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CLINICAL & BASIC RESEARCH

Neonatal Screening

Mean haemoglobin and red cell indices in cord blood from Omani neonates

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فرز (تحري) حديثي الولادة

المعدلات المرجعية لمؤشرات الخلايا الحمراء ومتوسط الهيموغلوبين في دم الحبل السري عند الأطفال العُمانيين حديثي الولادة

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الملخص: **الهدف:** التحقق من مؤشرات معدلات خلايا الدم الحمراء في فحص الدم العددي الكامل والاسْتشْرابُ السَّائل عالي الأداء في عينات من دم الحبل السري عند العُمانيين حديثي الولادة. **الطريقة:** تم فحص 7837 عينة من دم الحبل السري للأطفال حديثي الولادة وذلك بالقيام بفحص الدم العددي الكامل وفحص الاستشراب السائل عالي الأداء باستخدام البرنامج القصير للثالاسيميا "بيتا". تم إجراء التسلسل المباشر للعينات غير الطبيعية للهيموغلوبين (اس) و(دي) و(أي) و(سي) للتحقق من نتائج الاستشراب السائل عالي الجودة. إضافة لذلك فقد تم إجراء الفحص الجيني في الحالات التي وجد فيها كمية هيموغلوبين (أي) أقل من 10% لتأكيد وجود طفرة. **النتائج:** ثبت أن 4042 وليدا (51.58%) كان عندهم تحليل الاستشراب السائل طبيعيا (هيموغلوبين – أي 22.88±8.03، هيموغلوبين – اف 77.02±8.04)، بينما كانت النتيجة تؤشر بالإصابة بالثلاسيميا – ألفا في البقية (48.42%)، ولم نحصل على أية حالة لهيموغلوبين – أتش. كانت نتائج المجموعة السابقة كما يلي: متوسط الهيموغلوبين (15.38±2.04 جرام/ لتر)، وتعداد كريات الدم الحمراء (4.69±0.68 x 10^{12}/ لتر)، مكْدَاسُ الدَّم (50.5±7.18%)، الحَجْمُ الكُريويُّ الوَسَطيّ (107.66±7.75)، هيموغلوبينُ الكُرَيَّة الوَسَطيّ (33.31±4.07 بيكوجرام)، متوسط الهيموغلوبين الكريوي (30.98±3.44 جرام/ديسي لتر)، عرض توزيع الخلايا الحمراء (17.01±2.17%)، بينما كانت في المجموعة الثانية المصابة بالثالاسيميا (ألفا) كما يأتي وعلى التوالي: (14.79±2.90 جرام/ لتر) ;(5.09±0.77 x 10^{12}/ لتر) ;(49.7±7.40%) ;(97.29±13.8) ;(29.74±11.80 بيكوجرام) ;(30.39±3.6 جرام/ديسي لتر) ;(18.09±2.56%) تسلسل الحمض النووي للعينات للهيموغلوبين غير الطبيعي يمكن أن يؤكد نتائج فحص الدم العددي الكامل والاسْتشْرابُ السَّائل عالي الأداء في جميع الحالات. **الخلاصة:** هذه هي أول دراسة لمقارنة الهيموغلوبين ومؤشرات خلايا الدم الحمراء في دم الحَبل السري لحديثي الولادة العُمانيين مع مثيلاتها من بلدان أخرى في المنطقة، والتي تبين نتائج مماثلة لتلك التي ظهرت عند حديثي الولادة السعوديين. كما قمنافي هذه الدراسة أيضا بالتحقق من صحة تفسيرات فحص الدم العددي الكامل والاسْتشْرابُ السَّائل عالي الأداء لمؤشرات دم الحبل السري عند حديثي الولادة العُمانيين. كان وقوع الثلاسيميا (ألفا) التي تم تشخيصها عن طريق الهيموجلوبين في دم الحبل السري لحديثي الولادة 48.42%.

