CHAPTER 9

ENZYMOPATHIES

9.0 ENZYMOPATHIES

Enzymopathies, particularly those affecting the red cells occur at a high frequency in some populations. The most frequent of the red cell abnormalities is glucose-6-phosphate dehydrogenase deficiency. Other red cell enzyme deficiencies have been reported but at a considerably lower frequency.

9.1 Glucose-6-phosphate dehydrogenase deficiency

A case of primaquine induced haemolytic anaemia was first described as early as 1926 (Cordes, 1926). Later several additional cases were reported. Routine haematological examination of patients with primaquine-induced haemolysis failed to shed any light on the causative factors. However, radioactive labelling techniques revealed that the primaquine sensitivity was due to an intrinsic defect of the red blood cells. The familial nature of the defect was noted early and racial differences in susceptibility to pamaquine-induced haemolysis were recognized (Reviewed by El-Hazmi & Warsy, 1986).

Studies on red cells incubated with a variety of metabolic inhibitors turned the attention to the sulphydryl compounds of the red cells and it was shown that the red cells deficient in G-6-PD had a significantly lower content of reduced glutathione. In the beginning attention was directed towards glutathione reductase which is required for the conversion of the oxidized glutathione to reduced glutathione, using nicotinamide adenine dinucleotide phosphate (NADPH) as a hydrogen donar. Later it became apparent that the primaquine sensitivity was actually a result of G-6-PD deficiency which catalyses the reduction of $NADP^+$ to NADPH in the first step of the pentose phosphate pathway. Studies then concentrated on the estimation of G-6-PD level in the red cells and soon

G-6-PD deficiency was found to be common in the Mediterranean population and in black Americans and was associated with symptoms of variable severity. Further studies revealed molecular heterogeneity in the G-6-PD deficient phenotypes and this was shown to be due to the presence of several different G-6-PD variants, which differed not only in their physical characteristics but also in the clinical manifestations associated with the variants. Family studies revealed further genetic heterogeneity from one geographical area to another, and even within individual populations. Glucose-6-phosphate dehydrogenase deficiency was reported in the American Negroes, Greeks, Indians, Egyptians, Italians, Malays, Thais, Filipinos and Indonesian. In the Arabian peninsula and in several other African countries the presence of G-6-PD deficiency was identified and Beutler reported the frequency of G-6-PD deficiency in male population from several different countries (Beutler, 1978; 1990).

It was shown that the variants may differ in their electrophoretic mobility stability, pH optimum, specificity for glucose-6-phosphate and $NADP^+$ and utilization of substrate analogues. Though a number of variants had normal enzyme activity, but others had slightly or severely reduced activity, while still others had activity higher than the normals. On the basis of the residual activity, stability, clinical characteristics and susceptibility to haemolysis under different types of oxidative stresses, the G-6-PD variants were grouped into five main classes by Beutler and later this classification was adopted by the World Health Organization (WHO, 1985). The most common and normal form of the enzyme in all populations was found to be G-6-PD-B^+, and the most common variant was found to be G-6-PD-A^+, which is confined largely to the subjects of African ancesty and occurs in almost 18% of the male population. Female may have either A^+ or

B^+ or both A^+ and B^+. The common variants with significantly reduced activity are G-6-PD-Mediterranean and G-6-PD A^-. The former, though more prevalent in the Mediterranean countries, is also found in other parts of the world, while the later is frequent in the African populations, but occurs in other populations at a very low frequency.

The G-6-PD Mediterranean has the same electrophoretic morbidity as G-6-PD-B^+ (the normal enzyme), has less than 10% of the normal activity and has altered Km for glucose-6-phosphate and $NADP^+$. It has significantly reduced stability to heat and increased susceptibility to haemolytic anaemia particularly under certain oxidative stresses. It also predisposes to haemolytic anaemia upon ingestion of *Vicia fava* and produces "favism". The G-6-PD-A^- has electrophoretic morbidity faster than G-6-PD-B^+, the same as G-6-PD-A^+, but has lower activity in the range of 20%. Other than these there are a large number of 'private' variants which exist only in specific populations or in specific families (Beutler, 1978).

The G-6-PD gene (Gd) is inherited as a sex linked disorder and is transmitted as an X-linked recessive gene, where the Gd locus is linked tightly to the loci for color blindness and haemophilia. A wide variation is encountered in the G-6-PD activity in females and has led to the proposal that one X-chromosome is inactivated early in embryogenesis in the females, thus resulting in a mosaic of X-chromosome activity in the human females. Thus some cells transcribe genes on maternally derived and others on paternally derived chromosome. This is confirmed by the demonstration that females heterozygous for G-6-PD deficiency have two red cell populations, a deficient and a normal. This is referred to as the "Lyons phenomenon". If the normal X-chromosome is inactivated the heterozygous female may appear G-6-PD deficient and the reverse is true

if the deficient X-chromosome is inactivated. The G-6-PD locus has been extensively used as a marker to obtain the basic mechanism underlying the X-chromosome inactivation.

9.1.1. Pathophysiology of G-6-PD deficiency

The G-6-PD variants show a wide range of activity and hence a wide range of clinical and haematological presentations. Some G-6-PD deficients, show no sign of clinical or haematological abnormalities and may live all there lives without being aware of the defect. They are not anaemic under normal conditions, however, under special conditions, increased haemolysis of the G-6-PD deficient red cells may result. Generally, the young erythrocytes have a normal G-6-PD content, but as the cell ages the G-6-PD activity decline, and the NADPH/$NADP^+$ ratio is significantly reduced.

A number of abnormalities are associated with G-6-PD deficiency. These are listed in Table 9.1. There are several non-haemolytic abnormalities which have been reported in a few studies, though verification of these findings is awaited. These include higher incidence of cataract, reduced mental capacity, coronary artery diseases and decreased overall fitness. A statistically significant increase in the incidence of G-6-PD deficiency was reported in athletes compared to non-athletes (Reviewed by El-Hazmi and Warsy, 1986).

Among the haematological abnormalities, non-spherocytic haemolytic anaemia is encountered in some forms of G-6-PD deficiency (i.e. Class I variants) where the red cells are deprived of most functional G-6-PD activity. With other variants, the haemolysis may be elicited with certain factors which produce oxidative stress. ncluded amongst this groups are infections with certain organisms (Table 9.2), drugs (Table 9.3) and inhalation of pollen grains of *V. faba* or ingestions of these beans. This

Table 9.1: Abnormalities associated with G-6-PD deficiency

Haemolytic Abnormalities

- Non-spherocytic haemolytic anaemia.
- Haemolytic anaemia under oxidative stress caused by:
  - Drugs
  - Infections
  - Diabetic acidosis
  - Fava beans
- Favism

Non-Haemolytic Abnormalities

- Reduced fitness.
- Increased incidence of infection.
- Coronary artery disease.
- Sub-normal mental capacity.
- Cataracts.
- Neonatal jaundice with kernicterus.

Table 9.2: Some organisms implicated as haemolytic agents in G-6-PD deficient individuals

- Escherichia coli
- Pneumococcus
- Proteus
- Salmonella
- Streptococcus
- Staphylococcus
- Rickettsia
- Hepatitis B virus

Table 9.3: Some drugs implicated as haemolytic agents
in G-6-PD deficient individuals

- Primaquine
- Pamaquine
- Sulfanilamide
- Sulfacetamide
- Sulfa methoxazole
- Acetanilide

last type is referred to as "favism" and is one of the most serious clinical consequences of G-6-PD deficiency. It occurs commonly in the Greeks and Italians and has been reported in some parts of the Middle East especially Egypt, Iraq and Lebanon. The symptoms of favism appear 5 to 24 hours after ingestion of fava beans and are listed in Table 9.4. A schematic presentation of the haemolytic anaemia produced in G-6-PD deficient individuals is presented in Figure 9.1.

A number of therapies have been attempted experimentally for G-6-PD deficiency. These include administration of xylitol, isocitrate, ethylenediamine tetra acetic acid, corticosteroid and phenobarbital. However, none have proved to be satisfactory. Transfusion may be required for severe haemolytic episodes and to prevent renal damage. Splenectomy has been occasionally beneficial. Vitamin E administration over a prolonged period may protect the red cells against the haemolytic episodes in G-6-PD deficient red cells (Reviewed by Buetler, 1978; 1990; Weatherall et al, 1982).

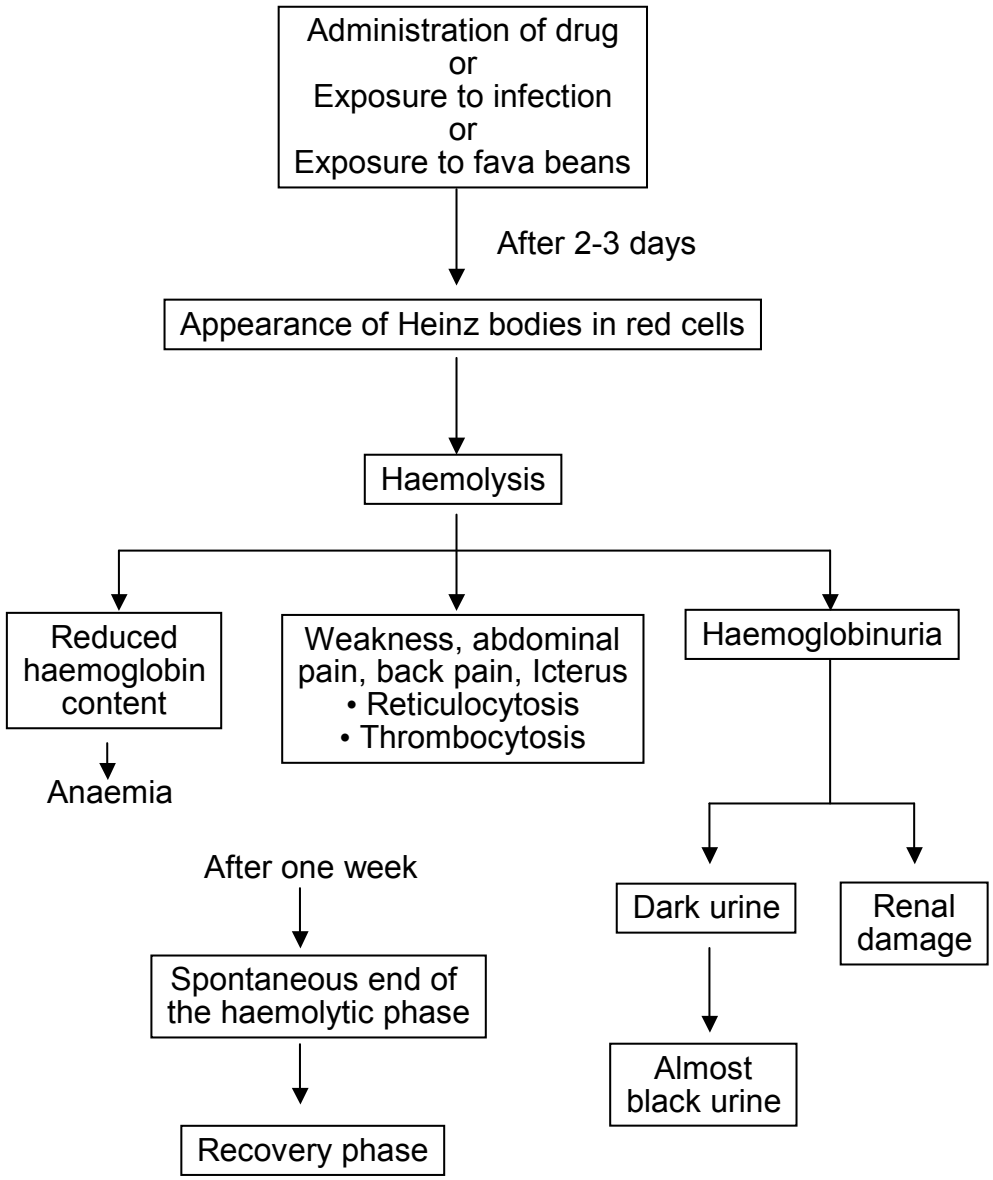
9.2 Frequency of glucose-6-phosphate dehydrogenase deficiency in Saudi Arabia

Glucose-6-phosphate dehydrogenase deficiency was first reported in the Saudi population in the eastern province by Gelpi (1965), who also showed that the frequency of G-6-PD deficiency varied significantly in the different villages in the Eastern Province. In the oasis populations, the G-6-PD deficiency was significantly higher compared to the frequency in the non-oasis population. Later, G-6-PD deficiency was reported in the western province of the country, though the frequency was found to be considerably lower compared to the frequency in the eastern population (Bayoumi et al, 1979). Screening studies were initiated in several regions of the country and the G-6-PD deficiency gene

Table 9.4: Signs and Symptoms associated with favism

- Severe haemolytic anaemia
- Pallor
- Dizziness
- Nausea
- Vomiting
- Malaise
- Chills
- Lumbar Pain
- Fever
- Jaundice
- Haemoglobinuria

Figure 9.1: Haemolytic anaemia in G-6-PD deficient red cells



was found to be widely distributed in the different provinces of the country, though at a variable frequency (El-Hazmi, 1982). These investigations also showed that the G-6-PD deficiency gene occurs at a high frequency in the areas where Hb S and the thalassaemia genes are also present at high frequencies.

