Identification of glucose 6-phosphate dehydrogenase deficiency in a population with a high frequency of thalassemia

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Abstract High frequencies of both thalassemia trait (5.2%) and glucose 6-phosphate dehydrogenase (G6PD) deficiency for only males (1.3%) have been observed in the Calabrian population. The G6PD activity measurement was carried out on 1239 samples of whole blood from Calabrian subjects of both sexes (age range 10–55) by a differential pH-metry technique which was quite suitable to determine the G6PD deficiency in mass screenings. The analyzed subjects showed: only the thalassemia trait; or only the G6PD deficiency; or only the total iron serum deficiency; or G6PD deficiency associated with the thalassemia trait or with the total iron serum deficiency. The G6PD heterozygous subjects have an enzymatic activity which is masked by both the thalassemia trait and the total iron serum deficiency. In a population showing high frequencies of both thalassemia trait and G6PD deficiency, the comparison of G6PD activity of heterozygous subjects also affected with the thalassemia trait is more reliable if referred to the enzymatic activity of the carriers of the latter inherited anomaly rather than to G6PD activity of normal subjects.

Key words: Glucose 6-phosphate dehydrogenase deficiency; Thalassemia; Glucose 6-phosphate dehydrogenase activity

1. Introduction

High frequencies of glucose 6-phosphate dehydrogenase (G6PD, EC 1.1.1.49; locus linked to the X chromosome) deficiency and thalassemia (a recessive autosomal disorder) have been observed in Calabria, southern Italy [1–3], as well as numerous genetic variants of both anomalies [4–7].

Studies of the activity of erythrocyte G6PD which were carried out on carriers of thalassemia (thal trait) and on thal trait subjects also showing G6PD deficiency ($G6PD^-$) have revealed two fundamental aspects: (a) microcytosis in subjects with the thal trait causes an increased activity of G6PD, expressed either as U/g hemoglobin (Hb) or as U/10^{12} red blood cells (RBC) [8,9]; (b) the activity of G6PD in young erythrocytes is increased [9]; this was confirmed by a study [10], carried out on 20 children affected with transitory erythroblastopenia, in which it was hypothesized that the activity is 100 times greater in young erythrocytes than in middle-aged ones. Although the hypothesis of such great activity was not confirmed by another study [11], performed in patients showing high reticulocytosis, it has been ascertained that the G6PD activity in young erythrocytes is undoubtedly higher.

The aim of the present study was to determine the activity of G6PD in the following Calabrian sample groups of subjects: normal subjects; thal trait subjects; $G6PD^-$ subjects; $G6PD^-$+thal trait subjects; $G6PD^-$+total iron serum deficiency (Fe^-) subjects; Fe^- females; and pregnant females. The clinical and physiological status of the latter two groups of subjects causes an alteration of their hematological condition. In order to analyze a statistically valid number of samples, we adopted the technique of differential pH-metry [12,13]; since it uses whole blood, it is able to provide equally precise results in a shorter time than the method recommended by the World Health Organization [14,15].

2. Materials and methods

The study of G6PD activity was carried out on 1239 samples of whole blood (anticoagulated with ethylenediaminetetraacetic acid) from Calabrian subjects of both sexes (age range 10–55): 844 normal subjects (386 females and 458 males); 127 thal trait (67 females, of whom 55 β-thal, 7 α1-thal, 'serious' α-thalassemia, 5 δβ-thal, and 60 males, of whom 50 β-thal, 3 α1-thal and 7 δβ-thal); 69 Fe^- females, of whom 12 were also $G6PD^-$; the latter group had mean values of total iron serum less than 35 μg/dl; 52 pregnant women (8–32 weeks of pregnancy); 147 $G6PD^-$ subjects (80 female heterozygotes, G6PD-heteroz, 17 of whom were thal trait too, and 67 male hemizygotes, G6PD-hemiz, 16 of whom were thal trait too).

The G6PD-hemiz subjects showing an enzymatic activity at least 5% less than the mean value (9.3 ± 1.0 U/g Hb) of the enzymatic activity of the normal male subjects were classified as G6PD Mediterranean (G6PD Med) phenotype, while those with values at least 5% greater than the same mean value of the enzymatic activity of the normal male subjects were classified as G6PD non-Mediterranean (G6PD non-Med). In the carriers of the thalassemia trait, both phenotypes were identified by means of a genetic-familial investigation.