مفتاح الكلمات: وليدي، فرز، تحري، تقصي، هيموغلوبين، متغيرات، ثتلاسيميا ألفا، عُمان.

ABSTRACT: ***Objectives:*** The aim of this study was to validate the interpretation of red blood cell indices in complete blood count (CBC) and high performance liquid chromatography (HPLC) results on cord blood samples in consecutive Omani neonates. ***Methods:*** Cord blood samples from 7,837 neonates, were analysed with CBC and HPLC using the β-thalassaemia short programme. Direct sequencing of abnormal samples with HbS, HbD, HbE and HbC was performed to validate the HPLC results. Additionally, in cases with HbA <10%, the β-globin gene was directly sequenced for β-thalassaemia mutation analysis. ***Results:*** Overall, 4,042 subjects (51.58%) had normal HPLC (HbA 22.88±8.03; HbF 77.02±8.04), whereas the presence of Hb Barts in the remaining 3,795 cases (48.42%) indicated the presence of α-thalassaemia. No case of HbH was detected. In the former subgroup respectively, the mean Hb (15.38±2.04 g/dl) red blood cell (RBC) count (4.69±0.68 x 10^{12}/l), Hct (50.5±7.18%), mean corpuscular volume (MCV) (107.66±7.75 fl), mean corpuscular haemoglobin (MCH) (33.31±4.07 pg), mean corpuscular haemoglobin concentration (MCHC) (30.98±3.44 g/dl), red cell distribution width (RDW) (17.01±2.17%) whereas, in the latter group with α-thalassaemia, it was (14.79±2.90 g/dl); (5.09±0.77 x 10^{12}/l); (49.7±7.40%); (97.29±13.8 fl); (29.74±11.80 pg); (30.39±3.6 g/dl), and (18.09±2.56%) respectively. DNA sequencing of samples with abnormal

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haemoglobin could validate the CBC and HLPC interpretations in all cases. ***Conclusion:*** This is the first study comparing the hemoglobin and red cell indices in the cord blood from newborn Omani subjects with those from other countries in the region, showing comparable results to those seen in Saudi neonates. The study also validates the CBC and HPLC interpretations of the cord blood red cell indices in the Omani neonate. The incidence of α-thalassaemia diagnosed by the presence of Hb Barts in cord blood of neonates was 48.42%.

Keywords: Neonatal; Screening; Reference range, Haemoglobin; Variants; Alpha-thalassaemia.

Advances in Knowledge

1. *This study validates the cord blood red cell indices obtained from Omani newborn subjects without any underlying haemoglobinopathy.*
2. *It validates the cord blood red cell indices in Omani newborn subjects with α-thalassaemia.*
3. *Finally, it validates the abnormal haemoglobins in Omani newborn subjects.*

Application to the patient care

1. *The results of this study will benefit patient care by comparative analysis of Omani cord blood red cell indices in subjects without any underlying haemoglobinopathy with those from other countries in the region.*
2. *It will improve patient care by comparative analysis of Omani cord blood red cell indices with α-thalassaemia with those from other countries in the region.*
3. *It will also improve patient care by comparing the influence of α-thalassaemia on cord red cell indices in the different subsets with variant haemoglobins amongst Omani newborn subjects.*

Several studies have shown that inherited haemoglobinopathies are widespread in Oman and are at a sufficiently high level to be of considerable national concern.[1,2] Specifically, Oman has a relatively high prevalence of α and β-thalassaemia, G6PD deficiency and sickle cell syndromes compared to other Arabian Gulf Countries.[3-6] A community based survey carried out in 1995 in Oman showed a significantly high prevalence of haemoglobinopathy.[5] The reported prevalence of sickle cell trait was 6% and β-thalassaemia trait was 2%, whereas the prevalence of homozygous sickle cell and β-thalassaemia was 0.2% and 0.07% respectively. The prevalence of other abnormal haemoglobins namely HbD, HbE and HbC were 0.6%, 0.3% and 0.02% respectively. Enzyme deficiency of G6PD was also prevalent (18%). Our own report in 2010 also showed comparatively high prevalence rates.[6] Interestingly, a high incidence of inherited haemoglobinopathies has also been reported in Mediterranean and Middle Eastern contries.[3,7,8] The prevalence of α-thalassaemia was reported as 39.99% and the sickle cell gene was seen in 23.4% of cord blood neonatal samples from Saudi Arabia.[8]