We initiated our screening studies in 1982 and screened several areas of Saudi Arabia in the Eastern, Western, Northern, and Central Provinces of the country. G-6-PD deficiency was identified in all regions of the country, though in the central province the frequency was significantly lower compared to the other provinces. The frequency of G-6-PD deficiency in the Saudi males and females in different regions are presented in Table 9.5. Individuals suffering from severe G-6-PD deficiency with activity less than 10% of the normal enzyme and individuals with only a partial deficiency of the G-6-PD activity, with activity ranging between 20-60% of the normal enzyme were identified in most regions. In each area, the actual number of G-6-PD deficient females was significantly higher than expected number calculated applying the Hardy-Weinburg Equilibrium (Table 9.6). There could be due to several factors contributing to disturb the Hardy Weinburg equilibrium in this population. These include:

(a) High rate of consanguinity in the Saudis. In a study recently reported the rate of consanguineous marriages was over 50% in the Saudi population. In addition, customs prevail whereby marriages generally occur between members of the same tribes.

and (b) High frequency of inactivation of the normal X-chromosome in the female heterozygotes, thus presenting a higher percentage of females as deficient.

Table 9.5: Frequency of G-6-PD deficiency in different regions of Saudi Arabia

Province	Region	Male		Female	
		No.	Frequency of G-6-PD Def.	No.	Frequency of G-6-PD Def.
Eastern	Al-Qateef	515	Severe 0.398 Partial 0.0272	445	Severe 0.214 Partial 0.0697
	Al-Hafouf	595	0.2325	900	0.125
	Hafr Al-Batin	355	0.84	459	0.43
Central	Riyadh	786* 1916*	0.071	678* 2078*	0.025 0.015
	Qaseem	426	Severe 0.0305 Partial 0.0094	589	Severe 0.0085 Partial 0.0102
	Buraida	547	0.0109	685	0.010
	Al-Russ	283	0.0035	370	0
	Al-Unaiza	117	0	238	0
	Al-Mesnab	126	0	161	0
	Bakeria	64	0.0156	95	0.0105
Western	Al-Ula	232	0.080	197	0.032
	Khaiber	457	0.220	206	0.160
	Yanbu	724	0.0179	315	0.0064
	Makkah	382	0.0576	307	0.0423
	Qunfuda	432	0.1275	200	0.1015
	Bisha	469	0.0767	351	0.054
	Najran	667	0.057	776	0.006
	Jaizan	753	0.204	432	0.048
	Sabya	289	0.107	353	0.045
	Samta	154	0.091	195	0.066
	AbuAreesh	169	0.106	211	0.0332
	Farasan	74	0.027	102	0.029
	Baish	115	0.026	163	0.0306
	Fifa	82	0.122	90	0.111
	Al-Baha	519	0.1275	328	0.1158
	Mahayel	361	0.1579	284	0.0352
	Abha	626	0.1597	482	0.0685
Northern	Hail	700	0.0171	946	0.0073
	Tabuk	412	0.0146	476	0.0126
	Arar	311	0.0064	359	0
	Al-Jouf	138	0.0217	142	0.0140

Table 9.6: Observed and expected number of G-6-PD deficient females in different regions of Saudi Arabia

Region	Area	No. investigated	Observed No.	Expected No. *
Eastern	Al-Qateef	445	95	70.49
	Al-Hafouf	900	112	48.7
Central	Riyadh	678	17	3.4
	Qaseem	589	5	0.548
Western	Al-Ula	197	6.1	1.26
	Khaiber	206	33	9.97
	Yanbu	315	2	0.1
	Makkah	307	13	1.0
	Qunfuda	200	20	3.25
	Bisha	351	14	2.06
	Najran	776	5	2.5
	Jaizan	432	21	17.98
	Al-Baha	328	38	5.18

* Calculated using Hardy-Weinburg equilibrium

9.3 G-6-PD phenotypes and their frequency in Saudi Arabia

The phenotyping of G-6-PD variants was carried out using electrophoretic techniques and specific staining from the G-6-PD activity on the gel. Figure 9.2 presents the electrophoretic separation of the various G-6-PD phenotypes commonly identified in the Saudi population. The normal enzyme in all areas of Saudi Arabia was G-6-PD-B^+, as in other parts of the world. G-6-PD-A^+ was the most common variant with normal G-6-PD activity. The most frequent G-6-PD deficient variant which produced a severe deficiency was G-6-PD Mediterranean which has the same electrophoretic mobility as G-6-PD-B^+ but has activity less than 10% of the normal enzyme. In most cases no G-6-PD band could be demonstrated on electrophoresis, while in others, a very faint band, was observed (Figure 9.1). Further confirmation of the G-6-PD variant was achieved by spectrophotometric estimation of the G-6-PD activity. The activity range of each of the G-6-PD variant obtained spectrophotometrically, is presented in Table 9.7.

G-6-PD Mediterranean was recognized in all areas of Saudi Arabia investigated, though at a variable frequency in the different regions. Partially deficient variant with same mobility as G-6-PD-B^+ but activity ranging between 20-60% was the next most frequent variant. Finally G-6-PD-A^-, the African variant was the third most frequent G-6-PD deficient variant. However, it was not present in every area, and occurred in other areas at a low frequency. The frequency of the various G-6-PD variants in the male and female population in the different regions of Saudi Arabia are presented in Tables 9.8 and 9.9 respectively. A brief discussion of the results from each province are presented below.

9.3.1 The Eastern Province

In Saudi Arabia, during the mid 1960s, the very first case of G-6-PD deficiency

Figure 9.2: Electrophoretic separation of G-6-PD variants on electrophoresis

1 2 3 4 5 6 7 8

Samples 2,4,6,8: Normal G-6-PD-$^{+}$

Sample 1 and 3: Partial deficient (activity 20-60% normal): G-6-PD-Med-like

Sample 7: G-6-PD deficient: G-6-PD-Med.

Sample 5: Heterozygous Hb A^{+}/G-6-PD-Med – female

(Hb A^{+} travels at the same position as G-6-PD-A^{+} but has low activity)

Table 9.7: Activity range (mU/10^9 erythrocytes) of the various Glucose-6-phosphate dehydrogenase variants identified in Saudi Arabia *

G-6-PD Phenotype	No. investigated	Mean±SD (mU/10^9 erythrocytes)
G-6-PD-B^+	1950	95.0 ± 35.0
G-6-PD-A^+	78	81.3 ± 15.0
G-6-PD-A^-	55	22.6 ± 10.6
G-6-PD-Med.	250	10.5 ± 4.6
G-6-PD-Med-like	190	37.5 ± 8.1

* Activity of G-6-PD determined spectrophotometrically using kits from Boehringer Mannheim

Table 9.8: Frequency of G-6-PD phenotypes in the male population in different regions of Saudi Arabia

Region/ Area	G-6-PD phenotypes					
	No. investi-gated	G-6-PD-B^+	G-6-PD-A^+	G-6-PD-A^-	G-6-PD-Med	G-6-PD-Med-like
Eastern						
Al-Qateef	515	0.5611	0.0136	0.0058	0.3922	0.0272
Al-Hafouf	234	0.679	0.021	0.021	0.176	0.130
Central						
Riyadh	651	0.838	0.043	0.010	0.046	0.064
Qaseem	426	0.944	0.0164	0.0023	0.0282	0.0094
Western						
Al-Ula	225	0.853	0.049	0.058	0.027	0.013
Khaiber	182	0.785	0.011	0.011	0.062	0.131
Yanbu	724	0.953	0.007	0.00	0.0179	0.0221
Makkah	382	0.717	0.029	0.003	0.0550	0.1963
Qunfuda	432	0.836	0.023	0.005	0.106	0.030
Bisha	469	0.844	0.004	0.00	0.0767	0.0746
Najran	262	0.702	0.046	0.015	0.046	0.191
Jaizan	109	0.602	0.037	0.028	0.176	0.130
Al-Baha	519	0.777	0.013	0.002	0.1255	0.817

Table 9.9: Frequency of G-6-PD phenotypes in the female population in different regions of Saudi Arabia

Region/ Area	G-6-PD phenotypes					
	No. investi-gated	G-6-PD-B^+	G-6-PD-A^+	G-6-PD-A^-	G-6-PD-Med	G-6-PD-Med-like
Eastern						
Al-Qateef	445	0.6854	0.0135	0.0112	0.2022	0.0697
Al-Hafouf	353	0.717	0.039	0.020	0.127	0.0.59
Central						
Riyadh	483	0.803	0.045	0.002	0.006	0.064
Qaseem	589	0.966	0.0068	-	0.0085	0.0102
Western						
Al-Ula	177	0.915	0.0	0.0056	0.011	0.011
Khaiber	162	0.841	0.045	0.006	0.023	0.107
Yanbu	315	0.971	0.010	0.00	0.0064	0.013
Makkah	307	0.645	0.0039	0.013	0.0293	0.261
Qunfuda	200	0.765	0.020	0.0065	0.095	0.095
Bisha	351	0.855	0.006	0.00	0.054	0.086
Najran	147	0.830	0.020	0.012	0.020	0.102
Jaizan	148	0.797	0.007	0.007	0.041	0.121
Al-Baha	328	0.722	0.003	0.003	0.113	0.131

was reported in the Saudi population in the eastern province. In this province, the population lives in nine urban centres and almost 60 villages, some of which are the oases which are scattered all over the province. The highest prevalence of G-6-PD deficiency was reported in the oasis population compared to the non-oasis population. Within this region, there were differences in the gene frequency with the highest frequency in the Al-Qateef population. The G-6-PD-A^+ and G-6-PD-A^- were identified though at a low frequency and G-6-PD-Mediterranean was the major cause of G-6-PD deficiency. A variant with mobility the same as G-6-PD-β^+ (the normal enzyme) and activity between 20-60% of the normal was identified at a high frequency in each of the area in the eastern province.