The activity of G6PD was measured by differential pH-metry [12,13], a technique characterized by simplicity of execution (absence of any pretreatment of the sample), rapidity of sample analysis (90 s/analysis), and high reproducibility (within-series CV 3.5%; between-series CV 2.9%) and accuracy ($r = 0.981$) [14]. Before the analysis of the 1239 subjects we excluded 22 (1.8%) because four out of these 22 (three females and one male) showed a number of platelets $> 400 \times 10^9$/l and 18 (14 females and one male) showed a number of leukocytes $> 12.0 \times 10^9$/l. The subjects described in Tables 1 and 2 do not include these 22 refused subjects.

Statistical analysis of the results was carried out with Student's *t*-test [16].

3. Results

Before presenting the results, it is necessary to consider two points: (a) there were no differences in the mean values of G6PD activity within the subjects with the β, α and δβ tha-

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Abbreviations: G6PD, glucose 6-phosphate dehydrogenase; Hb, hemoglobin; RBC, red blood cells

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Table 1
Values of principal hemometric parameters and of total iron serum in the examined groups of subjects

Sample group	Sex	*n*	Hb (g/dl)	RBC (10^{12}/l)	Mean corpuscular volume of erythrocytes (f/l)	Total iron serum (μg/dl)
Normals	M	458	14.6 ± 1.2	5.1 ± 0.4	83.7 ± 6.5	113 ± 12
	F	386	13.4 ± 0.9	4.7 ± 0.4	81.8 ± 7.6	96 ± 10
Pregnant women		52	12.5 ± 0.9	4.2 ± 0.5	86.4 ± 3.5	75 ± 12
Fe^-	F	57	9.2 ± 1.4	4.6 ± 0.6	68.6 ± 6.4	21 ± 7
Thal trait	M	60	12.0 ± 1.5	6.0 ± 0.5	67.0 ± 5.1	127 ± 16
	F	67	10.9 ± 1.7	5.5 ± 0.9	67.4 ± 4.8	112 ± 18
G6PD-hemiz		51	14.1 ± 1.1	4.9 ± 0.4	85.6 ± 4.9	106 ± 11
G6PD-heteroz		63	12.8 ± 1.3	4.4 ± 0.4	86.6 ± 5.3	93 ± 12
G6PD-heteroz+Fe^-		12	9.0 ± 1.6	4.4 ± 0.5	66.9 ± 6.0	19 ± 9
G6PD-hemiz+thal trait		16	12.2 ± 1.4	5.9 ± 0.6	85.0 ± 5.1	131 ± 18
G6PD-heteroz+thal trait		17	11.0 ± 1.2	5.3 ± 0.8	85.2 ± 7.0	115 ± 14

lassemia phenotypes, expressed either as U/g Hb or as U/10^{12} RBC; therefore, by the term 'thal trait' we mean the presence of any of these thalassemic phenotypes (Tables 1 and 2); (b) in agreement with other studies [9,14], the enzymatic activity of G6PD expressed as U/g Hb discriminates the different groups of subjects better than the activity expressed per number of erythrocytes; for this reason, the results that follow refer to the enzymatic activity expressed as U/g Hb (Table 2). The mean values of main hemometric parameters and total iron serum of all the groups are reported in Table 1.

The results of erythrocyte G6PD activity determination in all the subject groups analyzed are reported in Table 2.

The data concerning statistical analysis of differences are given in Table 3.

As expected, there were significant differences between the activity of G6PD of the normal subjects and those with the thal trait, in both males and females. In fact, the enzymatic activity of both thal trait groups of subjects was significantly increased with respect to the normal subjects. The same was true in the comparison of normal females with Fe^- females, because these latter show higher G6PD activity than normal females. However, the mean enzymatic activity of the latter group was not far from that of the thal trait females. This confirms the hypothesis [9,10] that the presence of a high number of young erythrocytes in the circulation, both in the thal trait subjects and in those with total iron serum deficiency, masks the true mean value of G6PD activity.