It has been postulated that the likelihood of children being born with a major haemoglobinopathy in Oman would be about 3 per 1,000 births.[5] Thus, with an annual birth rate of 35.76 births/1,000 population,[9] there would approximately be 106 new cases every year. Therefore, with this burden of haemoglobinopathy, the establishment of neonatal reference ranges is extremely important.

This study was undertaken under the auspices of His Majesty's Research directive to screen neonates by implementing a universal newborn screening at the Sultan Qaboos University Hospital (SQUH), Oman. Cord blood samples from newborns at SQUH (representing the Muscat Governorate), and from the Sohar Hospital (representing the Batinah costal region) were collected.

The objectives of our study were twofold. The first objective was to screen the newborn Omani subjects to establish the current prevalence of haemoglobinopathy and to see the effect of the decade long measures that were implemented following the first community study in 1995. The second objective was to validate the CBC and HPLC interpretations of the cord blood red cell indices in the Omani neonate. HPLC is a powerful tool for the simultaneous screening of newborn samples for haemoglobinopathies and to detect the presence of haemoglobin Barts.[10,11] It is extremely accurate, reliable with reproducible results and has thus become the method of choice due to its speed and precision. The objective of this study was therefore to validate the CBC and HPLC interpretations of the cord blood red cell indices in the Omani neonate and by a universal newborn cord blood screening in the Omani population.

Table 1: Comparative analysis of cord blood red cell indices (mean ± SD) in newborn Omani neonates compared with Saudi neonates[8]

