

Glutathione reductase deficiency in Saudi Arabia

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SUMMARY Glutathione reductase (GR) is a ubiquitous enzyme required for the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) concomitantly oxidizing reduced nicotinamide adenine dinucleotide phosphate (NADPH) in a reaction essential for the stability and integrity of red cells. Mutations in the GR gene and nutritional deficiency of riboflavin, a co-factor required for the normal functioning of GR, can cause GR deficiency. We conducted a study on 1691 Saudi individuals to determine the overall frequency of GR deficiency and to identify whether the deficiency results from genetic or acquired causes or both. The activity of GR was measured in freshly prepared red cell haemolysate in the presence and absence of flavin adenine dinucleotide (FAD) and the activity coefficient (AC) was determined. Samples with low GR activity (> 2.0 IU/g haemoglobin) both in the presence and absence of FAD and an AC between 0.9 and 1.2 were considered GR-deficient. Samples with AC \geq 1.3 were considered riboflavin-deficient. The overall frequency of partial GR deficiency was 24.5% and 20.3% in males and females respectively. In addition, 17.8% of males and 22.4% of females suffered from GR deficiency due to riboflavin deficiency. This could be easily corrected by dietary supplementation with riboflavin. No cases of severe GR deficiency were identified.

Introduction

Glutathione reductase (GR) is an ubiquitous enzyme associated with the hexose monophosphate shunt of glucose metabolism [1]. It catalyses the reduction of oxidized glutathione (GSSG) with the concomitant conversion of NADPH to NADP⁺.

Reduced glutathione (GSH) is involved in a number of reactions where it reduces several cellular components. In red cells, this reaction is of special significance because it is required for the stability and integrity of the red cells, and

low levels of GSH are associated with drug-induced haemolytic anaemia [2]. Flavin adenine dinucleotide (FAD) is an essential co-factor for maximal GR activity.

GR deficiency has been reported in several populations and may be due to either nutritional deficiency of riboflavin, which leads to an acquired deficiency of GR [3,4] or due to a mutation in the GR gene which results in GR variants with low activity [5,6]. We aimed to determine the frequency of GR deficiency in the Saudi population and to identify the cause of the deficiency.

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Subjects and methods

The study was carried out from 1984 to 1990. The study group included 1691 Saudi adults, randomly selected (1010 males and 681 females). The study group comprised individuals living in Gizan, Riyadh, Al-Hafouf and Khaiber.

Blood samples were drawn by venepuncture from each individual. The red cells were separated from the plasma by centrifugation and washed twice with cold physiological saline. Fresh haemolysate was prepared by addition of cold physiological saline and it was used for the measurement of GR activity according to a procedure published earlier [7]. The activity of GR was determined in the presence and absence of FAD using a spectrophotometric method, which measured the decrease in absorbance for 3 minutes at 340 nm in a test system at 30 °C containing 2 mL of 0.1M Tris-HCl buffer (pH 8.0), 0.5M EDTA, 2mM NADPH (0.1 mL), 1 mM oxidized glutathione (0.1 mL), and 1.0 mL of haemolysate. Using the GR activity in the presence and absence of FAD, the activity coefficient (AC) was calculated as follows:

$$AC = \frac{\text{Decrease in absorbance with FAD}}{\text{Decrease in absorbance without FAD}}$$

Chi-squared analysis was conducted using 2×2 contingency tables to determine the statistical significance of the difference in results obtained in males and females.

Results

The activity of GR was calculated using the decrease in absorbance at 340 nm. The mean activity and range of GR was $3.7 \pm$

Table 1 Total GR deficiency in Saudi Arabia

Sex	No. investigated	GR deficiency		χ^2 (P-value)*
		No.	%	
Males	1010	312	30.9	1.007
Females	681	227	33.3	(0.315)
Total	1691	539	31.9	-

*To determine the significance of the difference in results of males and females
GR = glutathione reductase

1.3 IU/mg haemoglobin (Hb) in the absence of FAD and 3.9 ± 1.2 IU/mg Hb in the presence of FAD. The samples were classified as GR-normal or -deficient. They were considered GR-deficient if the activity was less than 2.0 IU/mg Hb both in the presence and absence of FAD and the AC was between 0.9 and 1.2 [8]. On the other hand, if the activity was less than 2.0 IU/mg Hb and the AC was ≥ 1.3 , they were considered riboflavin-deficient [9].

The frequency of total GR deficiency was calculated (Table 1) and only partial GR deficiency was recognized. The difference in the frequency of GR deficiency in males and females was not statistically significant.

