Prevalence and Assessment of the Appropriate Laboratory Indices for Screening of Hemoglobinopathyies in Southern Iranian Newborns

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Abstract

Introduction:
Newborn screening is a systematic application of tests for early detection, diagnosis, and treatment of certain genetic or metabolic disorders that may lead to mortality and morbidity if untreated. As stated by WHO, each year over 330,000 babies are born worldwide with a severe form of hemoglobinopathy. Newborn screening for hemoglobinopathies could become one of the most important methods of decreasing mortality and morbidity and mitigating rising healthcare costs. The diversity and heterogeneous distribution of hemoglobin disorders make it necessary to develop strategies at the country level.

Materials and Methods:
Overall, 499 healthy full term Iranian neonates were screened for hemoglobinopathies who were originated from Fars province in southern part of Iran. The screening was performed on cord blood samples collected on EDTA. Following sample collection, complete blood cell count cell indices, osmotic fragility test and hemoglobin electrophoresis were performed.

Results:
Total prevalence of significant hematologic abnormal findings was 12.4% in this study, the most prevalent one was Alpha thalassemia (6.4%) followed by hereditary spherocytosis (4.8%), and sickle cell anemia (1.2%). The total analysis for detection of Alpha thalassemia by mean corpuscular volume (MCV) $\leq$ 94, mean corpuscular hemoglobin (MCH) $\leq$ 27, and hemoglobin level $\leq$ 14 indicated failure and these results were not appropriate to this discrete population.

Conclusion:
We suggested the new cut off points for neonatal screening programs of the Iranian population be MCV$\leq$96 and MCH $\leq$31 for Alpha thalassemia. A successful disease prevention strategy could lead to significant savings in spiraling healthcare costs and mitigate the scarcity of blood products. The healthcare budget savings realized from preventive screening justifies the spending on such a national thalassemia program. We recommended a neonatal screening program for southern Iranian population and also designed a new format for neonatal discharge summary.

Keywords:
Alpha thalassemia, Hemoglobinopathy, Nneonatal screening program, Hereditary spherocytosis, Sickle cell anemia.

Introduction

Newborn screening is a systematic application of tests for early detection and treatment of certain genetic or metabolic disorders. Early screening may causes reduction of mortality and morbidity in the longer term. The selection of screening methods has been based on several factors such as frequency of the disorder in the population, availability of a cost effective

screening test and the availability of treatment.

The prevalence of hemoglobinopathies vary between countries and now hemoglobin disorders are endemic in 71% of 229 countries and potentially affect 89% of births (1). The World Health Organization has indicated that each year over 330,000 babies are born worldwide with a different severe form of hemoglobinopathies (1). The high prevalence of hemoglobinopathies should result in screening for hemoglobinopathies that is mandated by state law (2).

Screening for sickle cell anemia, thalassemia and congenital spherocytic anemia (three common forms of hemoglobinopathies) would result in early detection of these abnormalities which would subsequently reduce the mortality and morbidity of these genetic disorders.

Early detection of sickle cell anemia, which is an autosomal recessive disorder, would lead to appropriate administration of prophylactic penicillin. Gaston and Verter presented children who received prophylactic penicillin and developed a significantly reduced rate of streptococcus pneumonia infections (2). Thalassemia, mutations or deletions of one or more of the globin genes, is another form of hemoglobinopathy. Two of the forms are more familiar, alpha and beta thalassemia, although there are several subtypes for these two groups. Early diagnosis of these abnormalities in combination with modern medical care can ensure long-term survival of patients (3). Congenital spherocytic anemia is a common disorder of the surface layer of red blood cells in which the affected cells overwhelm easily. The frequency is estimated about 1 in 2,000 to 5,000 persons and affected neonates are prone to severe hemolytic anemia crises, chronic hemolytic anemia, gallstone and marrow expansion. For this reason, early detection of this abnormality is critical in order to avoid further manifestations (4).

To sum up, newborn screening for hemoglobinopathies is an important method of decreasing mortality and morbidity. An accurate and early diagnosis must be made because each hemoglobinopathy requires specific management and carries a different prognosis. The pervasiveness, diversity and heterogeneous distribution of hemoglobin disorders make it necessary to develop strategies at the country level for early and preemptive care.