Haemoglobins	n	Hb gm/dl	RBC 10^{12}/L	HCT %	MCV fl	MCH pg	MCHC g/dl	RDW %	HbA %	HbF %	Ab. Hb S,D,E,C
Omani HbAF	3, 765	15.38 2.04*	4.69 0.68*	50.5 7.18*	107.66 7.75*	33.31 4.07*	30.98 3.44*	17.01 2.17*	22.88 8.03*	77.02 8.04*	--
Saudi HbAF[8]	243	15.1 1.65	4.5 0.52	47.4 5.32	106.0 8.0	33.6 2.36	31.8 1.7	17.9 1.7	27.2 7.1	72.8 7.7	
Omani HbAF Barts	3,505	14.79 2.9*	5.09 0.77*	49.7 7.4*	97.29 13.8*	29.74 11.8*	30.39 3.6*	18.09 2.56*	25.74 9.05*	73.96 8.91*	--
Saudi HbAF Barts[8]	136	14.3 1.98	5.03 0.68	46.4 5.9	92.5 9.9	28.6 2.9	30.7 2.2	19.2 2.0	28.1 8.2	67.8 8.3	--
Omani HbAFS	188	15.09 1.56	4.91 0.62	48.59 5.5	103.34 9.6*	32.92 2.9*	30.5 2.96*	16.65 1.5	13.13 7.8*	76.7 11.4*	8.18 2.9*
Saudi HbAFS[8]	57	14.97 1.47	4.66 0.5	47.1 5.2	101.2 6.2	32.3 2.1	31.9 1.7	17.7 1.4	18.5 3.8	73.6 5.4	7.9 2.57
Omani HbAFS Barts	218	15.02 2.0	4.73 0.66	47.0 7.6	96.51 8.04*	30.12 4.2*	30.26 3.51*	17.33 2.1	14.28 5.2 *	78.73 8.3*	6.97 3.4*
Saudi HbAFS Barts[8]	50	13.88 1.37	5.03 0.57	45.2 5.3	89.3 8.24	27.7 2.2	31.1 1.94	19.6 4.2	18.7 5.56	70.1 5.8	6.8 2.02
Omani HbFS	9	16.45 0.21*	5.06 0.37	48.5 8.09*	104.52 5.9*	32.55 2.8*	30.81 3.26	14.95 0.49*	--	89.55 3.78*	12.9 4.7*
Saudi HbFS[8]	3	14.13 0.1	4.26 0.1	44.8 3.6	105.2 7.8	33.2 0.57	31.7 2.7	17.5 0.5	--	89.2 3.8	10.8 3.8
Omani HbFS Barts	14	12.89 2.4*	4.40 1.04	45.57 5.5*	97.5 4.4*	30.63 6.8*	30.85 3.24	17.16 0.97*	--	86.85 5.2*	10.8 5.2*
Saudi HbFS Barts[8]	4	12.9 1.6	4.7 0.4	41.1 9.1	87.2 14.8	27.5 1.9	31.8 2.76	20.03 2.31	--	83.7 5.6	11.87 5.9
Omani HbAFD	45	14.86 1.3	4.4 0.39*	43.3 3.8	104.7 7.6*	33.84 3.5	30.77 2.66	16.75 2.01	13.91 7.41	78.76 8.3*	8.66 3.6
Omani HbAFD Barts	28	15.0 1.5	4.76 0.64*	49.47 7.5	101.4 10.23*	31.8 3.4	30.22 2.9	18.24 3.57	12.7 4.8	87.24 4.8*	8.42 3.8
Omani HbAFE	29	16.63 1.8*	5.31 0.91	57.6 5.07	104.77 7.88*	31.67 3.4	30.89 3.05	17.95 2.26	20.3 9.91*	75.36 6.5*	6.78 3.21
Omani HbAFE Barts	30	15.17 1.57*	4.9 0.99	50.9 11.0	99.6 6.8*	30.57 8.2	30.15 3.55	18.85 6.93	13.29 5.32*	86.7 5.32*	6.54 2.84
Omani HbAFC	3	15.7 1.19	4.24 0.78	42.12 6.78	101.5 7.8*	32.55 4.5	30.99 2.34	15.35 1.67	13.5 3.4	77.9 5.33*	7.8 2.67
Omani HbAFC Barts	3	15.05 1.62	4.62 0.8	44.9 9.32	99.75 5.6*	32.2 5.6	30.2 3.89	16.65 0.56	11.31 4.6	85.13 6.45*	6.21 3.24

*Legend: * P <0.05 between red cell indices, with and without α-thalassemia (Hb Barts) in Omani neonates; RBC = red blood cell; HCT = haematocrit; MCV = mean cell volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; HbAF = Hb A+F; HbAFS = Hb A+F+S; HbFS = Hb F+S; HbAFD = Hb A+F+D; HbAFE = Hb A+F+E; HbAFC = Hb A+F+C.*

Methods

This study was conducted under the auspices of His Majesty's Strategic Research Project initiated and funded between the years 2005 to 2008. The study was approved by the Institutional review board and conformed to the Declaration of Helsinki. The study enrolled a total of 3,740 subjects from SQUH in Muscat and 4,097 neonates from Sohar Hospital, Oman. All data was archived in a Microsoft Excel database. Statistical analysis was performed using the student's t test with a value <0.05 considered significant.

Between April 2005 and March 2007 and after informed consent was obtained, a total of 7,837 consecutive cord blood samples were

Table 2: Incidence of abnormal haemoglobinopathies observed in this study (n = 7,837)

Haemoglobins	Number (%)	High performance liquid chromatography (HPLC)		Direct Sequencing		Concordance
		Numbers	%	Numbers	%	%
Normal (A+F)	7,837 (100)	4042	51.57			
α-Thalassemia(A+F+Barts)		3795	48.42			
HbS (F+S+A)	773 (9.86)	429	5.47	428	5.46	99.8
HbD (F+D+A)		73	0.93	73	0.93	100
HbE (F+E+A)		59	0.75	59	0.75	100
HbC (F+C+A)		6	0.08	6	0.08	100
Low HbA,<10%		206	2.62	202	2.57	98.1

screened prospectively, for the presence of possible haemoglobinopathies by a full blood count followed by HPLC using the Biorad Variant II β-thalassaemia short programme.