The difference in mean enzymatic activity between the G6PD-heteroz subjects and the normal females is highly significant because the enzymatic activity of the first group of subjects is lower than the latter, while the difference between the latter group and the G6PD-heteroz+thal trait subjects is at the limit of significance. The difference between the G6PD-heteroz+thal trait subjects and the G6PD-heteroz ones is of greater significance due to the increase of the enzymatic activity of the latter group of subjects. Finally, comparison of the G6PD-heteroz+thal trait subjects with the thal trait females reveals the highest discriminant power due to the increase of the enzymatic activity of the latter group of subjects. The lack of a significant difference in mean activity between the G6PD-heteroz+thal trait and G6PD-heteroz+Fe^- groups confirms the role that thalassemia and total iron serum deficiency play in masking the true enzymatic activity in the female carriers of the G6PD deficiency (Table 3).

When the G6PD-hemiz and G6PD-hemiz+thal trait subjects are compared independently with the normal male subjects and with those with 'thal trait', the differences are highly significant because both G6PD-hemiz and G6PD-hemiz+thal trait subjects show a decreased enzymatic activity with respect to the normal male subjects (Table 3).

The distribution of the values of enzymatic activity of the G6PD-heteroz and G6PD-heteroz+thal trait subjects indicates that 76.2% of the first group and 29.4% of the second are clearly discriminated from normal females (Fig. 1).

The discrimination of the G6PD-hemiz and the G6PD-hemiz+thal trait subjects from the normal ones is 100% (Fig. 2).

The analysis of the male subjects affected with G6PD deficiency involved the study of the Mediterranean and non-Mediterranean phenotypes. The difference between the non-Med G6PD-hemiz subjects and the non-Med G6PD-hemiz+thal

Table 2
Mean value ± S.D. and relative values (%) of G6PD activity in whole blood measured by differential pH-metry in the examined groups of subjects

Sample group	*n*	Sex	G6PD activity			
			U/g Hb		U/10^{12} RBC	
			$\bar{x}$ ± S.D.	%	$\bar{x}$ ± S.D.	%
Normals	458	M	9.3 ± 1.0	100	266 ± 21	100
	386	F	9.6 ± 1.0	100	273 ± 23	100
Pregnant women	52		10.0 ± 1.3	104	294 ± 35	107
Fe^-	57	W	15.4 ± 4.1	160	305 ± 40	112
Thal trait	60	M	15.3 ± 2.0	164	307 ± 26	115
	67	W	16.2 ± 2.3	168	327 ± 43	119
G6PD-hemiz	51		0.7 ± 0.6	8	21 ± 16	8
G6PD-heteroz	63		5.6 ± 1.8	58	163 ± 50	59
G6PD-heteroz+Fe^-	12		8.1 ± 1.2	84	165 ± 32	60
G6PD-hemiz+thal trait	16		1.9 ± 1.0	20	40 ± 22	15
G6PD-heteroz+thal trait	17		7.9 ± 2.4	82	165 ± 48	60