If the GR deficiency was corrected in the presence of FAD, then it was classified as a nutritional deficiency. However, if GR activity still remained lower than normal in the presence of FAD, then it was classified as a genetic deficiency. The frequency of GR deficiency due to both genetic and nutritional causes in the Saudi males and females is shown Table 2. None of the males and females had complete GR deficiency. Only one female had GR activity less than 20% of normal and presented with mild anaemia. A high percentage of GR deficien-

Table 2 Frequency of GR deficiency as a result of genetic and acquired causes in Saudi Arabia

Sex	Total number	GR deficiency due to genetic cause ^a			GR deficiency due to riboflavin deficiency ^b		
		No.	%	χ^2 (P-value)	No.	%	χ^2 (P-value)
Males	1010	132	13.1	1.415	180	17.8	4.314
Females	681	75	11.0	(0.234)	150	22.0	(0.038)
Total	1691	207	12.2		330	19.5	

^aReduced GR activity and AC ranging between 0.9 and 1.2

^bReduced GR activity and AC \geq 1.3

GR = glutathione reductase AC = activity coefficient

Table 3 Frequency of partial GR deficiency due to genetic variants in different regions of Saudi Arabia

Region	Males			Females			χ^2 (P value)
	No. investigated	No. deficient	%	No. investigated	No. deficient	%	
Riyadh	270	20	7.3	211	11	5.2	1.326 (0.908)
Gizan	269	66	24.5	192	39	20.3	0.908 (0.340)
Hafouf	191	29	15.2	116	17	14.7	1.538 (0.968)
Khaiber	176	17	9.7	162	8	4.9	2.090 (0.147)
Total	1010	132	13.1	681	75	11.0	-

GR = glutathione reductase

cy in Saudis resulted from a nutritional deficiency of riboflavin. The difference in the results of genetic GR deficiency in males and females was not statistically significant. However, nutritional deficiency was significantly greater in females compared with males ($P < 0.05$).

The samples from the different regions were separated and the frequency of GR

deficiency due to genetic causes was calculated. The results are presented in Table 3. The highest frequency of partial deficiency of GR was in Gizan in both the males and females and the lowest was in the males in Riyadh and in the females in Khaiber. However, in all the regions the differences in the results of males and females were not statistically significant.

Discussion

The estimation of GR in the presence and absence of FAD and the calculation of AC has often been used to differentiate between nutritional and genetic GR deficiency. Individuals with an $AC \geq 1.3$ are considered riboflavin-deficient, while those with low activity and an AC value between 0.9 and 1.2 are considered GR-deficient because of genetic causes [9–12].

In our study, we found only partial GR deficiency as a frequently occurring problem in all areas of Saudi Arabia and the results in the males and females were not statistically different. This is expected as GR deficiency is an autosomal disorder and should occur at the same frequency in both sexes. Partial GR deficiency has been reported in the following populations: South Vietnamese, Iranian, Ethiopian [10], Sudanese [4] and Spanish [3]. The frequencies ranged from 0.3% to 22%. Thus GR deficiency is a common disorder in several populations [13–15], although reduced GR activity due to riboflavin deficiency is reported to be more common compared with deficiency due to genetically determined variants [15].

Riboflavin plays a significant role in the normal functioning of GR and deficiency of riboflavin in the diet is a common cause of reduced GR activity [5,6]. The AC value, which represents the degree of saturation of the apoenzyme with riboflavin, differentiates between cases of riboflavin deficiency and genetically determined GR deficiency and an AC range of 0.9–1.2 is considered normal [16].

As shown by the results of this study, riboflavin deficiency is a common cause of GR deficiency in the Saudi population. There may be several causes of this, the most common being nutritional habits and gastrointestinal problems. However, since

these problems were not assessed in our investigations, we were unable to confirm the actual cause of riboflavin deficiency in Saudis. The former leads to nutritional deficiency of riboflavin because of insufficient intake; the latter causes nutritional riboflavin deficiency because of decreased absorption in the gastrointestinal tract [17]. We believe that both these causes may play a role in affecting GR activity in Saudis and hence lead to a state of GR deficiency. Furthermore, riboflavin deficiency was significantly more prevalent in the females than males.

Some genetic variants of GR (phenotypes produced due to mutations in the GR gene) with reduced activity were also found in Saudis, although no case of complete deficiency was encountered. This would suggest that the GR variant in Saudis has only slightly reduced activity and hence may not be an important cause of haemolytic anaemia in this population. Several sporadic cases have been reported of individuals with partial GR deficiency suffering from haemolytic anaemias resulting from ingestion of sulfoxone or 8-amino-quinolines [2,18].

An interesting finding was the significant variation in the frequency of GR deficiency due to genetic causes in the different regions of Saudi Arabia. The greatest prevalence of GR deficiency was found in Gizan in both males and females, followed by Al-Hafouf in Eastern Province. Lower values were encountered in Riyadh and Khaiber. These differences may be due to the presence of genetic variations between these populations.

More detailed studies are required to investigate the clinical consequences of partial GR deficiency in Saudis and to determine the molecular basis of the defect in the Saudi population.

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