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Editorial note:

This is now the second year into the publication of **Topical Update – The Hong Kong College of Pathologists.** The article in this issue is contributed by the specialty of Haematology, on the subject of glucose-6-phosphate dehydrogenase deficiency, a common entity in Hong Kong. We welcome any feedback or suggestions. Please direct them to Dr. Janice Lo (e-mail: janicelo@dh.gov.hk) of the Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists. Happy reading!

Recent perspectives in glucose-6-phosphate dehydrogenase (G6PD) deficiency

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Background

G6PD catalyzes the conversion of glucose-6-phosphate (G6P) to 6-phosphogluconate concurrent with reduction of NADP to NADPH, which in turn acts through glutathione and catalase pathways to detoxify hydrogen peroxide, thus counteracting oxidative stress to the cell. In the body, red cells are most susceptible to oxidative damage because oxygen radicals are generated continuously as haemoglobin cycles from deoxygenated to oxygenated forms, as well as being readily exposed to exogenous oxidizing agents present in the blood. Hence G6PD deficiency is a prototype cause of haemolytic anaemia due to intrinsic red cell enzyme abnormality.

Deficiency of G6PD enzyme, an X-linked recessive disorder and the commonest inherited enzymopathy in humans, is prevalent in Southern China. In Hong Kong, the prevalence of G6PD deficiency is 4.47% for males and 0.27% for females based on data generated from neonatal screening. Clinical manifestations of G6PD deficiency range from neonatal jaundice and episodic haemolysis precipitated by drugs, fava beans and infection, to the more severe cases of chronic non-spherocytic haemolytic anemia (CNSHA) associated with Class I G6PD variants. Occasionally, neonatal jaundice if severe enough

may cause death or permanent neurological damage. Furthermore, patients with CNSHA may require intermittent blood transfusions. While more than 400 G6PD variants have been characterized using biochemical parameters, only around 129 variants have been deciphered at the molecular level [1]. Similar to inherited globin disorders, the spectrum of G6PD mutations is different between ethnic groups. The common G6PD variants previously reported in the Chinese, such as G6PD Canton (nt 1376 G→T), Kaiping (nt 1388 G→A) and Gaohe (nt 95 A→G) are associated with mild to moderate clinical severity, and are categorized as Class II – III variants.

Spectrum of G6PD variants in Hong Kong Chinese

Like other monogenic disorders, increasing knowledge on G6PD mutations paves the way for genotype phenotype studies. In the seven-year period from 1996 to 2002, a total of 181 consecutive cases of G6PD deficiency as detected by fluorescence spot test at the Haematology Laboratory of Queen Mary Hospital were accrued, in which G6PD enzyme assays were carried out and DNA samples were extracted for mutation analysis at the Department of Biochemistry, the University of Hong Kong. They comprised 139 males and 42 females. Most requests were ordered as routine screening before chemotherapy for haematological malignancies, drug prescription or marrow donation. Other indications for G6PD screening included investigation of jaundice (including neonatal jaundice), anaemia, movement disorder and confirmation of known history of G6PD deficiency. For males, the G6PD enzyme activity in mean ± standard error (S.E.) is 0.72 ± 0.09 IU/gHb (reference range: 6.35 – 10.33 IU/gHb), and the corresponding values for haemoglobin (Hb) level is 12.3 ± 0.27 g/dL. For females, the G6PD enzyme activity in mean ± S.E. is 3.57 ± 0.39 IU/gHb, and the corresponding values for Hb level is 10.8 ± 0.45 g/dL. As this cohort involved hospital patients, the G6PD enzyme activity may be affected by the degree of erythroid stress that in turn is reflected by the Hb level, since young red cells contain higher levels of G6PD than mature ones.

Seven G6PD mutations were detected. Three common variants namely G6PD Canton, Kaiping and Gaohe (also known as Gaozhou) accounted for approximately 70% of all cases. No CNSHA or Class I variant was encountered. The G6PD enzyme activity was correlated with mutation in males and females (Table 1). In males who were hemizygous for the G6PD mutation, the enzyme activity was very low as expected. Slightly higher enzyme activities were seen in G6PD Chinese-4 (nt 392 G→T) and G6PD Chinese-5 (nt 1024 C→T), both of which showed mean enzyme activity of above 1 IU/gHb. The mean G6PD enzyme activity ranged from 2.88 – 5.2 IU/gHb in heterozygous females. Both alleles were abnormal in two female subjects: one homozygous for G6PD Canton showing an enzyme activity of 0.94 IU/gHb, and another compound heterozygous for G6PD Canton and Chinese-4 showing an enzyme activity of 0.603 IU/gHb.

Only six subjects out of the 181 presented as acute haemolytic anaemia related to G6PD deficiency. Among five male patients with G6PD haemolysis, the precipitating factors were paracetamol overdose in suicide attempt, an infective episode, exposure to food dye and reactivation of hepatitis B infection (two patients). A 62-year old woman presented with acute haemolysis (Figure 1) after nitrofurantoin treatment for urinary tract infection. She was a heterozygous carrier of G6PD Canton with enzyme activity of 1.67 IU/gHb and her son, a 29-year old man, was confirmed to be G6PD Canton hemizygote with an enzyme level of 0.28 IU/gHb.