From ancient times, the eastern province has been highly endemic to malaria. The main vector responsible for transmission of malaria in this region is *Anopheles Stephens* and the main malarial parasites are *Plasmodium falciparum and Plasmodium vivax* (Malaria Control Program, Ministry of Health, 1985). The former is the cause of the largest proportion of human life threatening malaria in all areas of the world. Thus since G-6-PD deficiency is believed to provide protection against malaria, the geographic limits correspond precisely to the areas known to be hyperendemic for malaria. Within the provinces the difference in the frequency of G-6-PD deficiency could be related to the extent of malaria endemicity and other factors such as consanguinity and survival of the fittest (i.e. G-6-PD deficient).

9.3.2 The Northern Province

The northern province of Saudi Arabia has several oasis which have been endemic to malaria in the past. The main vector in this region in *Anopheles superpictus*, a vector of

Mediterranean origin. Another vector *Anopheles sergenti,* occurs frequently in the dry areas (Malaria Control Programme, 1985). In the past not many studies were reported from this region. However, one study conducted during the early 1960 reported a low frequency of G-6-PD deficiency gene. We extended our investigations and determined the frequency of G-6-PD deficiency in different areas of the northern province. The results of G-6-PD deficiency are presented in Table 9.5.

9.3.3. The Western Province

The western province, with its important coastal towns and industrial cities constitutes a very significant area. Foreigner inflow is high in this area, and the two holy cities i.e. Makkah and Al-Medina are both located in this region. In general, the population is cosmopolitan and from ancient times ethnic groups from different regions of the country and different parts of the world have settled in this area. The province has been endemic for malaria since ancient times and has still got a few persisting foci of malaria (Malaria Control Programme, 1985). The nomads from different malaria endemic regions, particularly Tehamat Asir in the south-western province, constantly introduces fresh malarial infection. In earlier studies the frequency of G-6-PD deficiency was reported as variable in the different tribes of the western province, with a range from 1.7 - 8.5%. We conducted screening studies in this province and found a very variable frequency of G-6-PD deficiency and phenotypes in the different areas. The results are presented in Tables 9.5, 9.8 and 9.9. As in the other regions, G-6-PD Mediterranean was the major cause of G-6-PD deficiency, though G-6-PD A^- was identified in each area.

The south-western province has two geographically and epidemiologically distinct areas which are physically separated by the mountain range of Asir. The eastern side is the

Asir plateau and on the western side are the low foothills known as Tehamat-Asir. The Asir area has been endemic for malaria, bur earlier studies showed a low frequency of G-6-PD deficiency (El-Hazmi, 1983). The Tehamat Asir region has areas which differ in malaria endemicity, ranging from the non-malarious to the hyperendemic regions. The main vector is *Anopheles arabinesis*. In some regions such as Najran, both *P. falciparum and P. vivax* infections are known to occur, however in Tehamat Asir, *P. falciparum* infection predominates. In earlier studies a wide range of G-6-PD deficiency was reported in the Tehamat-Asir areas ranging from < 0.1 to 12%. Considerably higher frequency was identified in the Jaizan area compared to Najran. We screened several areas in the south-western province and determined the frequency of G-6-PD deficiency and G-6-PD phenotypes. The results in the different areas of the south-western province are presented in Tables 9.5, 9.8 and 9.9. Interestingly, there were several significant differences in the gene frequency of the G-6-PD deficiency gene and in the frequency of the different G-6-PD phenotypes. In each area the major cause of the deficiency was G-6-PD Mediterranean while G-6-PD A^+, and A^- were identified at a considerably lower frequency.

The north-western province has several oases which are traditionally agricultural areas with poor drainage system. Due to valles, streams, springs, ditches and pools of stagnant water, there are many ideal sites for the breeding of the mosquitos and hence several of these areas including Khaiber and surrounding areas have been endemic for malaria since ancient times and may still have some persisting foci of malaria, though the Ministry of Health has conducted extensive successful programs for the eradication of the malarial parasites. The main vector found in the area is *A. sergente* and almost 80% of the

malaria is caused by *P. falciparum* while the rest is caused by *P. vivax* (Malaria Control Programme, 1985). In an earlier study a wide range of G-6-PD deficiency ranging from 0.1 to 23% was reported, where in some villages the frequency was as high as 24% while in others, the G-6-PD deficiency was almost non-existent. In a study conducted by us on 663 unrelated Saudis attending the out-patient clinics, the frequency of G-6-PD deficiency among the male population was found to be 23%.

During this study several areas were screened for G-6-PD deficiency and G-6-PD phenotypes and the results in each area are presented in Tables 9.5, 9.8 and 9.9. As in the other provinces, the frequency of G-6-PD deficiency and variants showed marked differences in the different areas investigated. The major cause of G-6-PD deficiency was G-6-PD Mediterranean. G-6-PD-A^- was identified but at a considerably lower frequency.

9.3.4 The Central Province

The central province is largely non-malaria endemic region. The hot dry weather and lack of pools of stagnant water prevent the growth and multiplication of mosquitos in this area and the only malaria cases encountered are those who have visited other areas with in or outside Saudi Arabia and have carried the infection to the central province. A study was conducted on 1462 unrelated individuals attending outpatient clinics and the frequency of G-6-PD deficiency was found to range from >1% to 7.1% in the overall male population and 3.2% in the blood donors (El-Hazmi et al, 1986; El-Hazmi and Warsy, 1986). In other studies on the population of Riyadh and the surrounding villages the G-6-PD deficiency range was 0.1% - 7.1% (El-Hazmi, 1983). We conducted extensive investigations in Riyadh, Al-Qassim and Sulayil in the central province of Saudi Arabia and the gene frequency of G-6-PD deficiency and of G-6-PD phenotypes was determined

in each of the area. The results are presented in Tables 9.5, 9.8 and 9.9. Unexpectedly, G-6-PD deficiency, was identified in these areas though at a lower frequency than the malaria endemic regions. The existence of G-6-PD deficiency in these non-malaria endemic regions suggests that selective forces other than malaria may be involved in influencing the establishment of the G-6-PD deficient variants. This may largely be due to genetic drifts and gene flow from other malarious regions which have a high frequency of G-6-PD deficiency. This could explain the existence of G-6-PD deficiency in Riyadh, which is the capital of the country and there is a constant inflow of population from other regions seeking jobs, business opportunities and educational facilities.

In summary, these extensive screening studies have shown the presence of G-6-PD deficiency in all regions of Saudi Arabia. In most of the malaria endemic areas, a correlation between malaria and G-6-PD deficiency is apparent, while in a few regions there are exceptions, where the frequency of G-6-PD deficiency is not very high, despite the area being a malaria endemic region. These include the northern province, Asir and Najran, which have been endemic to malaria from ancient times, yet have a lower frequency of G-6-PD deficiency compared to other malaria endemic regions. It is possible that in these regions, G-6-PD deficient trait may not have appeared by mutation or by migration to such an extent as to allow the G-6-PD deficient variants to become established at a high frequency by selective effect of malaria. Furthermore, it may be suggested that some additional selective force(s) are playing a role in increasing the frequency of this enzymopathy in some malaria endemic regions but not in the others. Several studies have shown that some populations in regions that are endemic for falciparum malaria have a very low frequency of G-6-PD deficiency. Included in this

category are Zoroastrians and the Armenians of Iran, the Ethiopians, several groups in New Guinea and the Sedong of Vietnam.

An interesting finding within each province which is classified as malaria endemic region, is that villages differ considerably in the frequency of the enzymo-pathy. This may be due to different villages having (i) different degree of malaria endemicity, (ii) genetic drifts, (iii) consanguinity and other forms of intermarriages.

Another interesting aspect which needs to be discussed is the inapplicability of the Hardy-Weinburg equilibrium in almost all regions of Saudi Arabia. In general, in non-random mating states, the frequency of G-6-PD deficiency in females is the square of the frequency of G-6-PD deficiency in the males and the expected number of G-6-PD deficient females may be calculated applying this rule. In our studies, in each area of Saudi Arabia, the number of expected G-6-PD deficient females was a lot lower than the observed number. This phenomenon shows that the Hardy- Weinburg equilibrium is disturbed (Table 9.6). This may be due to any of the following factors as discussed earlier: (i) high rate of consanguinity, (ii) preferential inactivation of the normal X-chromosome in the heterozygous females; hence a severe expression of G-6-PD deficiency in the heterozygous females, (ii) some other mechanism.

9.4 The "Normal Reference Value" for G-6-PD in Saudis

We established the 'normal reference' value for G-6-PD in the Saudi males and females and also conducted similar investigations on other population groups living in Saudi Arabia. The activity of the enzyme was estimated spectrophotometrically and the milli units/10^9 erythrocytes were calculated for each sample. The data was fed on the computers at the Computer Centre, King Saud University, Riyadh and the mean, median,

mode and standard deviation and percentile ranges were obtained. The normal reference range calculated as mean ± 2SD and 2.5th to 97.5th percentiles are presented in Table 9.10, for male and female from Saudi Arabia and different countries. A range of 95± 35 $mU/10^9$ erythrocytes for Saudi males and 100± 40 $mU/10^9$ erythrocytes for Saudi females was considered as the normal reference range. The frquency distribution histogram and the normal probability plot for G-6-PD in Saudis is presented as Figure 9.3.

9.5 Pyruvate kinase deficiency in Saudi Arabia

Pyruvate kinase catalyses the conversion of phosphoenol pyruvate to pyruvate in the Embden Meyerhoff pathway of glucose metabolism and its deficiency is the second most common enzymopathy known to affect the red cell metabolism (Beutler, 1978; Blume et al, 1968). The enzyme exhibits both partial and complete enzyme deficiency depending on the nature of the mutation. Patients with severe deficiency suffer from a severe non-spherocytic haemolytic anaemia, however, patients with partial deficiency exhibit a range of haematological abnormalities ranging from completely normal to a mildly anaemic conditions. Only a few population studies to determine the frequency of pyruvate kinase have been reported in literature, though there are several case reports of severely deficient patients. In one study on Spanish population, the PK deficiency was reported as 0.0024.