The CBC was performed using EDTA cord blood samples on Cell Dyn 4000™ automated blood cell counter (Abbot Diagnostics, Abbot Laboratories, IL, USA) within 4–12 hours of collection. HPLC was performed within 12–24 hours of collection using the β-thalassaemia short programme on the Bio-Rad VARIANT II™ instrument (Bio-Rad Laboratories, Hercules, CA, USA) using the manufacturer's instructions and controls. The samples were refrigerated from the time of collection up to the time of analysis. All samples were then processed to isolate and store mononuclear leukocytes for subsequent confirmatory molecular diagnostics.

Direct sequencing of abnormal samples with HbS, HbD, HbE and HbC was performed on the ABI Prism™ 3100 genetic analyser (Applied Biosystems, Foster City, CA, USA) to assign the genotype status to these subjects and validate the HPLC results. The DNA sequencing was performed by polymerase chain reaction (PCR)-amplified β-globin gene segment to look for the following mutations, namely HbS ($\beta^{6\ Glu\text{-}Val}$), HbD ($\beta^{121\ Glu\text{-}Gln}$), HbE ($\beta^{26\ Glu\text{-}Lys}$) and HbC ($\beta^{6Glu\text{-}Lys}$) as per the manufacturer's instructions and PCR conditions. Additionally, in samples with HbA below 10%, the β-globin gene was directly sequenced including the promoter, all exons and introns in these samples to look for all the known mutations reported for β-thalassaemia.

Results

On the basis of a CBC and HPLC, all samples were characterised as either normal (HbA+HbF), 4,042 cases; or α–thalassaemia, (HbA+HbF+Hb Barts) 3,795 cases. In the former group, 200 cases also had HbS, 45 cases had HbD, 29 cases had HbE, and 3 cases had HbC. Whereas in the latter group, 229 cases additionally also had HbS, 28 cases had HbD, 30 cases had HbE, and 3 cases had HbC.

Table 1 shows the comparative analysis of cord blood red cell indices (mean ± standard deviation [SD]) from newborn Omani neonates compared with Saudi neonates.[8] In a subset of the Omani neonates without any abnormal haemoglobin (HbA+HbF) (n = 3,765), the mean (±SD) Hb(g/dl), red blood cell count (RBC) count (x 10^{12}/L), haematocrit (Hct) (%), mean cell volume (MCV) (fl), mean corpuscular haemoglobin (MCH) (pg), mean corpuscular haemoglobin concentration (MCHC) (g/dl), red cell distribution width (RDW) (%), were 15.38±2.04; 4.69±0.68; 50.5±7.18; 107.66±7.75; 33.31±4.07, 30.98±3.44, and 17.01±2.17 respectively. Whereas in the subset of subjects with HbA, HbF, Hb Barts (n = 3,505), the study observed a 48.42% incidence of α-thalassaemia, based on low MCV and MCH on the CBC and significant amounts of Hb Barts on HPLC based on the manufacturer's cut-off limit. Their mean (±SD) Hb(g/dl), RBC count (x 10^{12}/L), Hct (%), MCV (fl), MCH (pg), MCHC (g/dl), RDW (%), were 14.79±2.90; 5.09±0.77; 49.7±7.40; 97.29±13.8; 29.74±11.80; 30.39±3.6, and 18.09±2.56 respectively. There was a statistically significant reduction in the MCV, MCH, MCHC, HCT, Hb and an increase in the RBC count. Furthermore, MCV was the best discriminator between the two

