Neonatal Screening for Hemoglobinopathies Using High-Performance Liquid Chromatography

Soad K. Al-Jaouni, MD, FRCPC; **Mohamad H. Gari,** MD, FRCPA; **Ghazi Damanhouri,** MD, FRCPA and **Mohamed F. Fadlallah,** MD, FRCPC
Department of Hematology and Pediatrics,
King Abdulaziz University Hospital, Jeddah, Saudi Arabia

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Introduction

Methods of screening for hemoglobin variants in neonatal samples are similar to those used for adults, but are subject to several constraints. Neonatal blood consists of ≐75% fetal hemoglobin (HbF). Any method used must therefore be sufficiently sensitive to detect low levels of normal and abnormal hemoglobins, as well as the absence of very small quantities of normal adult hemoglobin (HbA).

The primary aim of neonatal screening is therefore to identify infants with major significant hemoglobinopathies disorders, which include sickle cell anemia (HbFS), HbFSC disease, HbFSD, HbSO Arab, HbS/beta thalassemia and beta thalassemia major. These disorders identify by the absence of HbA and the presence of abnormal variant Hb [1, 3, 12, 21]. A secondary outcome is that they will also detect individuals with a variety of moderate and minor hemoglobin disorders, such as alpha thalassemia (α-thalassemia), heterozygotes for a Hb variant, or carriers of a clinically benign variant e.g. sickle cell trait (HbFAS).

Another outcome is the identification of the baby's own genetic risk, which at this stage can only be communicated to the parents and included in the primary healthcare record.

The conventional approach to qualitative and quantitative analyses of hemoglobin (Hb) molecules for diagnosis of hemoglobinopathies requires a

combination of tests. The preferred laboratory method in most hospitals using 'twin-tier' electrophoresis using citrate agar and cellulose acetate, with the second most common method being isoelectric focusing. The lack of a quantitative aspect to the isoelectric focusing methods and other technical limitations relating to lack of sensitivity or specificity for the twin-tiered method [1-4]. High-Performance Liquid Chromatography (HPLC) coupled with the development of an automated and rapid method [5], HPLC achieves excellent sensitivity and specificity, while adding the very important quantitative element to the analysis, [6-15], also has proven to be clinically accurate [16, 17].

Reviewing the literature it has become apparent that HPLC is the preferred method for screening that lead us to use an automated HPLC (VARIANT) system from BIO-RAD, to study alph-thalassemia and other hemoglobinopathies in our Newborn Screen In Jeddah.

Materials and Methods

The neonatal hemoglobinopathies screening program was designed to screen all Saudi babies born at King Abdulaziz University Hospital (KAUH) & King Fahad Arm Forces Hospital (KFAFH). Cord blood samples were collected in EDTA anticoagulated tubes from all the babies delivered in the two hospitals. All blood samples were labeled and transported to King Fahd Research Center at the hematology laboratory were all tests were performed. Samples were analyzed using HPLC instruments provided by Bio-Rad Laboratories and an analytical protocol developed by manufacture instruction and by the Genetic Disease Laboratory [6]. An autosampler and positional controls minimize sampling error. The test uses a Bio-Rad nonporous cation exchange resin, which allows rapid chromatographic separation

of the hemoglobin (Hb) variants, and is capable of running 500 specimens per cartridge with a run time of 2.5 min [7]. HbF, A,S,C,D, and E are detected as are unidentified variants (V) which occur at retention time between those of designated structural variants [19, 20]. Also, Hb Bart's is detectable by this method. All specimens were screened by α-thalassemia variant program. To detect Hb Barts, in spite of this program requiring a slightly longer run time, but provides a reliable quantitative Hb Bart. Cord blood of normal infants has less than 1% Hb Barts, but that of infants with α-thalassemia 2 has levels of 1-3% Hb Bart's. the blood of infants with α-thalassemia 1 has levels of >3-10% Hb Bart's [17, 18].

The α-thalassemia program was therefore developed and used to quantify Hb Bart's to detect alpha thalassemia genotypes in cord blood [17, 18, 20]. Table 1. represents the reference range for interpretation of α-thalassemia result.

Table 1: Variant α-thalassemia short program.