We conducted studies on pyruvate kinase in Saudi population to determine the frequency of PK deficiency. Spectrophotometric estimation of PK activity was carried out and normal reference values was established for the male and female population. The normal reference value for PK are presented in Table 9.11. No case of severe or complete PK deficiency was identified. Any sample with activity less than 60% of the normal range

Table 9.10: Normal Reference Range for G-6-PD in Saudi Arabia in different population living in Saudi Arabia

Nationality	Sex	No. Investi-gated	G-6-PD (mU/10^9 erythrocytes)				Normal range (mU/10^9 erythrocyte)	
			Mean	Median	Mode	SD	Mean ± SD	Percentile 2.5th - 97.5th
Saudi	M F	650 120	94.9 99.9	94.6 100.4	80.0 100.4	17.7 20.0	60.0 - 130.0 60.0 - 140.0	60.0 - 126.0 60.0 - 142.0
British & Americans	M F	44 42	105.5 97.8	104.8 96.2	104.8 98.3	18.2 17.6	69.1 - 141.9 62.6 - 133.0	68.8 - 139.9 67.7 - 125.2
Egyptian	M F	21 19	100.1 110.5	96.7 113.0	98.0 112.1	18.4 18.2	63.3 - 136.9 74.1 - 146.9	77.4 - 127.9 67.4 - 141.0
Filipinoes	M F	20 19	100.9 101.8	100.4 98.5	102.4 98.5	18.3 18.5	64.3 - 137.5 64.8 - 138.8	62.7 - 128.2 74.4 - 131.7
Pakistani	M F	17 19	94.0 96.8	95.3 96.9	95.3 96.9	15.0 15.5	64.0 - 124.0 65.8 - 127.8	66.9 - 113.0 61.9 - 128.6
Sudanese	M F	20 19	96.0 103.7	98.8 102.9	96.7 102.9	16.0 17.0	64.2 - 128.2 69.7 - 137.7	66.7 - 133.1 74.3 - 125.8
Total	M F	772 238	98.6 101.7	98.4 101.3	96.2 101.5	17.3 17.8	64.0 - 133.2 66.2 - 137.3	-

Figure 9.3: Frequency distribution histograms of G-6-PD in Saudis

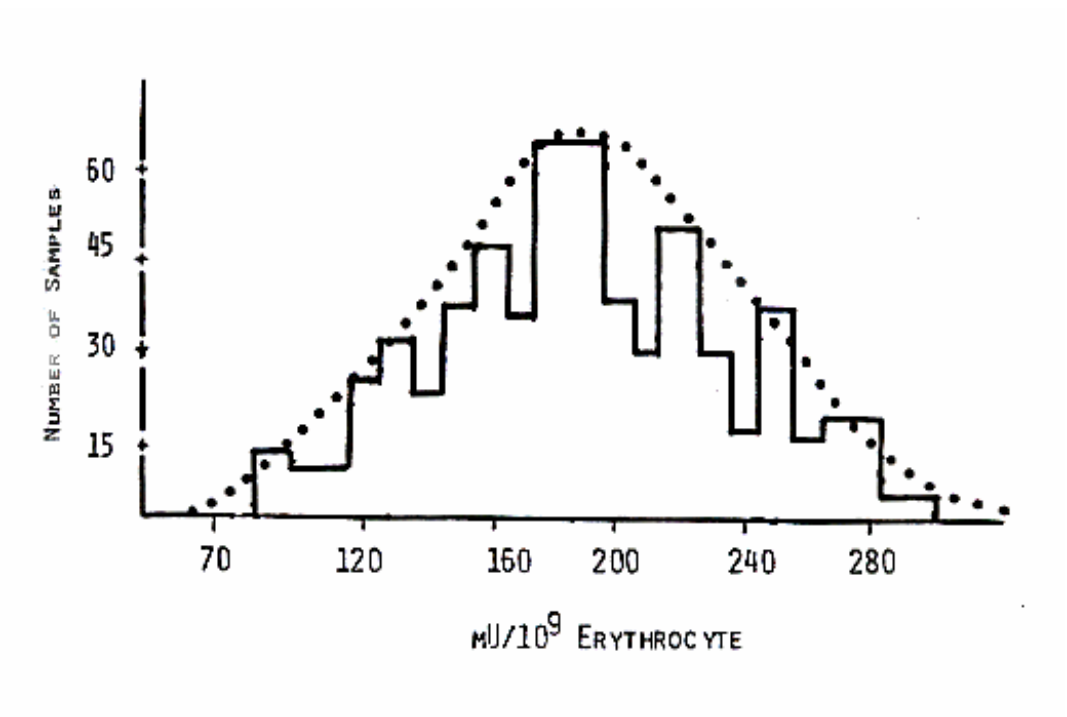


Table 9.11: Normal Reference Range for PK in Saudis

Nationality	Sex	No. Investi-gated	PK (mU10^9 erythrocytes)				Normal range (mU/10^9 erythrocyte)	
			Mean	Median	Mode	SD	Mean ± SD	Percentile 2.5th - 97.5th
Saudi	M	490	166.0	160.0	164.0	47.9	166.0± 95.0 (70-260)	68.0 - 259
	F	392	165.6	162.0	177.0	47.7	166.0± 95.0 (70-260)	77.0 - 258

was considered partially deficient. Only Partial deficiency of PK was encountered in the Saudis in different regions of Saudi Arabia and are presented in Table 9.12.

The frequency of PK deficiency obtained for Saudis are higher than those reported for other populations (Garcia et al, 1979; Blume et al, 1968). However, most of the other published reports are either isolated cases or clinical observations and population studies have not been conducted to determine the frequency of deficiency in most populations. It is possible that extensive population screening may reveal a higher frequency of these enzyme defects in other populations also.

9.6. Hexokinase deficiency in Saudi Arabia

Hexokinase (HK), an enzyme essential for the phosphorylation of hexoses in presence of ATP is an essential enzyme for glucose metabolism in the red cells. The deficiency of HK in relatively rare and a few cases with severe deficiency have been reported in literature. For the Spanish population frequencies of 0.005 was reported for HK deficiency (Gracia et al, 1979; Blume et al, 1968). Severe deficiency of HK produces non-spherocytic haemolytic anaemia, while partial deficiency may be generally assymptomatic.

We investigated the frequency of HK deficiency in Saudi population using a spectrophotometric method. Only partial HK deficiency was identified in three regions investigated. None of the samples analysed had complete or severe HK deficiency and partial deficiency was not associated with any haematological or clinical abnormality.The results are presented in Table 9.13.

9.7. Glutathione reductase deficiency in Saudi Arabia

Glutathione reductase (GR E.C. 1.6.4.2) plays an essential role in maintaining the

Table 9.12: Frequency of partial* PK deficiency Saudi population

Region	Sex	No. investigated	Frequency of Partial PK* deficiency
Al-Hafouf	Male	190	0.030
	Female	120	0.000
Khaiber	Male	457	0.066
	Female	206	0.050
Riyadh	Male	784	0.060
	Female	678	0.015
Jaizan	Male	119	0.008
	Female	147	0.000
Najran	Male	267	0.008
	Female	152	0.000

* PK activity less than 60% of the normal

Table 9.13: Frequency of partial hexokinase deficiency in different region
s of Saudi Arabia

Region	Sex	No. investigated	Frequency of Partial HK* deficiency
Riyadh	Male	784	0.020
	Female	678	0.010
Khaiber	Male	457	0.020
	Female	206	0.020
Al-Hafouf	Male	190	0.010
	Female	120	0.090

* HK activity less than 60% of the normal

stability of red cell membrane proteins and haemoglobin by catalysing the conversion of oxidized glutathione (GSSG) to reduced glutathione using either NADPH or NADH as a coenzyme. Dietary riboflavin level plays an important role in influencing the glutathione reductase activity and in several studies riboflavin deficiency has been shown to be associated with reduced GR activity (El-Hazmi and Warsy, 1985). In addition, genetic variants of glutathione reductase also occur which have a reduced activity. Furthermore, several unrelated clinical conditions have been described in which the glutathione reductase activity is abnormal. These include haemophilia B, leukaemia, the thalassaemias and homozygous Hb C-disease.

Blood samples from Saudi males and females were analysed to determine glutathione reductase deficiency in the population of Riyadh, Jaizan, Khaiber and Al-Hafouf in different province of Saudi Arabia. In addition, samples from Jaizan in south- western province of Saudi Arabia were also analysed to determine the frequency of acquired verses genetic deficiency of glutathione reductase. In all these samples glutathione reductase activity was estimated in presence and absence of flavin adenine dinucleotide (FAD) using the procedure described by Beutler (1975) and modified slightly by us (El-Hazmi & Warsy, 1985). The glutathione reductase activity coefficient (GR AC) was determined as follows:

$$\text{GRAC} = \frac{\text{GR units in presence of FAD}}{\text{GR units in absence of FAD}}$$

The normal range for GR AC was taken as 0.9-1.2. GRAC value >1.3 suggested riboflavin deficiency (such samples were not considered glutathione reductase deficient) and only samples with low glutathione reductase activity in presence and absence of FAD

were regarded as GR deficient. Only partial GR deficiency was encountered in the populations investigated.

The results obtained in Riyadh, Jaizan, Khaiber and Al-Hafouf are presented in Table 9.14. The haematological parameters in glutathione reductase deficient groups compared to the normal non-deficient population are presented in Table 9.15. Several cases were encountered in both groups that were α-thalassaemic. Samples of GR deficient and GR normal α-thalassaemic and non α-thalassaemic were separated and the results of haematological parameters are presented in Table 9.16.

In the south-western province cases with both genetic and acquired glutathione reductase deficiency were encountered. The frequency of the two types of glutathione reductase deficiency is presented in Table 9.17.

The results show that partial glutathione reductase deficiency due to either genetic or acquired causes, occurs at a variable prevalence in different regions of Saudi Arabia and influences the haematological parameters only slightly. However, in the presence of thalassaemia the patients suffer from microcytic hypochromic anaemia.

9.8 The level of erythrocyte glucose-6-phosphate dehydrogenase and pyruvate kinase in patients with acquired and genetic anaemias

Erythrocytes are non-nucleated cells that depend entirely on glycolysis to fulfill their nutritional requirements. In addition, about 10% of the glucose is metabolised by the pentose phosphate pathway which provides reduced NADPH and pentoses. The presence of sufficient level of NADPH is absolutely essential for the stability and integrity of the red cell.

During our investigations to determine the frequency of G-6-PD and PK

Table 9.14: Frequency of partial GR deficiency in different areas of Saudi Arabia

Area	Sex	No. investigated	Frequency of partial GR deficiency
Khaiber	M	176	0.096
	F	162	0.049
Al-Hafouf	M	191	0.150
	F	116	0.143
Riyadh	M	374	0.053
	F	211	0.052
Jaizan	M	269	0.245
	F	192	0.203

Table 9.15: Mean values of haematological parameters in non-thalassaemic partially GR deficient Saudi males and females in Riyadh

Sex	No. Investi-gated	Haematological parameters (Mean ± SD)							
		RBC ($x10^{12}$/l)	Hb (mmol/l)	PCV (l/l)	MCV (fl)	MCH (mmol/l)	MCHCH (mmol/l)	Hb A_2 (%)	Hb F (%)
GR deficient									
Male	17	5.01 ±0.54	9.74 ±0.49	0.38 ±0.03	84.00 ±2.50	1.93 ±0.21	25.50 ±1.88	3.00 ±0.23	0.55 ±0.25
Female	8	4.40 ±0.53	7.88 ±0.61	0.38 ±0.04	87.60 ±4.00	1.81 ±0.09	20.85 ±0.02	3.10 ±0.25	0.50 ±0.25
Normal GR									
Male	354	5.70 ±0.44	9.55 ±0.74	0.44 ±0.05	84.20 ±2.50	1.76 ±0.09	20.47 ±0.62	3.00 ±0.30	0.80 ±0.25
Female	200	4.90 ±0.44	8.44 ±0.68	0.40 ±0.03	84.20 ±2.50	1.76 ±0.09	20.54 ±0.62	3.00 ±0.25	0.55 ±0.22

Table 9.16: Value of haematological parameters in α-thalassaemic and non-α-thalassaemic GR deficient individuals

Sex	GR Deficiency	No. Investi-gated	Haematological Parameters (Mean ± SD)					
			RBC ($x10^{12}$/l)	Hb (mmol/l)	PCV (l/l)	MCV (fl)	MCH (mmol/l)	MCHCH (mmol/l)
Male	Thalassaemic	3	5.15 ±0.48	7.59 ±1.70	0.33 ±0.06	68.00 ±8.50	1.40 ±0.31	22.54 ±2.58
	Non-Thalassaemic	17	5.01 ±0.54	9.74 ±0.49	0.38 ±0.03	84.00 ±2.50	1.93 ±0.21	25.50 ±1.88
Female	Thalassaemic	3	4.54 ±0.40	6.86 ±0.25	0.33 ±0.012	74.30 ±5.00	1.51 ±0.12	20.47 ±0.62
	Non-Thalassaemic	8	4.40 ±0.53	7.88 ±0.61	0.38 ±0.04	87.60 ±4.00	1.81 ±0.09	20.85 ±0.62

Table 9.17: Frequency of partial GR deficiency due to genetic or acquired causes to the south-western province of Saudi Arabia

Sex	No. investi-gated	Frequency of GR deficiency			
		Due to variant		Due to Riboflavin def.	
		No.	Frequency (%)	No.	Frequency (%)
Male	269	66	24.8	48	17.8
Female	192	39	20.3	43	22.4

deficiency in the Saudi population, we came across a number of anaemic Saudi individuals who had levels of G-6-PD several times higher than the normal. In these individuals we correlated the PK and G-6-PD level to the values of haematological parameters. The results showed significant positive or negative correlation between G-6-PD level and the haematological parameters (Table 9.18; Figures 9.4 to 9.7) and the PK level and the haematological parameters in anaemic and non-anaemic individuals (Table Table 9.19 and Figures 9.8 to 9.11).