Table 3: Reference range for various haemoglobins (%) in cord blood from newborn Omani subjects (n = 7,837)

	HbA % (n = 7837)	HbF % (n = 7837)	HbS % (n = 429)	HbD % (n = 73)	HbE % (n = 59)	HbC % (n = 6)
Mean	22.88	77.02	13.6	8.88	6.64	6.21
Standard Deviation	8.04	8.05	6.96	4.87	2.98	3.41
Median	22.0	78.0	12.6	9.15	5.8	5.7
Range	4–50	25.1–96	2.1–21.9	3.7–19.1	2.2–14.6	2.7–11.6
95% confidence interval	22.62–23.13	76.75–77.29	12.7–14.5	6.94–9.83	5.47–8.05	2.64–9.79

groups ($P = 7.0^{e-118}$).

Since HPLC cannot diagnose the presence of the β-thalassaemia gene at birth, in samples with HbA below 10%, the β-globin gene was directly sequenced including the promoter, all exons and introns in the abnormal samples (as per the manufacturer's technical report) [Table 2]. In 105 of the 206 cases, an underlying known and previously reported mutation described in β-thalassaemia cases could be documented in this cohort of newborn cases.[6] On complete analysis of the remaining cases, amongst the 206 cases we could demonstrate a known β-thalassaemia mutation in a total of 202 cases (98.1%). Additionally, in cases with an abnormal haemoglobin on HPLC, direct sequencing of abnormal samples with HbS (n = 429), HbD (n = 73), HbE (n = 59), and HbC (n = 6) was also performed on an ABI Prism 3100 genetic analyser to assign the genotype status to these subjects and validate the HPLC results [Table 2 and Figure 1]. In 428 of the 429 cases with HPLC showing HbS, the $\beta^{6Glu-->Val}$ mutation could be documented by direct sequencing of the relative region of the β-gene, (99.76%) whereas, in the single sample with HbS in cord blood, but no $\beta^{6Glu-->Val}$ mutation, we found an abnormality in codon 16 of the delta chain. Furthermore, direct sequencing results in all cases of HbD, HbE and HbC showed complete concordance (100%) [Table 2].

Population reference range for various haemoglobins (%) in cord blood from newborn Omani subjects (n = 7,837) are shown in Table 3. The mean HbA, HbF, HbS, HbD, HbE, HbC (±SD) were 22.88±8.04; 77.02±8.04; 13.6±6.96; 8.88±4.87; 6.64±2.98, and 6.21±3.41 respectively. The overall incidence of other haemoglobinopathies was 9.86% (n = 773), with 5.46% (n = 428) incidence of sickle haemoglobin.

Discussion

Oman is a country with a population comprising a wide range of ethnic groups, and high rates of consanguinous marriages.[12] This is a significant reason for the increased prevalence of haemoglobinopathies, which is of growing importance as knowledge of the population structure can be a unique aid in planning genetic services.

HPLC is a powerful tool to screen newborns for haemoglobinopathies. Cord blood sampling offers an easy, simple and practical method for demonstrating the coexistence of α-thalassaemia by detecting the presence of Hb Barts. We were able to identify these subjects very well with the Biorad Variant II© system, using the β-thalassaemia short programme which works on the principle of cation exchange high pressure liquid chromatography. Since each haemoglobin has a characteristic retention time, Hb Barts being a fast moving haemoglobin, is easily separated and eluted from the other haemoglobins present in the neonatal cord blood samples. The precision is further improved by running a chromatographic calibrator with an assigned value for Hb Barts at the beginning of each run. Thus, using the presence of Hb Barts as an indicator of α-thalassaemia, we found that 51.57% subjects had normal HPLC (absence of Hb Barts).

