

## NEONATAL SCREENING FOR SICKLE CELL DISEASE, GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND $\alpha$ -THALASSEMIA IN QATIF AND AL HASA

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**Background:** Screening programs to determine the frequency of sickle cell, glucose-6-phosphate dehydrogenase deficiency and  $\alpha$ -thalassemia gene are available in Saudi Arabia, although not used frequently. Greater use of these programs will decrease the morbidity and mortality of Saudi children affected by these disorders.

**Patients and Methods:** Neonatal hemoglobin electrophoresis and glucose-6-dehydrogenase fluorescent spot tests were performed on newborn babies delivered between December 1992 and December 1993 at the Qatif Central Hospital and at the King Fahad Hospital in Al Hasa. Cord blood samples were collected from babies born in these two hospitals. Babies born in other hospitals had blood collected in their first visit to Qatif primary care centers at the time of vaccination. All specimens were sent to Dammam Central Laboratory. The diagnosis of sickle cell and  $\alpha$ -thalassemia was based on cellulose acetate electrophoresis and confirmed by agar gel electrophoresis, and glucose-6-phosphate dehydrogenase was confirmed by fluorescent spot test.

**Results:** A total of 12,220 infants, including 11,313 Saudis (92.6%), were screened over a 12-month period. The common phenotypes detected in these infants included AF, AF Bart's, SFA, SFA Bart's, FS and FS Bart's. In the Saudi infants, homozygous sickle cell disease was detected in 2.35% and 1.08% in Qatif and Al Hasa, respectively. The frequencies of sickle cell gene were 0.1545% and 0.1109% in Qatif and Al Hasa.  $\alpha$ -thalassemia gene based on an elevated level of Hb Bart's were 28% and 16.3% in Qatif and Al Hasa. The screening for G6PD deficiency revealed a high prevalence of 30.6% and 14.7% in Qatif and Al Hasa. In the non-Saudi infants, the frequencies were low.

**Conclusion:** The outcome of this study indicates that the Saudi populations in Qatif and Al Hasa are at risk for hemoglobinopathies and G6PD. Neonatal screening programs are essential and cost effective and should be maintained as a routine practice.

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**Key Words:** Neonatal screening, hemoglobinopathies, G6PD.

Hemoglobinopathies and glucose-6-phosphate dehydrogenase deficiency (G6PD) are among the major health problems of Saudi Arabia and constitute the most common genetic disorders among people originally belonging to the Western side of the Arabian Gulf (Qatif and Al Hasa Provinces).<sup>1-5</sup> The programs for diagnosis of hemoglobin disorder by electrophoresis at birth by cord blood have been available for the past 15 years.<sup>6,7</sup> These are effective programs that identify and confirm the diagnosis of all infants with significant hemoglobinopathy as early as two months of age. The use of agar gel

electrophoresis at acid pH will make identification of Hb S more accurate due to the fast mobility of Hb F, so even if there is a large component of Hb F in a newborn, it will not obscure Hb S.<sup>6</sup> These affected cases should be followed in special clinics that provide comprehensive follow-up by knowledgeable staff. This will decrease the mortality and morbidity of homozygous sickle cell disease, which is highest in the first three years of life,<sup>8,9</sup> and is a consequence of increased susceptibility to severe bacterial sepsis and meningitis, particularly due to *Streptococci pneumoniae*.<sup>10,11</sup>

Although  $\beta$ -thalassemia is indistinguishable from normal electrophoresis done at early infancy, the diagnosis of clinically significant  $\alpha$ -thalassemia syndrome is suggested by the presence of greater than 2% of Bart's hemoglobin, which aids in the differential diagnosis of hypochromic microcytic anemia late in infancy.

In Saudi Arabia, this neonatal program has been routinely provided only to employees of Aramco Oil

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TABLE 1. Numbers of newborns from participating centers.

Hospital/Center	Saudi	Non-Saudi	Total
Qatif Central Hospital	1943 (91.6%)	217 (8.4%)	2160
Qatif Primary Health Center	485 (98.6%)	7 (1.4%)	492
KFHH	8269 (92.9%)	667 (7.1%)	8936
Al Hasa Primary Health Center	616 (97.5%)	16 (2.5%)	632
Total	11,313 (92.6)	907 (7.4%)	12,220

KFHH=King Fahad Hospital Hofuf.

TABLE 2. Nationality and sex of newborns screened.

Nationality	Al Hasa			Qatif		
	Male	Female	Und.	Male	Female	Und.
Saudi	4410	4378	97	1254	1172	2
Non-Saudi	334	344	5	116	107	1
Total	4744	4722	102	1370	1279	3

Und.=undefined sex.

TABLE 3. Percentage of electrophoresis pattern in newborns screened.