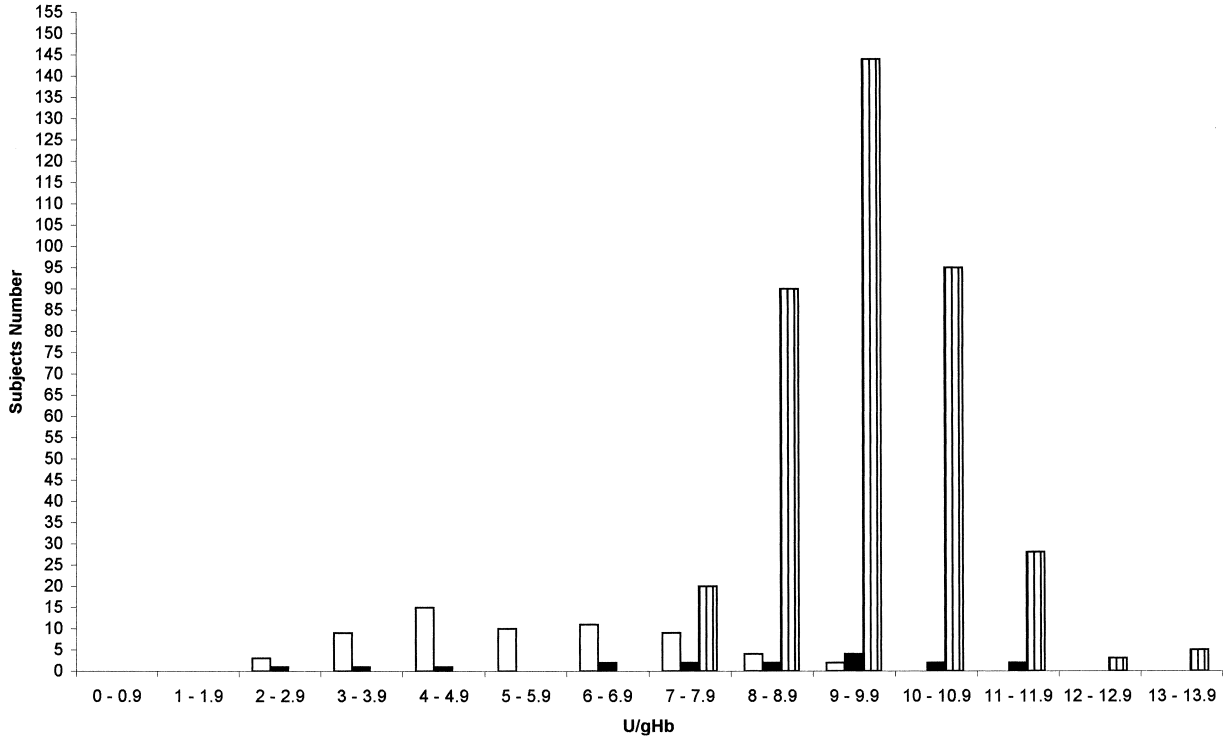

Fig. 1. Distribution in classes of the values of G6PD activity in: ▥ normal females; □ heterozygous females; ■ heterozygous female carriers of the thalassemia trait.

trait ones is at the limit of significance. In contrast, the difference between the Med G6PD-hemiz subjects and the Med G6PD-hemiz+thal trait ones is highly significant because the latter group shows an increased enzymatic activity (Table 3).

4. Discussion

The Calabrian population has a mean frequency of thalassemia trait of 5.2% [1] and of G6PD deficiency, referred only to males, of 1.3% [2,3]. Therefore, the probability of inheriting both anomalies in the heterozygous form is 0.13% in females and 0.07% in males.

The results confirm the real difficulty, already highlighted by other authors [9], of identifying female heterozygotes for G6PD deficiency when they are also carriers of thalassemia. This is easily understood when one considers both the role of random inactivation of the X chromosome [17–19], which per se renders it impossible to discriminate all heterozygous subjects from normal females, and the role that thalassemia plays in masking the true values of G6PD activity in these subjects. In confirmation of this, our careful genetic-familial investigation allowed us to identify the 'false negative' heterozygous carriers and non-carriers of thalassemia whose values of enzymatic activity fell within the normal range (Fig. 1); these subjects were considered 'obligate' carriers of the enzyme deficiency because they were mothers or daughters of a hemizygous subject.

In light of our results, it is clear that in a population like

Table 3
Statistical significance of the differences in mean values of G6PD activity between the examined groups of subjects by Student's *t*-test

Normal F	vs.	Thal trait F	Student's $t = 23.10$	$P < 0.001$
Normal F	vs.	Fe^- F	Student's $t = 10.63$	$P < 0.001$
Thal trait F	vs.	Fe^- F	Student's $t = 1.31$	$P < 0.2$
Normal F	vs.	G6PD-heteroz	Student's $t = 17.26$	$P < 0.001$
Normal F	vs.	G6PD-heteroz+thal trait	Student's $t = 2.91$	$P < 0.01$
G6PD-heteroz	vs.	G6PD-heteroz+thal trait	Student's $t = 3.68$	$P < 0.001$
G6PD-heteroz+thal trait	vs.	Thal trait F	Student's $t = 12.84$	$P < 0.001$
G6PD-heteroz+Fe^-	vs.	G6PD-heteroz+thal trait	Student's $t = 0.54$	$P < 0.6$
Normal F	vs.	G6PD-heteroz+Fe^-	Student's $t = 4.28$	$P < 0.001$
G6PD-heteroz	vs.	G6PD-heteroz+Fe^-	Student's $t = 6.05$	$P < 0.001$
G6PD-heteroz+Fe^-	vs.	Fe^- W	Student's $t = 11.33$	$P < 0.001$
Normal M	vs.	Thal trait M	Student's $t = 22.88$	$P < 0.001$
Normal M	vs.	G6PD-hemiz	Student's $t = 89.40$	$P < 0.001$
Thal trait M	vs.	G6PD-hemiz	Student's $t = 53.80$	$P < 0.001$
Normal M	vs.	G6PD-hemiz+thal trait	Student's $t = 29.10$	$P < 0.001$
Thal trait M	vs.	G6PD-hemiz+thal trait	Student's $t = 37.30$	$P < 0.001$
G6PD-hemiz non-Med	vs.	G6PD-hemiz non-Med+thal trait	Student's $t = 1.92$	$P = 0.05$
G6PD-hemiz Med	vs.	G6PD-hemiz Med+thal trait	Student's $t = 3.91$	$P < 0.001$