Before the availability of molecular diagnostic methods for the diagnosis of α-thalassaemia, there was good evidence that the presence of Hb Barts in the neonatal period indicated the presence of α-thalassaemia.[13,14] However, the relationship between the amounts of Hb Barts and the underlying molecular defect was not clear. Most surveys using assays that detect 0.5% to 1% Hb Barts in cord blood detect a large proportion of neonates with α-thalassaemia, but not all; hence, surveys solely based on the presence of Hb Barts in cord

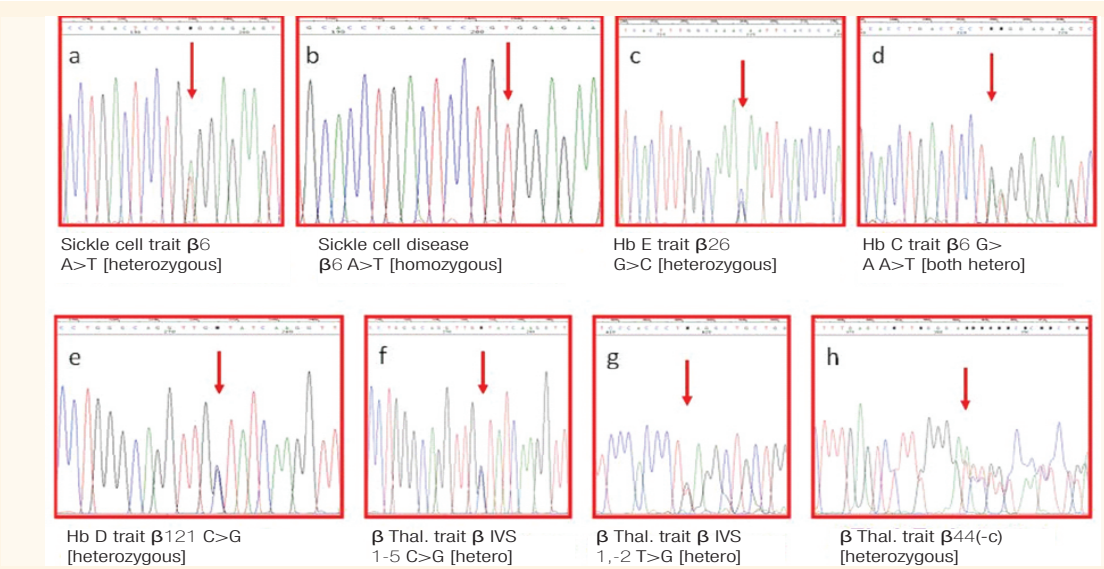

Figure 1: Capillary electropherogram results of DNA sequencing by PCR-amplified β-globin gene segment to document HbS ($\beta^{6\ Glu\text{-}Val}$) (a,b), HbD ($\beta^{121\ Glu\text{-}Gln}$) (e), HbE ($\beta^{26\ Glu\text{-}Lys}$) (c), HbC ($\beta^{6\ Glu\text{-}Lys}$) (d) and the common β-thalassemia mutations (f,g,h).

blood consistently under-reported the frequency of α-thalassaemia.[15] Moreover, although the levels of Hb Barts are related to the degree of α-chain deficit, there is no way to distinguish the various α-thalassaemia syndromes solely on the basis of HPLC.[16]

A comparative analysis of the red cell indices in these two groups revealed that α-thalassaemia resulted in a lower mean Hb, Hct, MCV, MCH, MCHC and HbF whereas it resulted in a higher mean red cell count and HbA concentration [Table 1]. Furthermore, all these differences were statistically strongly significant. The best discriminator was found to be MCV followed by red cell count, MCH and Hb concentration. Similar observations have been also made by other investigators; however, the degree of abnormality varies amongst these parameters.[7,8] The greatest difference reported has been seen in MCH as individuals with α-thalassaemia clearly make less haemoglobin per cell than their normal counterparts. However, subjects with α-thalassaemia trait maintain adequate haemoglobin levels, the main compensatory mechanism being via the increased red cell numbers.