Materials and Methods

This study was conducted in Hafez hospital nurseries in Shiraz from April 2010 to December 2011 and the local ethical committee approved the protocol. Overall, 506 healthy Iranian full term neonates were screened for hemoglobinopathies. Newborns with prematurity, congenital or obvious abnormalities were excluded. The screening was performed on cord blood samples collected on EDTA tubes. Prior to blood collection, the umbilical cord was wiped with gauze to reduce maternal blood contamination. Following blood collection, initial hematologic analysis was done immediately at the Hafez hospital laboratory using an automated blood cell (Symex Le800) and then all samples were referred to referral laboratory for hemoglobin electrophoresis and an osmotic fragility test. Samples were analyzed by isoelectric focusing on cellulose acetate gels (pH=8.6) and agarose gels (pH=6) (5). The results were positive for alpha thalassemia by detection of Bart's hemoglobin and positive for sickle cell anemia by detection of hemoglobin S. The osmotic fragility test was performed according to the manufacturer's guidelines of Parpart Ah et al. (6 and more than 0.50 grams per liter was significant for the marking of positive results. Indexes of complete cell count such as hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were used as screening methods for early detection of hemoglobinopathies. Subsequently, all data were analyzed with SPSS version 17.

Results

Four hundred ninety-nine enrolled neonates fulfilled our inclusion criteria in order to be analyzed for hemoglobinopathies and others were excluded. A total of 61(12.4%) newborns screened positive for haemoglo- binopathies; 6.4% were female and 5.8% were male. The prevalence of alpha thalassemia, hereditary spherocytosis and sickle cell anemia was 6.4%, 4.8% and 1.2% respectively.

Table 1: The Prevalence of Hemoglobinopathies in the Population

Hemoglobinopathies Sex	Normal hemoglobin	Alpha thalassemia	Sickle cell anemia	Hereditary spherocytosis
Male	225 (45.2%)	14 (2.8%)	1 (0.2%)	14 (2.8%)
Female	212% (42.2%)	18 (3.6%)	5 (1.0%)	10 (2.0%)

According to a former study in Thailand, MCV ≤94 Femto Liter (Fl) was appropriate for detection of alpha thalassemia as a screening method with specificity and sensitivity about 70% and 80% (7,18). However, in our population, this level caused missing about 56% of neonates who were positive for alpha thalassemia with hemoglobin electrophoresis therefore we analyzed the higher level of MCV systematically. MCV≤96 FL were appropriate for our population with a detection of 82% in cases of alpha thalassemia (P<0.482) (Table 2).

Alternatively, MCH≤ 27 Pico gram (Pg) was presented as a screening method for alpha thalassemia however only 25% of neonates who were positive for these hemoglobinopathies in our population were diagnosed with this level. Different levels of MCH were evaluated for best sensitivity and specificity and had been summarized in table 3.
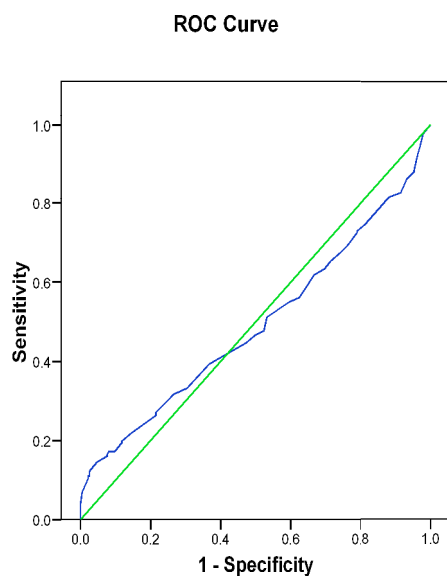
Table 2: The Sensitivity and Specificity of Different Levels of MCV for Detection of Alpha Thalassemia in Iranian full Term Neonates.

MCV levels		94 Fl	95 Fl	96 Fl	97 Fl	98 Fl
Sensitivity		57%	75%	78%	63%	81%
95% Confidence Interval	Lower Limit	0.393899	0.573695	0.606013	0.63918	0.63918
	Upper Limit	0.740476	0.882567	0.903687	0.923836	0.923836
Specificity		0.946352	93%	93%	87%	90%
95% Confidence Interval	Lower Limit	0.920754	0.903441	0.903441	0.876813	0.876813
	Upper Limit	96%	0.951842	0.951842	0.931702	0.931702
Positive predictive value		43%	43%	44%	38%	38%
Negative predictive value		96%	98%	98%	98%	98%

Table 3: The Sensitivity and Specificity of Different Levels of MCH (Pg) for Detection of Alpha Thalassemia in Iranian Full Term Neonates.

| MCH level		27 Pg	29 Pg	31 Pg
Sensitivity		24%	60%	78%
95% Confidence Interval	Lower Limit	0.117433	0.422384	0.595582
	Upper Limit	0.426305	0.76572	0.900559
Specificity		98%	93%	93%
95% Confidence Interval	Lower Limit	0.962326	0.913291	0.905495
	Upper Limit	0.990546	0.958992	0.953438
Positive predictive value		47%	41%	44%

Hemoglobin level and MCHC were presented as screening indexes for hereditary spherocytosis. Hb ≤ 14 was acceptable for other populations (7) however in our study about 56% of neonates who were positive for hereditary spherocytosis with the osmotic fragility test showed Hb level higher than 14. For that reason, we assessed higher levels of hemoglobin and at level of 16 about 91.3% of cases were diagnosed but with unacceptable sensitivity and specificity and all levels are presented in ROC curve in figure 1.