Patient State	Newborn Hb Barts Level	> 1 year of age Hb Bart's level
Normal	Newborn < 1%	none
α-thalassemia 2	Newborn 1-3%	none
α-thalassemia 1	Newborn > 3-10%	none
Hb H Disease	Newborn 15-30%	1-5%
Hydrops Fetalis	Newborn 80-100%	none applicable

Although β-thalassemia is indistinguishable from normal screen done at early infancy, one of the limitation of most screening methods including HPLC for sickle cell disease is that there is often confusion between sickle cell anemia and sickle cell β-thalassemia. A similar screening limitation is also true of other compound conditions such as E/ β-thalassemia {8}. HPLC is a good program to be used for

neonatal screening which determines the need to consistently demonstrate the presence or absence of small quantities of HbA, which can be detected at 0.5%. The common variants, HbS, HbC, HbE and HbD were also detected consistently at the level of 1% [21].

Results and Interpretation

A total of 834 newborns were screened between May 2001 to January 2002. 441 (52.88) neonate showed a normal screen with a HbFA. While 393 (47.12%) showed abnormal result, the majority of them were α-thalassemia with percentage 39.57%. Table 2. illustrate the result. Normal human Hb has four α-globin genes. Mild α-thalassemia mutations due to deletion or inactivation of one α-globin gene e.g., α-thalassemia 2 (α α /-α) which are extremely common in our study with a percentage of 35.86%, other mild form of α-thalassemia where two of α-globin gene deletion e.g., α-thalassemia 1 (αα/--). Luckily this form is less common among our population which presented with a percentage of 3.6%. this form of inheritance needs follow-up by genetic counseling to prevent a birth of a sever form of Hydros Fetalis where there is deletion of four genes which are incompatible with life and cause still births. HbH disease is a moderate form of the disease where there is deletion of 3 α globin gene (--/-α) which is a quite common seen inheritance. For both parents having the mild form of α-thalassaemia, there is a 25% chance of bearing a child with HbH disease in each pregnancy.

Sickle cell anemia is a quite common problem, which can cause a burden on health if there is one affected child in a 1000 newborn births.

Table 2: the Distribution of Hemoglobinopathies in Saudi Neonates by HPLC

Newborn Pattern	Screening result		Male	Female
Total number of Neonate screened	834	100%	422	412
Normal Screen (Hb FA)	441	52.88%	289	245
Abnormal Result	393	47.12%	175	218
Total α-thalassemia (Bart's)	330	39.57%	143	187
α-thalassemia 2 (α α/ - α)	299	35.86%	133	166
α-thalassemia 1 (- -/α α)	30	3.60%	10	20
Hb H Disease (- -/ - α)	1	0.12%	0	1
Sickle cell Trait (Hb FAS)	37	4.44%	18	19
Sickle cell anemia (HbFS)	1	0.12%	1	0
Hb E Trait (Hb F A E)	16	1.92%	7	9
Hb C Trait (Hb F A C)	1	0.12%	1	0
B-thalassemia Trait (Hb F A A_2)	8	0.96%	5	3

Discussion

The programs for diagnosis of hemoglobin disorder using electrophoresis at birth by cord blood have been available for the past 15 years in the Kingdom [22, 23]; also recent studies recommend that neonatal screening programs are essential and should be maintained as a routine practice [24]. Previous studies in our region

showed that hemoglobinopathies is one of the major health problems and most common genetic disorder among people originally in this area [28, 29, 30, 31].

We tested the possibility of deploying a modern high performance liquid chromatography is a successful approach from efficiency and cost effectiveness point of view in detecting neonates who are carriers or patients of genetic hemoglobin disorders and in line with other studies it confirms the occurrence of various genetic hemoglobin disorders at varying rate. In developing communities with a cultural preference for consanguinity gene variants are spread among families and tribes. Hence an affected child is a marker for a group at a high genetic risk and such studies are warranted to detect carriers are high risk prior to clinical presentation and reproduction [25, 26].

Our results indicates that the use of high performance liquid chromatography is a successful approach from efficiency and cost effectiveness point of view in detecting neonates who are carriers or patients of genetic hemoglobin disorders And. in line with other studies it confirms the occurrence of various genetic hemoglobin disorders at varying rate.

At this region of Saudi Arabia the percentage of children among the study group with alpha thalassemia 2 is significantly high (35,86%), followed by sickle cell trait (4,44%). Altogether the neonates who were found to have genetic hemoglobin disorders are (47,13%) of the study group, this is fairly alarming value that necessitates the establishment of a nation wide prevention program high speed, efficient and cost effective approach to pick up cases at an early stage of life, hopefully this should enable health planners to put forward the appropriate plans for

the community health services and adjust it to the real figures of the patients and carriers. This approach too shall provide the adequate level of standardization and in the future replace the premarital testing proposed nowadays to prevent the appearance of new cases due to marriage of undetected carriers, this approach of premarital testing may remain effective till the more comprehensive newborn screening program is established for years.