Pattern	Saudi (%)		Non-Saudi (%)	
	Qatif	Al Hasa	Qatif	Al Hasa
AF	58.8	75.5	92.9	97.4
AF-Bart's	8.3	3.0	0	0.4
AFS	10.9	6.9	4.4	0.6
AFS-Bart's	18.9	13.1	1.8	1.3
FS	1.6	0.8	0.1	0
FS-Bart's	0.7	0.26	0	0
Others	0.7	0.36	0.9	0.3

Company since 1981.<sup>7</sup> Al Awamy et al. had the unique opportunity of screening some of the newborn babies in hospitals in Qatif, Al-Khobar and Dammam between 1982 and 1987.<sup>1</sup> These early studies did not adequately represent people living in Qatif. No studies were done in Al Hasa or any other area in Saudi Arabia. The need for more representative studies to generate reliable data became obvious in early 1990. This study forms the first step in continuous neonatal diagnosis of hemoglobinopathies in Qatif and Al Hasa.

### Patients and Methods

The neonatal hemoglobinopathies' screening program was designed to screen the newborn population in the Eastern Province. The target population included all babies born in Qatif Central Hospital, Qatif, and King Fahad Hospital, Al Hasa, from December 1992 to December 1993. In addition, babies delivered at home in the Qatif and Al Hasa areas, and coming to primary health care centers for vaccination, were also included. Cord blood samples were collected in EDTA anticoagulated tubes from all the babies delivered in the two hospitals. Babies delivered elsewhere but coming to the primary care centers for vaccination had heel prick and blood samples collected in EDTA microtainers tubes.

Samples from Qatif Central Hospital and primary health care centers of Qatif area were transported daily to the regional laboratory in Dammam in cooled containers. Samples collected at King Fahad Hospital, Al Hasa, and from the primary health care centers in the Al Hasa area were initially stored in King Fahad Hospital Laboratory at suitable temperatures. These samples were taken to the regional laboratory in Dammam in cooled containers at temperatures less than 8°C. The time between collection of the specimen and analysis was less than five days.

All samples were subjected to electrophoresis at alkaline pH (pH 8.6-8.8) on cellulose acetate plates. Agar gel hemoglobin electrophoresis at acid pH (pH 6.0-6.3) was performed on all samples in which Hb S was identified.

Samples for G6PD deficiency were analyzed using fluorescent screening technique. Fluorescence is produced due to reduction of NADP to NADPH. This reaction is coupled with oxidation of glucose-6-phosphate to 6 phosphogluconate and catalyzed by G6PD. Specimens with G6PD activity of less than 20% of normal do not fluoresce, as the small amount of NADPH formed is re-oxidized by the glutathione present in the reagents. The reports of screening of cord blood samples were placed in the files of the babies' mothers.

Parents of babies with FS electrophoresis pattern were contacted by phone by a team of social workers and informed of the diagnosis. The need for follow-up was explained to them. Appointments were arranged for at-risk babies to confirm the diagnosis and for regular further follow-up. The screening program is still continuing in Qatif.

### Results

During the 12-month period, 12,220 infants were screened, of whom 11,313 (92.6%) were Saudis. The distribution of newborns according to institutions and nationality is summarized in Table 1, which shows most of the sample to be from the two main hospitals. The numbers of boys and girls were nearly equal (Table 2).

Common hemoglobin electrophoresis patterns are AF, AFS, FS, AF Bart's and AFS Bart's. There were 47 samples (0.4%) with rare hemoglobin variants. These were not characterized further (Table 3).

The prevalence of sickle cell disease in Saudi infants was 2.35% and 1.08% in Qatif and Al Hasa, respectively. In non-Saudi infants, the prevalence rates were 0.45% in Qatif and 0 in Al Hasa (Table 4). The prevalence of  $\alpha$ -thalassemia (Hb Bart's >2%) in Saudi infants was 28% and 16.3% in Qatif and Al Hasa, respectively. In non-Saudi infants, it was 1.8% and 1.9% in Qatif and Al Hasa, respectively (Table 5).

Glucose-6-phosphate dehydrogenase-reduced activity (Table 6) in Saudi infants was found in 30.6% and 14.6% in Qatif and Al Hasa, respectively. In non-Saudis, the rates

were 5.8% and 2.6% in Qatif and Al Hasa, respectively. In Qatif, the deficiency was in 35% of the male and in 21% of the female subjects. These were lower in Al Hasa infants, with 19.4% in male and 8.2% in female subjects.