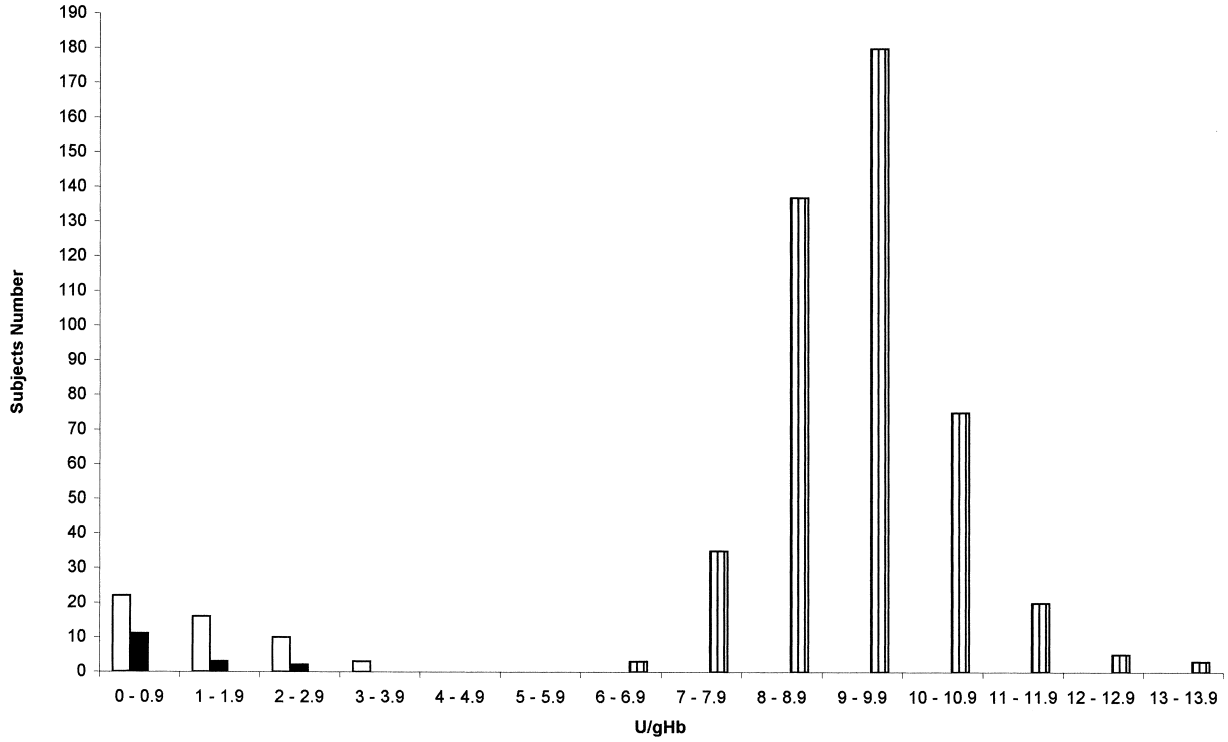

Fig. 2. Distribution in classes of the values of G6PD activity in: ▥ normal males; □ hemizygous males; ■ hemizygous male carriers of the thalassemia trait.

that of Calabria, which presents relatively high frequencies of both the thalassemia trait and G6PD deficiency, the comparison of the G6PD activity of heterozygous subjects also affected with the thalassemia trait is more reliable if performed with respect to the enzymatic activity of the carriers only for the latter inherited anomaly rather than with the G6PD activity of normal subjects (Table 3). In particular, in carriers of thalassemia, one must bear in mind the opportunity to distinguish those with the α1 phenotype from those with the α2 (or 'mild' thalassemia) phenotype. The latter subjects have hemometric values within the normal range; thus they do not show the thalassemia because they show normal values of G6PD activity.

Finally, the use of a rapid, precise, reproducible and accurate technique like differential pH-metry [12–14] has not only allowed us to study a large number of subjects, but has proved to be an appropriate means of investigation in mass screening for the identification of G6PD deficiency [2,3].

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