In our opinion this study and similar studies are of more than local relevance, since the prevalence rates genetic hemoglobin disorders in the Middle Eastern countries is about 30% and in Pakistan it is 40%. [26, 27].

The screening program Should in the near future be supplemented by screening modalities like the extended family studies that produces a high yield of information on carriers and couples at risk particularly in countries where the consanguineous marriage is common [25], and the more specific molecular and genetic testing to identify the causative mutations if known, or describe it if still unknown, as well as the education of the public on the importance of carrier detection in the prevention of these life threatening disorders rather than enforcing the premarital testing.

In summary the outcome of this study illustrates the distribution of the various hemoglobinopathies and indicates that the Saudi population in Jeddah are at risk of developing these disorders. Neonatal screening programs are essential as well as cost effective and should be implemented in the prevention program as a routine practice. HPLC provides a comprehensive and sensitive screening tool eliminating the need for a second screening test.

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استخدام جهاز قياس انسيابية السوائل عالي الأداء (HPLC) في اكتشاف حالات اعتلالات خضاب الدم الوراثي لدى المواليد

سعاد الجاعوني ، محمد قاري ، غازي دمنهوري و محمد فضل الله
كلية الطب - جامعة الملك عبد العزيز - جدة ، المملكة العربية السعودية

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المستخلص : يقدم برنامج فحص المواليد معلومات ديموغرافية هامة تساهم في تأسيس برنامج شامل للوقاية من اعتلالات خضاب الدم الوراثية، وتقليل المراضة والوفيات الناجمة عن هذه الاعتلالات. ونظرا لما يقدمه جهاز قياس انسيابية السوائل عالي الأداء (HPLC) من امكانيات مثل القياس الكمي والدقة والحساسية المتناهية فانه يعتبر من أفضل وسائل القياس المسح الشامل لاعتلالات خضاب الدم الوراثي.

تم دراسة ما مجموعه ٨٣٤ مولودا من السعوديين، منهم ٤٢٢ ذكورا و ٤١١ اناث وذلك في الفترة مابين شهري مايو ٢٠٠١ و يناير ٢٠٠٢ بكل من مستشفى جامعة الملك عبد العزيز ومستشفى الملك فهد العسكري بجدة. وبعد جمع عينات الحبل السري من المواليد تم ارسالها الى مركز الملك فهد للبحوث الطبية لاجراء اختبار انسيابية السوائل عالي الأداء (HPLC).

أظهر الفحص أن ٤٤١ (٥٢،٨٨%) من العينات تعود لأصحاء، و ٣٩٣ (٤٧،١٢%) تعود لحالات مختلفة من اعتلالات خضاب الدم الوراثي مقسمة كما يلي: ٣٣٠ حالة (٣٩،٥٧%) ألفا ثالاسيميا، منها ٢٩٩ حالة (٣٥،٨٦%) من النوع ألف ثالاسيميا II، و٣٠ حالة (٣،٦%) ألفا ثالاسيميا I. وحالة واحدة من مرض خضاب الدم H (٠،١٢%)، أما حالات الحاملين للصفة الوراثية للأنيميا المنجلية فقد شكلت ٣٧ حالة (٤،٤٤%) وحالة واحدة لمريض أنيميا منجلية (٠،١٢%). وهناك ١٦ حالة من حاملي الصفة الوراثية لخضاب الدم E (١،٩٢%).

يتبين من نتائج هذه الدراسة التوزيع الكمي لاعتلالات خضاب الدم الوراثي، وأن السكان في المنطقة التي يشملها المسح معرضون للاصابة بهذه الاعتلالات. ان المسح الخاص بالمواليد يعد ضروريا من الناحية الصحية والاقتصادية لذا يجب ادراجه كفحص روتيني ضمن برنامج وقائي شامل للكشف عن الأمراض الوراثية. كما أن افحص انسيابية السوائل عالي الأداء يتميز بحساسية مرتفعة وبقدرته على العمل منفردا كأداة مسح شامل دون الحاجة لاختبارات اضافية أخرى.

كلمات دالة : فحص المواليد، اعتلالات خضاب الدم الوراثي، فحص انسيابية السوائل عالى الأداء.