### Discussion

A large number of neonates with sickle cell disease are born annually in Qatif and Al Hasa. These have high risks of mortality and morbidity, mainly during infancy.<sup>6</sup> The incidence of sickle cell trait in the Saudi neonate is very high, 28.21% and 20.02% in Qatif and Al Hasa, respectively. This figure is different from that reported by Al Awamy,<sup>12</sup> based on births in Qatif General Hospital (old hospital), with a prevalence rate for sickle cell trait of 17.9%, but later corrected to approximately 27% after adding babies from Qatif that were born in Dammam and Al-Khobar.<sup>1</sup>

In the absence of a cure for sickle cell disease, successful management involves prevention and early intervention of complications associated with the disease. Neonatal screening objectives should be: 1) early identification of these sickle cell disease infants that could ensure their early placement into appropriate health care programs, and the education of families about early fetal complications; 2) provision of the appropriate prophylaxis against pneumococcal infection and *Hemophilus influenzae*<sup>10</sup>; and 3) the early identification of sickle cell trait that would decrease the incidence of the disease in the future through genetic counseling before marriage.

In the sickle cell clinic, the parents of the affected infants were tested for abnormal hemoglobin. They were provided with non-directed genetic counseling so that they could make decisions about future reproductive activity. All other siblings were tested to detect previously undiagnosed hemoglobin disease, and parents were informed about the result in the next clinic visit. The percentage of newborns with sickle cell trait is very high (28.21% and 20.02% in Saudi newborns in Qatif and Al Hasa, respectively), so for the time being cases of sickle cell trait were not informed by phone, but information was left in the mother's chart and parents were informed usually during their postnatal visits. Our aim is that this information, which may not affect the child during his childhood, will influence his or her later search for a spouse, to avoid producing children with sickle cell disease.

The prevalence of  $\alpha$ -thalassemia gene in our study of Saudi infants was very high (28% and 16.3% in Qatif and Al Hasa, respectively). We believe this figure is even less than the actual figure, due to the method we used for the diagnosis of  $\alpha$ -thalassemia (presence of more than 2% of Hb Bart's) which is known to miss many cases. If non-screening methods such as restriction enzyme analysis were used, the results could be as high as 50%.<sup>2</sup> The information about  $\alpha$ -thalassemia is included in the

TABLE 4. Numbers of newborns with sickle cell disease and trait.

Disease	Qatif	Al Hasa
Saudi		
Sickle cell disease	57 (2.35%)	96 (1.08%)
Sickle cell trait	685 (28.21%)	1779 (20.02%)
Gene frequency of HbSS	0.1645	0.1109
Non-Saudi		
Sickle cell disease	1 (0.45%)	0
Sickle cell trait	14 (6.2%)	13 (1.9%)
Gene frequency of HbSS	0.0357	0.0095

TABLE 5. Numbers of newborns with  $\alpha$ -thalassemia.

Nationality	Qatif	Al Hasa
Saudi	680 (28%)	1451 (16.3%)
Non-Saudi	4 (1.8%)	3 (1.9%)

TABLE 6. Numbers of newborns with G6PD deficiency.

Infants with G6PD deficiency	Qatif	Al Hasa
Saudi		
Male	475 (37.9%)	910 (20.6%)
Female	267 (22.8%)	385 (8.8%)
Total	744 (30.6%)*	1305 (14.5%)
Non-Saudi		
Male	8 (6.9%)	12 (3.6%)
Female	5 (4.7%)	6 (1.7%)
Total	13 (5.8%)	18 (2.6%)
Total male	483 (35%)	922 (19.4%)
Total female	272 (21%)	390 (8.3%)

\*There will be some discrepancy in total figures due to undefined sex of some patients. Refer to Table 2.

mother's chart or referred to the primary care center of the affected infant with the aim of helping to differentiate it from hypochromic microcytic anemia, and preventing an inappropriate investigation or iron therapy in the future.

The phenotype glucose-6-phosphate dehydrogenase of Mediterranean variant is the major phenotype in our area.<sup>2</sup> The fluorescence in the spot test of G6PD test gives a prevalence in Saudi infants in Qatif and Al Hasa of 30.6% and 16.7%, respectively. Although these figures are very high, they are less than the actual prevalence, since some cases will be missed due to the short lifespan of erythrocytes at the neonatal age, high fetal hemoglobin and increased activity of G6PD enzyme in the neonates.<sup>13</sup>

The gene for G6PD is sex linked. In the homozygous female, the level of erythrocyte enzyme is low and it will be easily detected by the screening test, but female heterozygotes have variable proportions of red cells with normal and low enzymatic activity, and since fluorescence in the spot test reflects the overall enzymatic activity of the examined sample,<sup>14</sup> heterozygous females may be missed in our study.

The cost of treatment of neonatal hemoglobinopathy is very high for Saudis in these areas. The cost of equipment and materials needed by the technicians involved in this program is about 10 Saudi Riyals (US\$2.67) per specimen, with about one-fifth of this cost for G6PD test. We believe that for this screening program to be successful, it must be

linked to a primary health service, and our future plan is to computerize all sickle cell gene carriers detected by this program in the coming years, so that information will be available in future for premarital counselling.

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