G6PD deficiency in females

The majority of females with detectable G6PD deficiency are in fact heterozygous carriers of G6PD mutations, with the enzyme deficiency being manifested due to extreme lyonization. These subjects are also at risk of drug-induced haemolysis if the enzyme level is sufficiently low. The diagnosis of heterozygous females using screening tests and enzyme assays, however, is unreliable. Although most heterozygous females tend to show an intermediate G6PD activity, the

range of lyonization has to be taken into account, so that normal G6PD enzyme activity does not exclude heterozygosity. Molecular analysis is the best way to be certain about the G6PD status of a female subject. Clinically, the level of activity gives a good guide to the severity of G6PD deficiency in heterozygous females. With extreme lyonization and an enzyme activity level that falls into the hemizygous range, the risk is anticipated to be the same as an affected male subject. Moreover, homozygous or compound heterozygous females will not escape detection, as their enzyme activity is very low and comparable to that seen in deficient males.

Since the skewing of X-chromosome inactivation has been reported to increase with age, an intriguing contention is that elderly females who are heterozygous for G6PD mutations may present with biochemical enzyme deficiency [2]. Importantly, these subjects will not be detectable at birth despite population based neonatal screening programs for G6PD deficiency. To address this issue, a recent local study identified 18 G6PD heterozygotes among 173 elderly Hong Kong Chinese women with median age of 90 years [3]. Three heterozygotes were biochemically G6PD deficient with enzyme levels of 1.3, 3.6 and 4.7 IU/gHb respectively owing to skewed X-chromosome inactivation affecting the wild type allele, and at 1.73% (3/173) the prevalence in females was significantly higher than that obtained from population screening at birth. Fifteen heterozygotes, with skewing apparently affecting the mutant alleles, showed G6PD enzyme level within normal limits but still significantly lower than females not harbouring any G6PD mutation. Based on this experimental evidence, and the fact that female patients with bona fide G6PD haemolysis are encountered in clinical practice, it is therefore prudent to check the G6PD status of female subjects, at least in the elderly, before prescribing drugs with oxidizing properties at our locality.

Summary

The spectrum of G6PD mutations in Hong Kong is similar to other Chinese populations. Common G6PD variants are G6PD Canton, Kaiping and Gaohe (Gaozhou), which are of mild to moderate severity. Female heterozygotes may also present with biochemical G6PD deficiency. Due to age-related skewing of X-chromosome inactivation, elderly females who are heterozygous carriers of G6PD mutations are particularly at risk of manifesting G6PD deficiency. In addition to males, a case can be made to screen the G6PD status of females, at least in the elderly, before prescribing drugs with oxidizing properties.

References

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3. Au WY, Lam V, Pang A, Lee WM, Chan JL, Song YQ, Ma ES, Kwong YL. Glucose-6-phosphate dehydrogenase deficiency in female octogenarians, nanogenarians and centenarians. J Gerontol A Biol Sci Med Sci 2006; 61: 1086 – 1089.

Figure 1

Peripheral blood smear showing a hemi-ghost cell (arrow) during acute G6PD haemolysis in this patient. Wright stain x 1,000

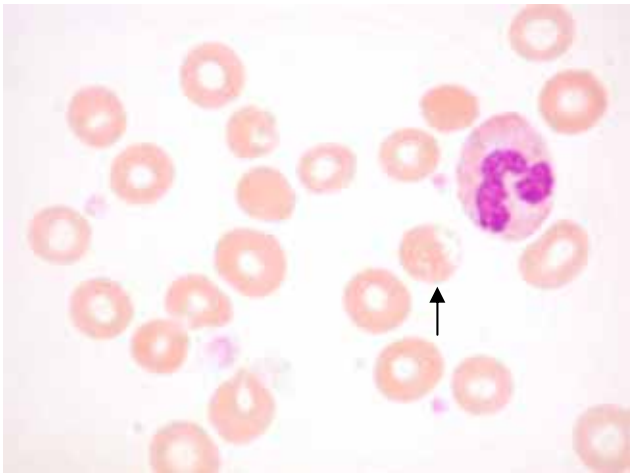

Table 1: The spectrum of G6PD mutations in Hong Kong Chinese

G6PD variant	Male subjects				Female subjects*			
	No.	Age (yrs)	Haemoglobin (g/dL)	G6PD activity (IU/gHb)	No.	Age (yrs)	Haemoglobin (g/dL)	G6PD activity (IU/gHb)
G6PD Canton (nt 1376 G→T)	40	37 ± 4	12.8 ± 0.5	0.43 ± 0.09 (0 – 3.3)	16	43 ± 5	11.0 ± 0.5	3.19 ± 0.82 (0.29 – 9.3)
G6PD Kaiping (nt 1388 G→A)	46	37 ± 3	11.3 ± 0.5	0.90 ± 0.23 (0 – 8.6)	7	35 ± 10	11.7 ± 2.1	4.94 ± 0.48 (3.05 –6.37)
G6PD Gaohe (nt 95 A→G)	14	38 ± 7	12.8 ± 0.7	0.60 ± 0.19 (0 – 2.36)	5	38 ± 10	10.9 ± 1.0	5.20 ± 1.89 (0.6 – 12.1)
G6PD Viangchan (nt 871 G→A)	9	21 ± 6	12.3 ± 0.8	0.42 ± 0.12 (0.02 – 1.03)	5	46 ± 10	9.6 ± 1.2	2.88 ± 0.77 (0.29 – 4.98)
G6PD Chinese-4 (nt 392 G→T)	7	33 ± 13	13.2 ± 0.8	1.05 ± 0.19 (0.18 – 1.82)	0			
G6PD Union (nt 1360 C→T)	4	59 ± 15	13.5 ± 0.7	0.22 ± 0.13 (0.045 – 0.6)	0			
G6PD Chinese-5 (nt 1024 C→T)	2	42 (mean)	12.2 (mean)	1.95 (mean)	0			
Unknown	9				4			
Poor DNA quality	8				3			
Total	139				40			

Key: *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. Age, haemoglobin level and G6PD activity are tabulated in mean ± standard error, with range of enzyme activity in parenthesis.