We further attempted to investigate the G-6-PD and PK levels in patients with different types of acquired and genetic individuals. Regression analysis was carried out and correlation coefficient were obtained between the enzyme level and the haematological parameters in each anaemic group separately.

The red cell enzyme levels in different anaemic states are presented in Table 9.20. The results of regression analysis and the correlation coefficient and the P values between PK and the haematological parameters are presented in Table 9.21, while those between G-6-PD and the haematological parameters are presented in Table 9.22.

Both enzymes showed statistically significant negative correlations with RBC, total Hb and PCV in patients with sickle cell anaemia, α- and β-thalassaemia, iron deficiency anaemia and in the total anaemic group, while a positive correlation was obtained between the enzyme levels and the red cell indices and WBC. The increase in the level of G-6-PD and PK in anaemia could be due to an increase in the level of reticulocytes and young erythrocytes which have high enzyme levels. Another possibility is that in the anaemia states a true elevation of the enzyme activity is taking place in an attempt to fulfill the extra needs for energy and NADPH requirements of the red cells.

Table 9.18: Regression analysis and correlation coefficient ® between glucose-6-phosphate dehydrogenase and haematological parameters

Correlation of G-6-PD with	r	Intercept	Slope	P
RBC ($x10^{12}$/l)	0.4222	184.961	-21.22	0.0001
PCV (l/l)	0.4475	202.119	-3.6	0.0001
Hb (g/dl)	0.4550	217.48	-11.28	0.0001
Retics (%)	0.4762	71.988	2.272	0.0001
WBC ($x10^{9}$/l)	0.289	74.215	3.017	0.0001
MCV (fl)	0.2894	-49.72	2.029	0.0001
MCH (pg)	0.1378	72.63	1.24	0.068
MCHC (g/dl)	0.074	71.48	0.967	0.98
Hb A_2 (%)	0.0167	103.85	1.225	0.830
Hb F (%)	0.045	144.83	0.46	0.728

Figure 9.4: Correlation between G-6-PD and haemoglobin level in anaemic and non-anaemic individuals

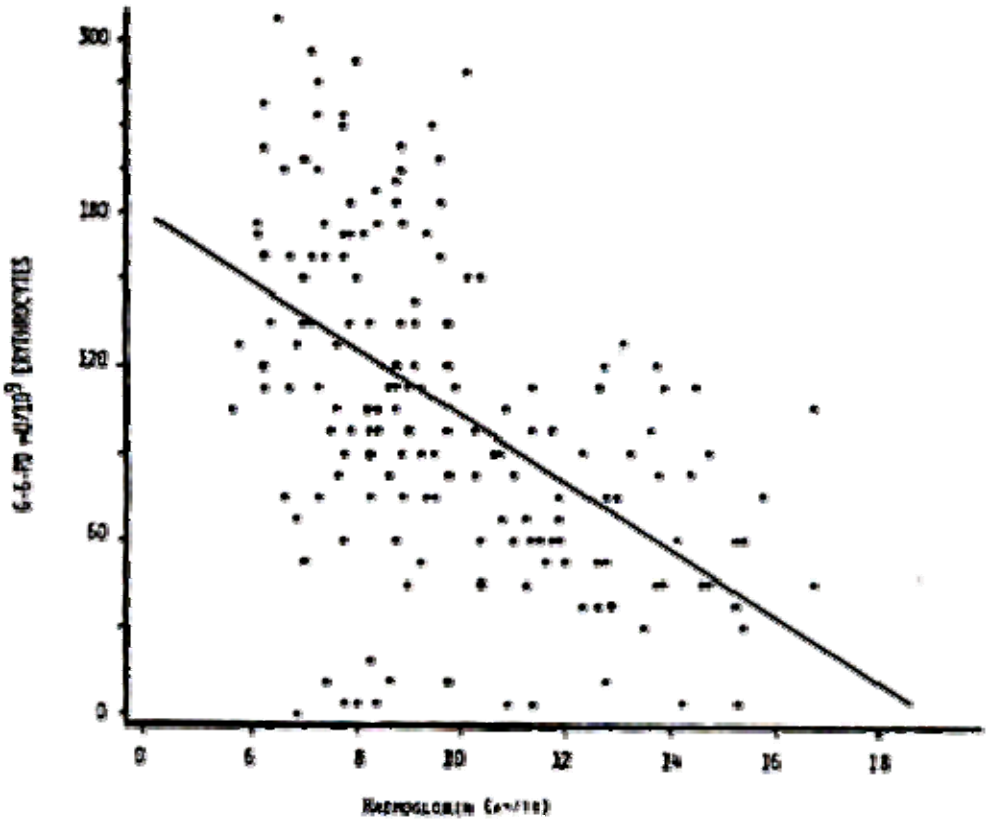


Figure 9.5: Correlation between G-6-PD level and RBC in anaemic and non-anaemic individuals

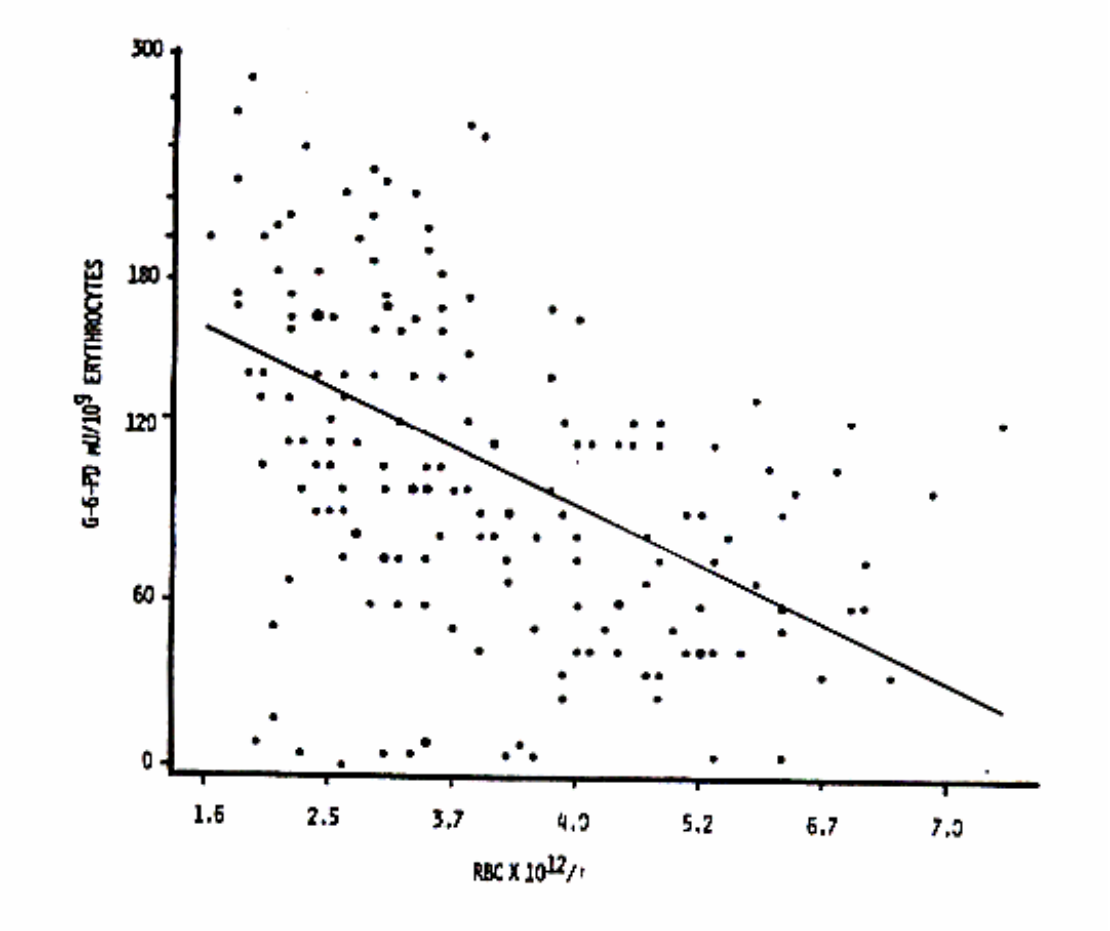


Figure 9.6: Correlation between G-6-PD level and PCV in
anaemic and non-anaemic individuals

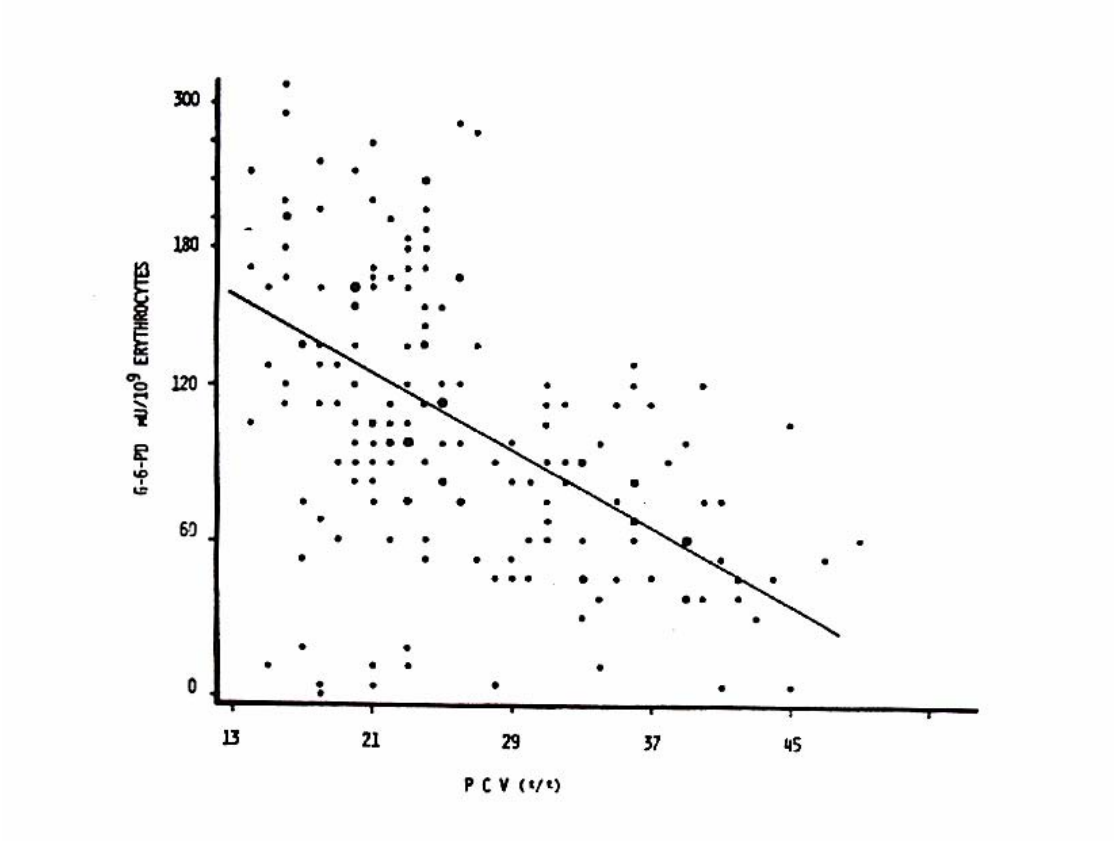


Figure 9.7: Correlation between G-6-PD level and reticulocyte counts in anaemic and non-anaemic individuals

