 (1.2%)	0	0
Unknown	7 (16.3%)	17 (10.6%)	3 (7.9%)	24† (13.4%)

* Chiu et al[3]—samples from United States, Taiwan and China; Huang et al[4]—cord blood samples from one hospital in Taipei, Taiwan; Ainoon et al[5]—cord blood samples from maternity hospital in Kuala Lumpur, Malaysia; Present study—includes data on male subjects and female heterozygotes; one female subject homozygous for G6PD Canton and another female subject compound heterozygous for G6PD Canton and Chinese–4 were excluded

† Includes cases with G6PD mutations unknown and those with poor DNA quality for study

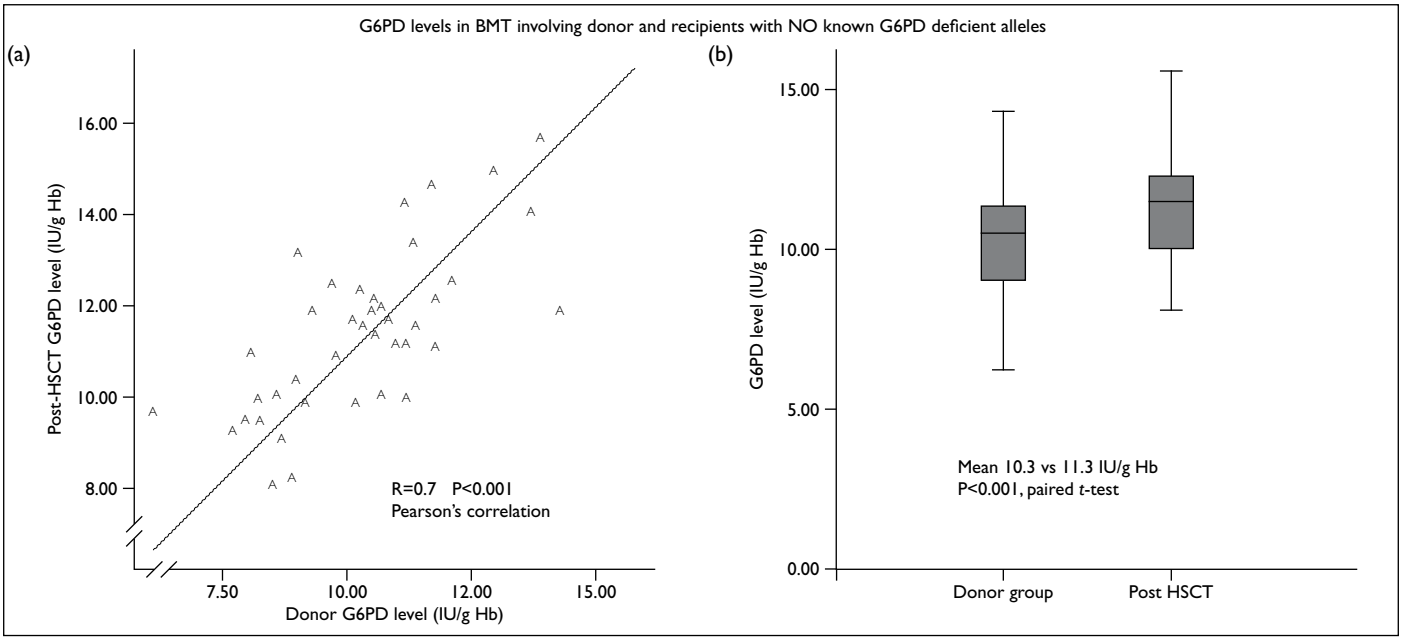

FIG. (a) Correlation between donor and post–haematopoietic stem cell transplantation (HSCT) recipient glucose-6-phosphate dehydrogenase (G6PD) levels in 40 consecutive adult myeloablative HSCT cases. (b) Box and whisker plot of donor and post-HSCT G6PD levels, showing increased mean G6PD in recipients than their respective donors

Chinese patients with haematological malignancies since oxidising medications (eg cotrimoxazole-trimethoprim, rasburicase) may be used. A G6PD level is more reliable than a simple screening, since patients with low-normal G6PD levels (mostly heterozygous females) may still suffer from massive intravascular haemolysis under severe oxidative stress (eg a combination or high dose of oxidising medications).[8] Furthermore, any haemolytic challenge may not be well-tolerated with concomitant marrow suppression and renal impairment. Hence, it may be advisable to use alternative medications (eg pentamidine inhalation) unless the G6PD level is well within normal range.