For MCHC, we compared former data with our results and MCHC ≥ 35 was acceptable level in the Thailand study, nonetheless in our population, different levels of MCHC were analyzed for detection of the best cut off point for this index. Data are presented as a ROC curve below.
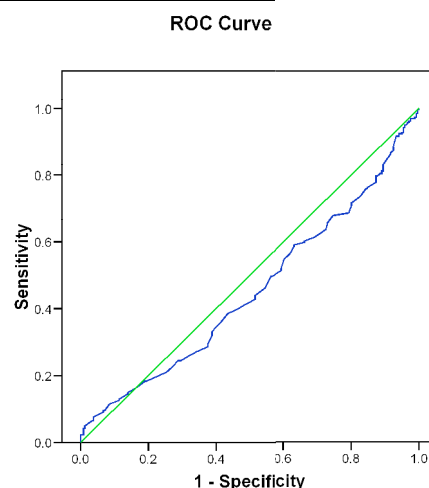
Area	Std. error(a)	P-value	95% Interval	Confidence
			Lower Bound	Upper Bound
0.454.	0.26.	0.074	0.403	0.504

Fig2: ROC Curve for Sensitivity and Specificity of MCHC Level in Detection of Hereditary Spherocytosis

Area	Std. error(a)	P-value	95% Interval	Confidence
			Lower Bound	Upper Bound
0.494	0.26	0.823	0.443	0.545

Fig1: ROC Curve for Sensitivity and Specificity of Hemoglobin Level in Detection of Hereditary Spherocytosis

Discussion

Total prevalence of hemoglobinopathies in our population as a sample of souther Iranianian population was 12.4% although the heterogeneous reports are available for the prevalence of hemoglobinopathy in

Iran. This prevalence was 15% in north area, 8-15% in south, 8% in Isfahan, and 28% in Booshehr (8). The prevalence of hemoglobinopathies is heterogeneous in other countries had been stated such as 1.4% in Saudi Arabia (9), 0.04% in United Arab Emirates (10), 2.1% in Bahrain (11), 0.4% in Oman (12). This diversity in the results is likely evidence of several factors such as genetic, environmental and migration patterns on the epidemiology of hemoglobinopathies. Consideration of regional epidemiology of these abnormalities is necessary when evaluating the necessity and cost effectiveness of screening neonates for hemoglobinopathies. Since the population in the Iran are at high risk for hemoglobinopathies, all infants should be tested in the neonatal period by means of a national screening programs (13,16). Selection of the best technique for screening is crucial in a location-specific neonatal screening program. Moreover, the effectiveness of any screening program depends on the test sensitivity, cost effectiveness and the availability of method chosen to test discrete populations. Different opinions exist about the adequacy of universal or selective screening of newborns for hemoglobinopathies (14,15,17,19). The costs of selective screening are much less than for universal screening but failure to detect all cases is a defect of this cost effective method so universal screening is recommended, particularly in a high prevalence regions. The techniques used for neonatal screening must have a high rate of sensitivity and specificity for the identification of newborns with a clinically significant hemoglobinopathies. Our target was to present the best indices for screening of these disorders with the usage of a simple complete cell count test.

As mentioned in our results, another study by Tritiposm in Thailand recommended MCV≤94 Fl and MCH≤ 27 Pg as acceptable levels for screening alpha thalassemia but in our population, these levels are not appropriate because after hemoglobin electrophoresis, we found that we missed a significant group of neonates who were positive for alpha thalassemia. With that in mind, we analyzed our data for detection of the best test levels for our population and all neonates who are positive for alpha thalassemia were assessed again with MCV ≤ 96 FL as acceptable sensitivity (78%) and specificity (93%) for the detection of alpha thalassemia. MCH is another index for screening of alpha thalassemia but in our study the threshold level of this index was not accurate for our population. Thus, we concluded that MCH ≤ 31 Pg is the best level for a screening program for the detection of alpha thalassemia instead of MCH ≤ 27.