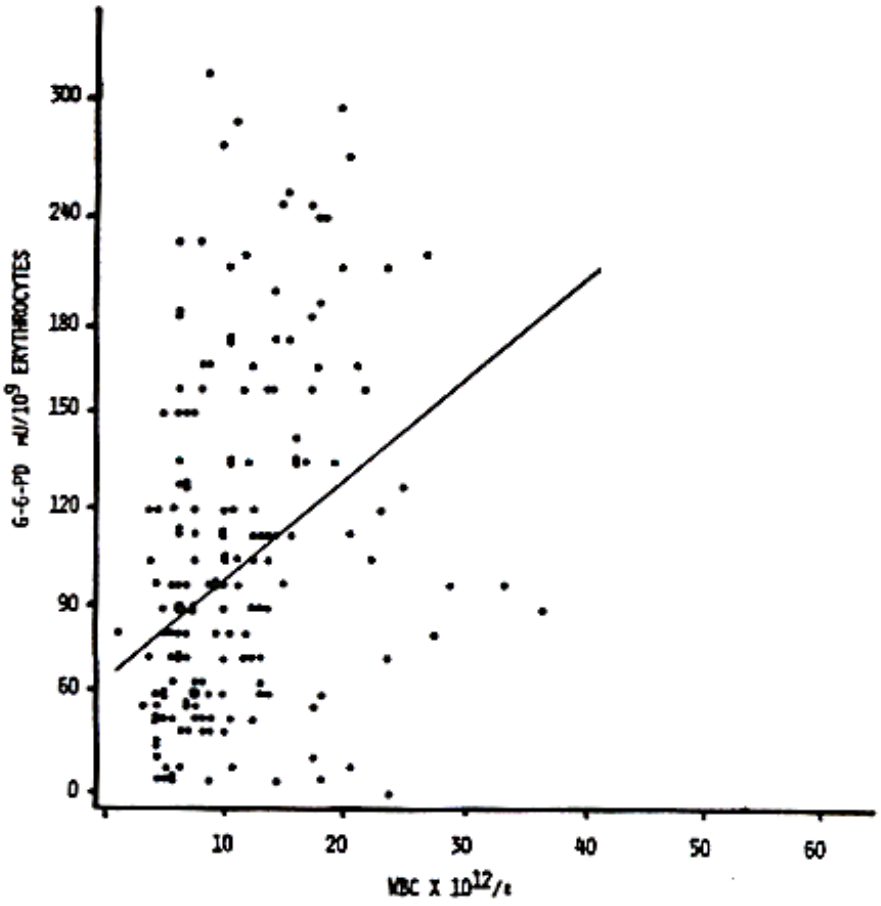


Table 9.19: Regression analysis and coefficient (r) between PK and haematological parameters in anaemic and non-anaemic cases

Correlation between PK and	No. investigated	Haemoglobin Status	r	Intercept	Slope	P*
RBC ($x10^{12}$/l)	170	Anaemic	-0.481	597.86	-82.89	0.0001
	53	Non-Anaemic	-0.504	322.04	-31.90	0.0001
Hb (g/dl)	170	Anaemic	-0.405	717.37	-44.50	0.0001
	53	Non-Anaemic	-0.173	258.06	-6.90	0.001
PCV (l/l)	170	Anaemic	-0.436	667.70	-14.62	0.0001
	52	Non-Anaemic	-0.381	305.49	-3.79	0.005
WBC ($x10^{9}$/l)	170	Anaemic	0.196	279.58	3.84	0.05
	53	Non-Anaemic	-0.153	184.08	3.242	0.273
MCV (fl)	169	Anaemic	0.452	-304.97	8.167	0.0001
	53	Non-Anaemic	0.250	29.78	1.687	0.007
MCH (pg)	170	Anaemic	0.283	136.3	6.8	0.0002
	53	Non-Anaemic	0.402	15.49	5.18	0.002
MCHC (g/dl)	170	Anaemic	0.205	57.84	7.19	0.005
	53	Non-Anaemic	0.385	-39.06	5.45	0.005
Hb A_2 (%)	166	Anaemic	-0.054	356.36	-8.83	0.484
	49	Non-Anaemic	-0.011	164.95	-0.91	0.941
Retics (%)	167	Anaemic	0.535	217.9	6.53	0.0001
	49	Non-Anaemic	-0.146	172.41	-3.78	0.496

*P value less than 0.05 was considered statistically significant

Figure 9.8: Correlation between PK and RBC in
anaemic and non-anaemic individuals

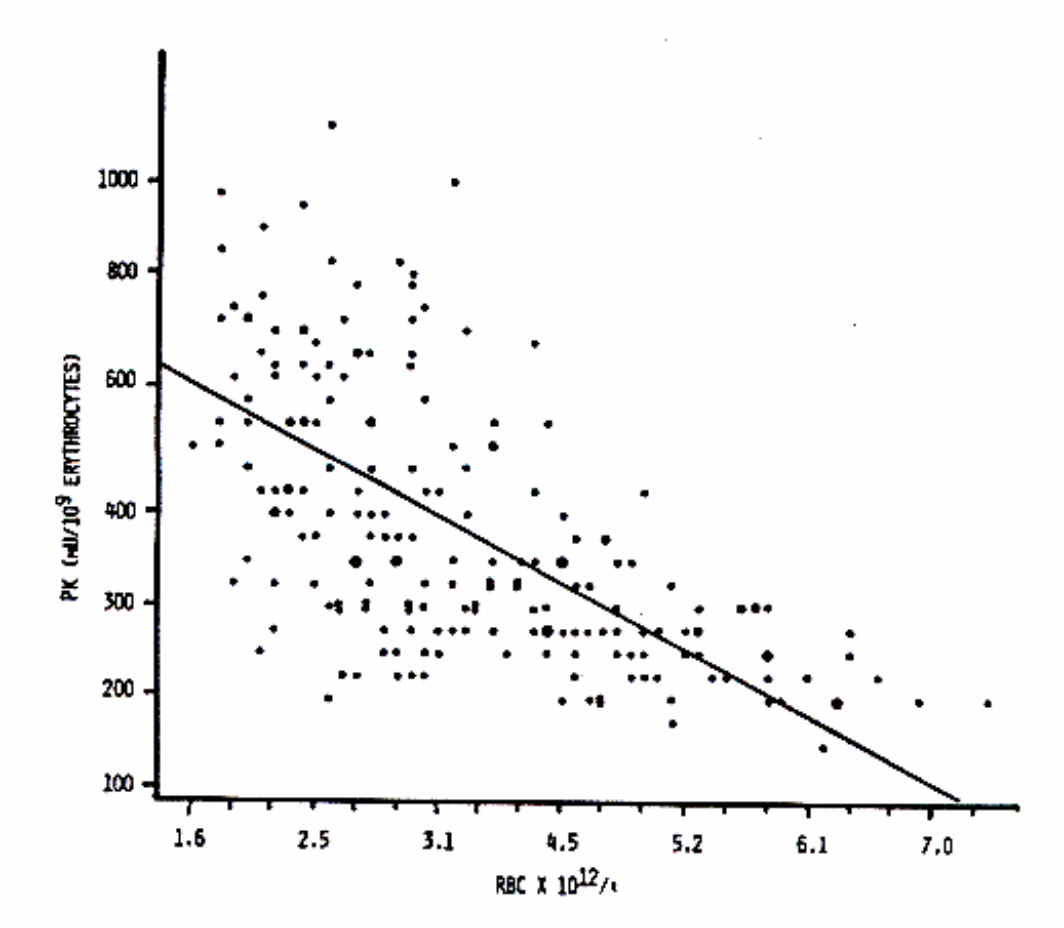


Figure 9.9: Correlation between PK and PCV in
anaemic and non-anaemic individuals

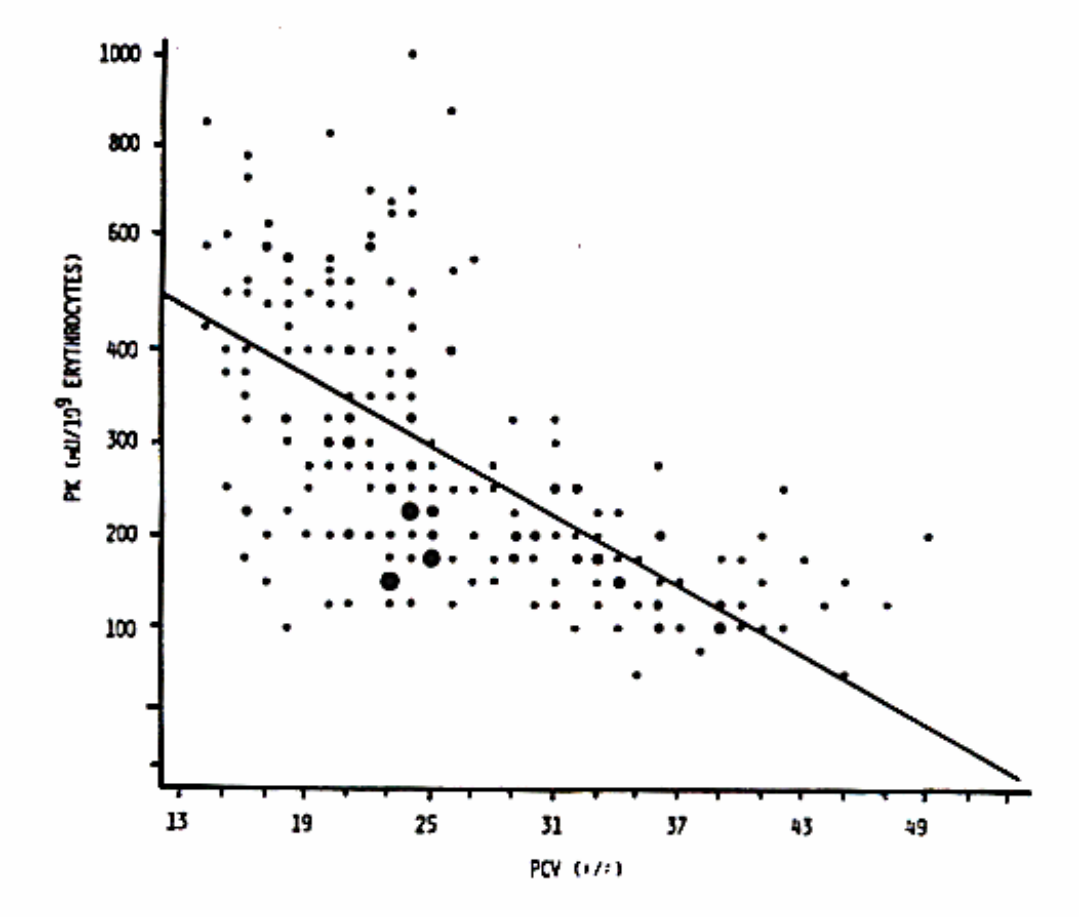


Figure 9.10: Correlation between PK and reticulocyte count in anaemic and non-anaemic individuals