Many physicians may also have a low index of suspicion in screening female patients for G6PD deficiency. This is because of the low incidence rate at birth. However, up to 10% of females in some populations are heterozygotes and age-related pronounced skewing of randomly lyonized X-chromosomes will give an increasing frequency of G6PD deficiency.[9] Haematopoietic stem cell transplantation (HSCT) is increasingly performed for older patients, and the incidence of G6PD deficiency in females over 50 years old is low but not negligible.[9,10] Furthermore, by definition, the incidence of G6PD deficiency in female patients with clonal myeloid haemopoietic disorders (eg myelodysplasia and chronic myeloid leukaemia) will be the same as that in male population.[11] Hence, irrespective of previous birth screening history, a repeat G6PD screening is obligatory at HSCT work-up.

Glucose-6-phosphate dehydrogenase–deficient donors

Most adult donors (either matched sibling or unrelated voluntary donors) will be screened for G6PD deficiency as part of the work-up procedure. Deficiency per se should not be a factor affecting donor eligibility or donor choice. The rate of engraftment, transfusion requirement and post-HSCT Hb level do not differ between recipients receiving G6PD-deficient or normal adult stem cells.[11] Similar to the recipient screening process, we would advise a G6PD level rather than a fluorescent screen test to identify donors with low G6PD levels.[6,8] These will mostly be female heterozygote carriers, and due to the possibility of increased skewing towards the deficient allele after HSCT,[12] repeat G6PD assays in the recipient after HSCT is mandatory.

In countries where G6PD deficiency is screened at birth, the biochemical G6PD status of cord blood units are also known. Many public voluntary cord blood banks exclude such units for processing and banking. On the other hand, most commercial and hybrid banks accept such units. Our experience of using unrelated cord blood units with G6PD deficiency showed no competitive disadvantage in terms of engraftment.[13] This applies to both adult and paediatric HSCT, with single or double cord blood units. Hence, our current policy is that G6PD deficiency per se is not a factor affecting donor cord unit choice or eligibility for storage.

Glucose-6-phosphate dehydrogenase levels after haematopoietic stem cell transplantation

Patients receiving stem cells from known G6PD-deficient donors will have acquired G6PD deficiency and should receive the same precautions as normal G6PD-deficient individuals. On the other hand, G6PD-deficient patients receiving normal marrow will be cured of their enzymopathy. With complete engraftment, the G6PD level should convert to the donor status by 3 to 6 months' post-HSCT and can actually be used as a surrogate marker for red cell chimerism and engraftment status.[10]

Normal patients receiving stem cells from donors with normal G6PD levels are unlikely to have G6PD deficiency problems after HSCT. This is particularly the case if the donor is male (or female without common G6PD-deficient alleles [Table 2]). Our retrospective correlation data showed that the 'normal' post-HSCT G6PD level correlates with 'normal' donor level (Fig a). Hence, polygenic determinants of 'normal' G6PD enzyme levels are passed on via an HSCT.[14] Further paired analysis showed that G6PD levels are significantly more likely to be higher in recipients (by 1 IU/g Hb) than in their donors (Fig b). This minor element of environmental influence may be due to a shorter red cell survival or a selective effect of GVHD, infections or medications on red cell enzyme expression.

For known or suspected female heterozygotes, there is a possibility of marked changes in G6PD levels in recipients after HSCT. This is due to the stochastic skewing of random X-inactivated chromosomes at engraftment, due to the limited numbers of stem cells transplanted and engrafted.[15] Such skewing can occur either way, and does not favour the normal allele. One problem from such skewing is that occult heterozygote females with completely normal (or even high-normal) G6PD levels may generate G6PD-deficient recipients, if the deficient allele becomes predominant.[10] In the absence of universal and infallible genetic screening, the only practical solution will be to repeat G6PD screening at 6 months for all HSCT recipients from Chinese female donors.

Conclusions

Most Chinese physicians are conversant with the clinical implications of G6PD deficiency. However, with increasing worldwide patient migration and stem cell traffic, HSCT involving Chinese donors and patients may take place in any part of the world. Similarly, G6PD deficiency is also prevalent in other ethnic groups (eg Italians, Africans) but with a different genetic spectrum of mutations.[2] With clinical prudence, iatrogenic haemolytic problems associated with G6PD deficiency can be avoided in the HSCT setting.

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