The presence of α-thalassaemia was found to reduce the amount of HbA both in HbS heterozygous as well as homozygous HbSS subjects [Table 1]. Thus the presence of α-thalassaemia not only resulted in anatomically smaller RBCs, but also a cell which carried less HbA making it rheologically more adapted to flow through the small capillaries, thereby increasing the disease severity.[7,8,17]

In subjects with HbD, the presence of α-thalassaemia also resulted in lower HbA, HbD, MCV, and MCH; whereas the Hb, red cell count, Hct and HbF were higher. The differences in RBC count, MCV and HbF were statistically significant [Table 1].

In subjects with HbE the presence of α-thalassaemia resulted in lower HbA, HbE, MCV, MCH, Hb, RBC counts and Hct, whereas only the HbF was higher [Table 1]. The differences in Hb, MCV and HbF were statistically significant.

Overall, α-thalassaemia appears to influence all the red cell indices when it is the only abnormality. In the presence of abnormal haemoglobin, its influence was marginal unless the abnormal haemoglobin was present in a homozygous state, as in subjects who showed homozygous HbS. Thus with the presence of α-thalassaemia leading to a reduction in α-chains, the additional presence of a β structural variant will lead to a variable situation the outcome of which depends on the net globin chain synthesis rate. Some β-globin variants like HbE are synthesised less efficiently than HbA and represent less than 50% of the haemoglobin in the heterozygote. Furthermore, the rate of assembly of the αβ-chain complexes also would affect the final product. Therefore, the formation of the αβ-dimer is the rate limiting step in the assembly of haemoglobin.

Direct sequencing of samples with abnormal haemoglobin was used to validate the interpretation of the CBC and HPLC results and was found to be quite accurate [Figure 1]. All cases with HbD, HbE, HbC were documented to show HbD (β^{121} Glu-Gln), HbE (β^{26} Glu-Lys) and HbC (β^{6}Glu-Lys) mutation respectively. All cases with HbS were shown to carry the HbS (β^{6} Glu-Val) except in one case which had the delta chain codon 16 mutation, and is known to show a small abnormal haemoglobin band in the HbS window on HPLC in the β-thalassaemia short programme HPLC runs. Furthermore, in cases with low HbA (below 10%), 98.1% of subjects were documented to have one of the known mutations described as causative of β-thalassaemia, consistent with the recommendations of the manufacturer (Bio-Rad Laboratories, Hercules, CA, USA).

Thus, the significantly high prevalence of haemoglobinopathies in newborns from Oman emphasises the value of neonatal cord blood screening. This should be implemented as the first step in the national strategy towards total management of haemoglobinopathies—including early diagnosis, comprehensive clinical care and counselling of the affected families. In the light of the results of the current study, this initiative is being taken forward to encompass all the regions of Oman. The results of this large study would indicate that using HPLC is a cost effective method (<2 US $ per sample).[18]

Conclusion

In this study, for the first time, we were able to establish and validate the neonatal cord blood red cell indices and the prevalence of underlying abnormal haemoglobin by a universal newborn cord blood screening in the Omani population. The study observed that approximately 10% of the population still carries an abnormal haemoglobinopathy with significant clinical consequences, like Hb S, Hb D, Hb C and the β-thalassaemia gene. Furthermore, we have also established the prevalence of α-thalassaemia in this cohort and its potential to alter the red cell indices as well as its ameliorating effect when co-associated with the presence of other haemoglobin variants, which are highly prevalent in Oman. The comparative analysis of cord blood red cell indices and mean haemoglobins showed similar results to those seen with neonates from other countries in the region.

Therefore, as a direct result of this study, it is suggested that "targeted screening" with prescreening of both parents, and then selecting only the samples of neonatal cord blood from newborns with one parent having an underlying genetic trait for haemoglobinopathy, would result in a huge cost saving compared to the universal neonatal cord blood screening undertaken in this study.

CONFLICT OF INTEREST

The authors reported no conflict of interest.

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