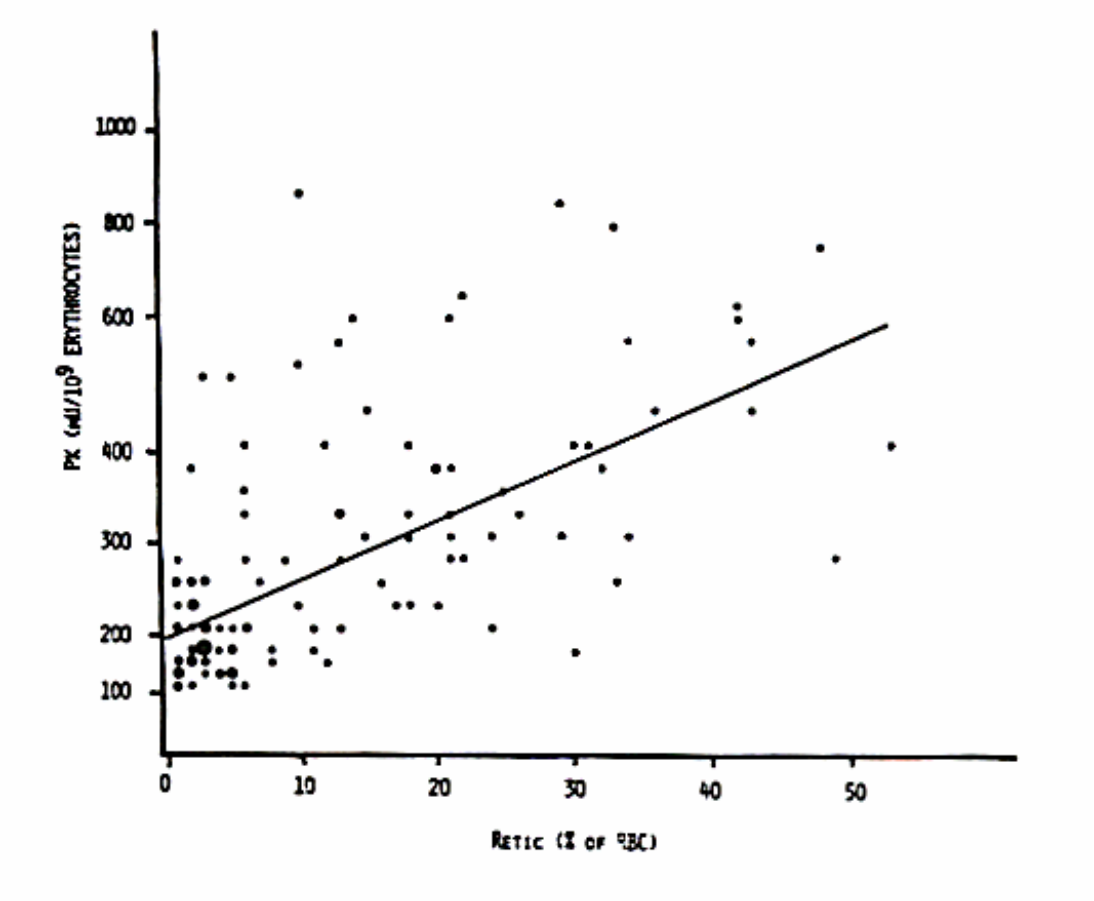


Figure 9.11: Correlation between PK and WBC in
anaemic and non-anaemic individuals

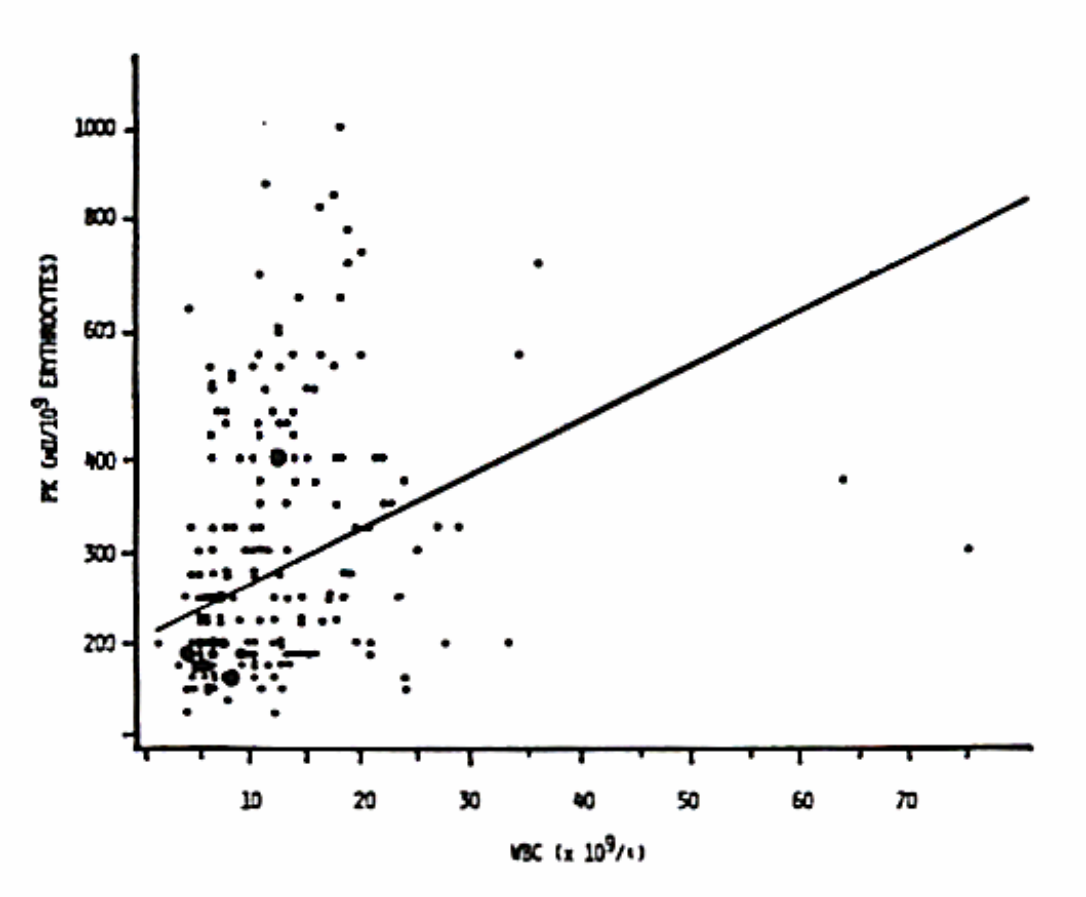


Table 9.20: Red cell enzyme levels in different anaemic states

Sample	No. Investigated	Enzyme level (Mean ± SD)	
		G-6-PD mU/10^9 Erythrocyte	PK mU/10^9 Erythrocyte
Normal	49	62.5 ± 30.5	161.7 ± 54.1
Anaemic	157	117.1 ± 64.6	329.6 ± 175.6
Sickle cell anaemia	72	138.3 ± 68.8	404.0 ± 185.8
α-Thalassaemia	27	109.3 ± 41.7	301.1 ± 151.4
β-Thalassaemia.	50	75.48 ± 35.02	211.8 ± 92.56
Iron Deficiency	30	111.8 ± 77.03	223.67 ± 81.22

Table 9.21: Regression analysis and correlation coefficient (r) between PK and haematological parameters in patients with different abnormalities

Correlation of PK with	Abnormalities	No. investi-gated	r	Intercept	Slope	P*
RBC ($x10^{12}$/l)	Normal	53	-0.504	322.04	-31.9	0.0001
	Anaemic (Total)	170	-0.481	597.86	-82.89	0.0001
	SS	78	-0.4522	685.31	-91.71	0.0001
	α-Thal.	27	-0.490	534.72	-64.89	0.0001
	β-Thal.	50	-0.709	435.33	-50.98	0.0001
	Fe Def.	30	-0.704	485.58	-61.06	0.0001
Hb (g/dl)	Normal	53	-0.173	258.06	-6.90	0.001
	Anaemic (Total)	170	-0.405	717.37	-44.5	0.001
	SS	78	-0.350	845.77	-52.68	0.001
	α-Thal.	27	-0.436	536.77	-25.35	0.001
	β-Thal.	50	-0.659	483.75	-25.2	0.0001
	Fe Def.	30	-0.284	318.01	-9.1	0.0001
PCV (l/l)	Normal	53	-0.381	305.49	-3.79	0.005
	Anaemic (Total)	170	-0.436	667.70	-14.62	0.0001
	SS	78	-0.384	762.22	-15.87	0.0005
	α-Thal.	27	-0.438	499.38	-8.1	0.0005
	β-Thal.	59	-0.720	465.67	-8.7	0.0001
	Fe Def.	30	-0.592	458.12	-8.94	0.001
WBC ($x10^{9}$/l)	Normal	53	0.153	184.08	3.242	0.010
	Anaemic (Total)	170	0.196	279.58	3.840	0.050
	SS	78	0.059	385.49	1.241	0.605
	α-Thal.	27	0.305	257.20	3.51	0.01
	β-Thal.	50	0.390	148.95	6.98	0.01
	Fe Def.	30	0.173	267.91	4.96	0.09
MCV (fl)	Normal	53	0.250	29.78	1.689	0.07
	Anaemic (Total)	169	0.452	-304.97	8.167	0.0001
	SS	78	0.455	-259.37	8.33	0.001
	α-Thal.	27	0.251	3.77	3.87	0.01
	β-Thal.	50	0.436	-193.37	5.72	0.005
	Fe Def.	30	0.346	-95.2	4.60	0.01

Correlation of PK with	Abnormalities	No. investi-gated	r	Intercept	Slope	P*
Retics (%)	Normal	49	-0.146	172.41	-3.78	0.0496
	Anaemic (Total)	167	0.535	217.9	6.53	0.0001
	SS	78	0.208	317.37	2.95	0.010
	α-Thal.	27	0.209	232.8	2.42	0.014
	β-Thal.	50	0.563	141.6	10.8	0.003
	Fe Def.	30	-0.712	418.0	-79.0	0.01

Table 9.22: Regression analysis and correlation coefficient (r) between G-6-PD and haematological parameters in patients with different abnormalities

Correlation of PK with	Abnormalities	No. investi-gated	r	Intercept	Slope	P*
RBC ($x10^{12}$/l)	Normal	49	0.173	31.90	6.03	0.233
	Anaemic (Total)	157	-0.367	193.1	-23.63	0.0001
	SS	72	-0.332	217.6	-26.33	0.004
	α-Thal.	27	-0.431	159.56	-13.51	0.014
	β-Thal.	50	-0.355	130.31	-11.45	0.039
	Fe Def.	30	-0.896	332.39	-50.59	0.0001
Hb (g/dl)	Normal	49	-0.194	122.88	-4.31	1.181
	Anaemic (Total)	157	-0.390	254.90	-15.76	0.0001
	SS	72	-0.267	262.53	-14.68	0.02
	α-Thal.	27	-0.474	166.70	-6.31	0.01
	β-Thal.	50	-0.535	177.13	-9.21	0.001
	Fe Def.	30	-0.857	322.03	-22.56	0.001
PCV (l/l)	Normal	49	-0.334.	139.77	-1.99	0.018
	Anaemic (Total)	157	-0.355	218.28	-4.32	0.0001
	SS	72	-0.301	240.73	-4.50	0.010
	α-Thal.	27	-0.463	156.07	-1.88	0.007
	β-Thal.	50	-0.463	152.47	-2.49	
	Fe Def.	30	-0.974			
WBC ($x10^{9}$/l)	Normal	49	-0.074	68.64	-0.89	0.613
	Anaemic (Total)	157	0.194	92.57	2.02	0.015
	SS	72	0.164	108.69	2.12	0.010
	α-Thal.	27	0.674	41.76	6.33	0.030
	β-Thal.	50	0.0065	79.30	0.056	0.970
	Fe Def.	30	0.008	110.17	0.177	0.981
MCV (fl)	Normal	49	-0.200	108.12	-0.587	0.167
	Anaemic (Total)	156	0.350	-70.32	2.395	0.0001
	SS	72	0.313	-50.49	2.39	0.007
	α-Thal.	27	0.018	102.54	0.07	0.962
	β-Thal.	50	0.043	61.93	0.25	0.811
	Fe Def.	30	0.434	-199.25.	4.45	0.465

Correlation of PK with	Abnormalities	No. investi-gated	r	Intercept	Slope	P*
Retics (%)	Normal	49	0.007	140.16	0.038	0.964
	Anaemic (Total)	156	0.4590	217.48	-11.28	0.0001
	SS	70	-0.0078	140.16	0.036	0.964
	α-Thal.	27	0.333	78.18	3.718	0.03
	β-Thal.	50	0.440	46.20	3.53	0.04
	Fe Def.	30	0.434	-199.25	4.45	0.465

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Since the red cell enzymes are not synthesised in the mature erythrocytes, either an increased synthesis could have occurred in the pre-erythrocyte stage or the activity of these enzymes might be elevated by some positive modulator.