For hereditary spherocytosis, a hemoglobin level ≤ 14 and MCHC ≥ 31 were used as screening levels but this method had not been found sensitive in our population. According to statistical analysis and the ROC curve presented in figure 1 and 2, it is not possible or appropriate to use acceptable levels of Hb and MCHC as a screening method for hereditary spherocytosis.

Conclusions and recommendations

The prevalence of spherocytosis and hemoglobinopathies in this newborn screening study, suggests that neonatal screening for hemoglobinopathies should be considered mandatory at the national level and integrated into neonatal screening program. A successful disease prevention strategy could lead to significant savings of spiraling healthcare costs and mitigate scarcity of blood products thus making early screening cost effective in the longer term. Another aspect of this study was to present the modified cut off points when screening for alpha thalassemia in our population.

References

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008 Jun 86(6):480-7.

2. Gaston MH, Verter JI, Woods G, Pegelow C, Kelleher J, Presbury G, et al. Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. N Engl J Med. 1986 Jun 19; 314(25):1593-9.

3. Vichinsky E, MacKlin E, Waye J, Lorey F. Changes in the epidemiology of thalassemia in North America: a new minority disease. Pediatrics.2005:Vol. 116 No. 6.

4. Buchanan G, Crary SH, Neunert C. Hereditary spherocytosis, a guide for the primary care physician .pediatric hematology-oncology department, children's medical center Dallas. ccbdinfo@childrens.com.

5. Dacie JV, Lewis SM. Investigation of haemoglobinopathies in Edinburgh, Churchill Livingstone. Practical Hematology. 1984:179-98.

6.Parpart Ak, Lorenz Pb, et al. The osmotic resistance (fragility) of human red cells. J Clin Invest. 1947 Jul; 26(4):636-40.

7. Tritipsombut J, Sanchaisuriya K, Fucharoen S, Fucharoen G, Siriratmanawong N, Pinmuang-ngam CH. Hemoglobin Profiles and Hematologic Features of thalassemic Newborns. Arch Pathol Lab Med. 2008; 132:1739–45.

8. Movahed A. Prevalence of hemoglobinopathies according to hemoglobin level and its relation with mean corpuscular volume In students residing in Booshehr 1386. The Persian Gulf biomedical research institute magazine,1388; 1: 54-9.

9. Hazmi MA. Haemoglobinopathies, thalas-saemias and enzymopathies in Saudi Arabia: the present status. Acta Haematol. 1987; 78 (2-3):130-4.

10. Al Hosani H, Salah M, Osman HM, Farag HM, Anvery SM. Incidence of haemoglo-binopathies detected through neonatal screening in the United Arab Emirates. La Revue de Santé de la Méditerranée orientale. 2005; Vol. 11, No 3.

11. Sheikha Salim Al Arrayed, Haites N. Features of sickle-cell disease in Bahrain. Eastern Mediterranean Health Journal. 1995. Volume 1, Issue 1; 112-19.

12. Pratibha Nair, Eiman Ibrahim, Tasneem Obeid, Abeer Farid, Ghazi Omar Tadmouri. Genetic disorders in Oman:A CTGA Perspective. Sultan Qaboos Univ Med J. 2010 Apr; 10(1):23-30.

13. Tongsong T, Wanapirak Ch, Sirivatanapa P, Sanguansermsri T, Sirichotiyakul S, Piyamongkol W. Prenatal control of severe thalassaemia: Chiang Mai Strategy. Prenat Diagn 2000; 20: 229-34.

14. Le Galès A, Galactéros F. Economic analysis of screening newborns for sickle cell in metropolitan France. Rev Epide miol Sante Publique 1994; 42:478–92.

15.Tsevat J, Wong J, Pauker S. Neonatal screening for sickle cell disease: a cost-effectiveness analysis. J Pediatr 1991; 118: 546–54.

16. Bouva MJ, Mohrmann K, Brinkman HB, Kemper-Proper EA, Elvers B, Loeber JG, et al.A national neonatal screening programme for hemoglobinopathies in the Netherlands. J Med Screen. 2010 Jun; 17(2): 58-65.

17. Chui DH, Fucharoen S, Chan V. Hemoglobin H disease: not necessarily a benign disorder. Blood, 2003,Volume 101, number 3.

18. Yin SH, Chong QT, Mei LA, Lin TP, Fraser A, Chuan S Et al. A Molecular Epidemiologic Study of Thalassemia Using Newborns' Cord Blood in a Multiracial Asian Population in Singapore. J Pediatr Hematol Oncol 2004; 26: 817–19.

19. Michlitsch J, Azimi M, Hoppe C, Walters MC, Lubin B. Newborn Screening for Hemoglobinopathies in California. Pediatr Blood Cancer 2009; 52: 486–90.