These results clearly showed that in anaemic patients red cell enzyme deficiencies, i.e. G-6-PD and PK deficiency, could be masked due to an elevation of the level or activity of the enzymes as a consequence of anaemia. It is, therefore, recommended that during determination of G-6-PD and PK status of the red cells, care

must be taken to remove the reticulocytes and the WBCs prior to enzyme estimation. Furthermore, it must be remembered that in anaemic state a normal enzyme level, may not rule out an associated genetic deficiency.

9.9 Microheterogeneity of G-6-PD

The microheterogeneity of G-6-PD was estimated using isoelectric focussing in LKB Ampholine PAG plates pH 5.5-8.5. The ready-made gel was obtained from LKB Productor and was prefocussed at 1600V, 50 mA and 25W for 25 min. The samples (10µl) were applied on small filter strips (5x3 mm) and placed about 1 cm from the cathode using 0.1M NaOH as cathode solution and 0.4M HEPES as anode solution. Isoelectric focussing was carried out at 8°C for 2.5 hours applying 1600V, 50mA and 25W. The gel was stained specifically for G-6-PD using a stain prepared by mixing 0.2M Tris-HCl buffer, pH 8.0, 5mM glucose-6-phosphate, 2mM nicotinamide adenine dinucleotide phosphate ($NADP^+$), 0.23mM phenazine methosulfate (PMS) and 0.1mM tetrazolium bromide (MTT). After incubating the gel with the stain at 37°C for 30-60 min., the gel was washed, placed in 1% acetic acid and preserved in glycerol.

The isoenzyme patterns obtained for the different G-6-PD phenotypes are shown

Figure 9.12: Microheterogeneity of glucose-6-phosphate dehydrogenase seen on isoelectric focusing

1 **2** **3** **4**

in Figures 9.12. G-6-PD-B^+ was composed of 5 distinct isoenzyme bands which were numbered I-V from the cathodic to anodic end. Bands II, III and IV showed stronger activity compared to the band I & V. Three minor bands more anodic to band V were also observed. In the different G-6-PD variants the isoenzyme pattern was significantly different. In G-6-PD-Mediterranean no isoenzymes were identified in majority of the cases. However, in a few, a weak band No. 1 was observed. In G-6-PD-A^+ the isoenzyme No. IV & V were much stronger than I, II & III and similarly bands VI, VII & VIII were also stronger than in G-6-PD-B^+. On the other hand, G-6-PD-A^- showed only 2 isoenzymes. These were No. IV & V and the bands cathodal and anodal were missing. For G-6-PD-Mediterranean-like, different isoenzyme patterns were obtained in samples with different G-6-PD activity. In some individuals band II was stronger in intensity, while in others band III was stronger. These results revealed significant microheterogeneity in G-6-PD variants and showed that each variant (phenotype) of G-6-PD has several isoenzyme types, which may result from different modifications of the same enzyme and though they have the same charge and are not separated on electrophoresis, the separation is possible by isoelectric focussing as these isoenzymes differ in their isoelectric point. An interesting finding is that the variants differed in these isoenzymes pattern.

9.10 Clinical, haematological and biochemical findings in G-6-PD deficient individuals

Glucose-6-phosphate dehydrogenase deficiency was one of the most frequently identified red cell genetic defect and was encountered in a large percentage of the Saudi population. Generally, the G-6-PD deficient individuals did not show any significant clinical or haematological abnormalities and were assymptomatic. The episodes of acute

haemolytic anaemia were faced by almost all patients under the attack of different factors including drugs, infections, or *Vicia faba*. Favism was identified in several individuals but the exact frequency of favism could not be obtained, as different patients differed in their susceptability to the fava beans. Some patients had eaten the beans several times without any clinical consequences, but an isolated exposure precipitated severe haemolysis while others had no susceptibility to the breans. Since majority of the severe deficiency was caused by G-6-PD Mediterranean in this population, it is expected that the frequency of favism will be high in the Saudi population.

Table 9.23 presents the haematological parameter values in the individuals with G-6-PD deficiency and Table 9.24 presents the biochemical parameter values in these patients.

As can be seen from these results, no major haematological or biochemical abnormality occurs in the G-6-PD deficient individuals under the steady state condition and thus the condition may go undiagnosed, until exposure to an environmental factor which would precipitate the haemolytic attack which may be severe. Since G-6-PD deficient individuals have lower NADPH level which is needed for the synthesis of fatty acid and cholesterol. It was suggested that these individuals may have lower level of plasma lipids. However, no significant differences could be observed between the G-6-PD deficient and G-6-PD normal individuals in the levels of the plasma lipids.

It is recommended that in the high risk areas and in high risk families, all members and new borns must be screened for G-6-PD deficiency and each G-6-PD deficient individual must be given a card stating their G-6-PD deficient state, and listing drugs and foods to be avoided, in order to prevent severe haemolytic episodes.

Table 9.23: Haematological findings in individuals with G-6-PD deficiency and normal H-6-PD

Haematolo-gical Parmeters	G-6-PD-Mediterranean			Normal		
	Male	Female	Children	Male	Female	Children
Age (years)	33.0 ±18.0	26.0 ± 6.6	6.6 ± 3.7	330.11.0	26.0 6.6	6.6 ± 3.0
RBC ($x10^{12}$/l)	5.7 ± 0.5	4.0 ± 0.46	5.1 ± 0.56	4.6 ± 0.8	4.0 ± 0.45	3.0 ± 0.6
Hb (g/dl)	14.7 ± 2.2	10.7 ± 1.9	12.9 ± 1.8	12.9 ± 2.8	11.7 ± 1.8	8.0 ± 2.1
PCV (l/l)	0.44 ±0.06	0.35± 0.06	0.38± 0.05	0.40 ±0.09	90.35±0.06	60.75±0.07
WBC ($x10^{9}$/l)	5.0 ± 1.2	5.6 ± 2.0	7.5 ± 2.9	5.6 ± 2.0	5.6 ± 2.0	5.6 ± 2.3
MCV (fl	83.0 ± 4.5	86.6± 13.6	76.0± 8.2	85.5 ± 9.5	86.6 ± 13.6	85.0 ± 7.0
MCH (pg)	27.0 ± 1.7	26.7 ± 3.4	25.4 ± 3.3	28.0 ± 3.8	26.7 ± 3.3	27.4 ± 5.9
MCHC (g/dl)	33.0 ± 1.7	31.3± 3.5	33.7 ± 1.5	33.0 ± 5.2	31,0 ± 3.4	31.0 2.6
Hb F (%)	1.2 ± 0.5	0.4 ± 0.2	1.0 ± 0.5	1.1 ± 0.5	0.4 ± 0.3	0.3 ± 0.3
Hb A_2 (%)	3.0 ± 0.5	2.5 ± 0.36	3.0 ± 0.4	2.6 ± 0.5	2.5 ± 0.4	2.9 ± 0.2

Table 9.24: Haematological findings in individuals with G-6-PD defcieincy and normal G-6-PD

Haematological Parmeters	G-6-PD-Mediterranean			Normal		
	Male	Female	Children	Male	Female	Children
A. Liver Function Profile						
T. Prot (g/l)	76.0 ± 1.0	76.5± 9.6	70.0 ± 9.4	74.5± 4.5	76.0 ± 9.6	72.0 ±14.0
Albumin (g/l)	47.0± 1.0	41.4 ±0.86	43.1± 3.5	43.5 ± 3.5	41.4 ± 4.6	37.4 ±9.7
T. Bil (mmol/l)	6.8 ± 2.4	4.2 ± 2.2	5.1 ± 2.6	9.3 ± 7.8	4.2 ± 2.2	3.6 ± 2.9
D. Bil (mmol/l)	1.7± 0.1	2.4 ± 1.8	1.7 ± 0.1	3.5 ± 1.8	2.4 ± 1.8	2.8 ± 1.0
SGOT (U/l)	35.3 ±10.4	20.1 ± 4.8	31.3± 12.9	24.5± 9.9	20.0 ± 4.8	23.6 ± 5.9
SGPT (Ul/l)	21.7 ±10.9	12.1± 7.7	8.0 ± 5.4	13.5 ±11.3	12.1 ± 7.6	10.4± 11.7
B. Bone Profile						
Ca++ (mmol/l)	2.4 ± 0.1	2.3 ± 0.17	2.3 ± 0.2	2.4 ± 0.1	2.3 ± 0.17	2.3 ± 0.3
PO_4" (mmol/l)	0.89 ± 0.1	1.2 ± 0.2	1.1 ± 0.27	1.3 ± 0.2	1.2 ± 0.2	1.1 ± 0.4
Alt (U/l)	72.0 ± 6.1	76.1 ± 28.0	151.0 ±76.0	96-0± 66.0	76.1±28.0	150.0±65.0
C. Renal Profile						
Urea (mmol/l)	4.7 ± 1.5	3.9 ± 0.9	3.5 ± 0.8	4.1 ± 1.3	3.9 ± 0.9	3.6 ± 0.9
Creatinine (mmol/l)	88.0 ±20.0	65.3 ± 14.3	52.8 ± 14.3	81.1 ± 20.0	65.2 ± 13.4	74.2 ± 18.0
D. Electrolytes						
Na== (mmol/l)	139±.0 1.1	136.1 ± 3.0	136.1± 3.0	140.6 ±5.4	141.0 ± 2.6	142.0 ± 2.7
K+ (mmol/l)	4.0 ± 0.2	4.4 ± 0.6	4.4 ± 0.6	5.0 ± 2.0	4.6 ± 0.7	4.9 ± 0.8
Cr (mmol/l)	102.0± 0.6	102.5 ±3.5	102.0 ± 3.5	102.0 ± 4.7	103.0 ± 2.4	107.0 ± 2.5
E. Lipids						
Cholesterol (mmol/l)	4.8 ± 2.0	3.9 ± 0.9	3.9 ± 0.9	3.8 ± 0.9	4.5 ± 0.8	3.8 ± 0.5
Truglycerides (mmol/l)	1.7 ± 0.8	1.1 ± 0.4	1.0 ± 0.4	1.0 ± 0.3	1.4 ± 0.6	1.4 ± 0.9
F. Miscellaneous						
Uric Acid (mmol/l)	0.246 ±0.05	0.252±0.065	0.252± 0.06	0.288±0.06	0.244± 0.08	0.376± 0.15