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NEONATAL SCREENING FOR ANAEMIA IN HEALTHY, FULL-TERM IRANIAN NEWBORNS: IS THERE ANY INDICATION

Mahdi Shahriari, Narjes Pishva*, Reza Bahrami, Alimohammad Alemansuri, Amir Naghshzan

Neonatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

***Author for Correspondence: Narjes Pishva**
Neonatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.

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ABSTRACT

Background: The high incidence of alpha thalassemia, G6PD deficiency and spherocytosis are three reasons that newborn screening should be routine in all delivery rooms in order to decrease morbidity. The object of this study was neonatal screening for anaemia in healthy, full-term Iranian new-borns. Materials and Methods: One thousand neonates were screened over a nearly three-year period for detection of the cause of anaemia. All neonates were Iranian and lived in Fars Province, southern Iran, and were healthy and full term. This study was conducted in Zeinabiieh and Hafez Hospitals affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. The screening was performed on cord blood samples collected on EDTA and the method was approved by the Ethical Committee. After sample collection, complete blood cell count, osmotic fragility test and haemoglobin electrophoresis were done for each sample. Results: Total prevalence of causes of anaemia in this neonatal screening programme was 12.2 %; the most prevalent one was alpha thalassemia (6.4 %), followed by hereditary spherocytosis (4.8 %) and sickle cell anaemia (1.2 %). The total analysis for detection of alpha thalassemia suggested that screening methods by mean corpuscular volume (MCV) ≤ 97 fl, mean corpuscular haemoglobin (MCH) ≤ 29, and haemoglobin level ≤ 16 g/dl plus MCHC > 35, were the appropriate cut-off points for our population. Conclusion: Our study does not endorse previously described cut-off points for the mean corpuscular volume, or the mean corpuscular haemoglobin as indexes for screening of alpha thalassemia, or haemoglobin as a screening index for hereditary spherocytosis.

KEYWORD: Haemoglobinopathy, neonatal screening programme, alpha thalassemia, hereditary spherocytosis, sickle cell anaemia.

INTRODUCTION

Newborn screening is the systematic application of tests for early detection and treatment of certain genetic or metabolic disorders. In time, effective screening causes a reduction of the mortality and morbidity of these disorders. Selection of screening methods based on several factors such as frequency of the disorder in the population, availability of an effective screening test that is cost effective and the availability of treatment.

The World Health Organization has reported that each year over 330,000 babies are born worldwide with different severe forms of haemoglobinopathy.[1] The prevalence of haemoglobinopathies is different between countries and now haemoglobin disorders are endemic in 71 % of 229 countries, potentially affecting 89 % of births, although this prevalence was 75 % of births previously.[2] These reports on high prevalence of haemoglobinopathies lead to the recommendation that screening for haemoglobinopathies be mandated by state law.[3]

Sickle cell anaemia, thalassemia and congenital spherocytosis are three common disorders that screening methods are focused on; the early detection of these abnormalities is sought in order to reduce the mortality and morbidity of these genetic disorders.

Early identification of affected individuals with sickle cell anaemia, an autosomal recessive disorder, leads to appropriate administration of folic acid, prophylactic penicillin and pneumococcal vaccination. Gaston and Verter showed that children who received prophylactic penicillin had a significantly reduced rate of infection with streptococcus pneumonia infections.[3]

Thalassemia, mutations or deletions of one or more of the globin genes, is another form of haemoglobinopathy, and has two forms that are more familiar: alpha and beta thalassemia, although only alpha thalassemia is diagnosable at birth. Early diagnosis of these abnormalities in combination with modern medical care ensure long-term survival of patients and less misdiagnosis of alpha thalassemia as iron deficiency due to the normalization of Hb electrophoresis after neonatal

period; beta thalassemia, by contrast, is diagnosed after six months of life.

Congenital spherocytosis is a common disorder of the red blood cell membrane and the affected cells can break open easily. Its estimated frequency is about 1 in 2,000 to 5,000 persons,[4] and affected neonates are prone to more severe haemolytic anaemia crises, chronic haemolytic anaemia, gallstone and marrow expansion,[5] and thus early detection of this abnormality is beneficial.

Newborn screening for haemoglobinopathies is an important method for decreasing mortality and morbidity and an accurate and early diagnosis must be made, because each haemoglobinopathy requires specific management and carries a different prognosis, although the diversity and heterogeneous distribution of haemoglobin disorders make it necessary to develop strategies at the country level, especially if its frequency is more than 6 % or if delay in diagnosis may cause severe morbidity.[6]

MATERIAL AND METHODS

This study was conducted in two hospital nurseries in Shiraz, Iran, between February 2009 and December 2011. One thousand and six neonates were screened for spherocytosis and haemoglobinopathies; all neonates are healthy, full-term Iranian newborns. Preterm newborns and newborns with other congenital or obvious abnormalities were excluded. The screening was performed on cord blood samples collected on EDTA tubes and before blood collection; the umbilical cord was wiped with gauze to reduce maternal blood contamination and the local Ethical Committee approved all processes. After blood collection, initial haematological analysis was done immediately at the hospital laboratory using an automated blood cell (Symex Le800) and then all samples were sent to a referral laboratory for haemoglobin electrophoresis and osmotic fragility test.

Samples were analysed by isoelectric, focusing on cellulose acetate gels (pH=6) and agarose gels (pH=8.6) (4). Results were recorded as positive for alpha thalassemia by detection of Bart's haemoglobin and positive for sickle cell anaemia by detection of haemoglobin S. The osmotic fragility test was done according to the manufacturer's instructions of Parpart et al.,[6] and more than 0.50 gram per litre was significant for marking out positive results.

Indexes of complete cell count such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were used as screening methods for early detection of spherocytosis and haemoglobinopathies. All data were analysed with SPSS program, version 17.

RESULTS

One thousand enrolled neonates were analysed for haemoglobinopathies and six were excluded. A total of 61 (12.2 %) newborns screened positive for haemoglobinopathies, of which 6.4 % were female and 5.8 % male. Prevalence of alpha thalassemia, hereditary spherocytosis and sickle cell anaemia was 6.4 %, 4.6 % and 1 %, respectively.

According to a previous study in Thailand, MCV $\leq$ 94 fl was appropriate for detection of alpha thalassemia as a screening method with specificity and sensitivity about 70 % and 80 %.[14] However, in our population this level missed about 56 % of neonates who were positive for alpha thalassemia with haemoglobin electrophoresis, so we analysed the higher level of MCV systematically. MCV $\leq$ 97 fl was appropriate for our population, with detection of 82 % of cases of alpha thalassemia (P-value < 0.482).

On the other hand, MCH $\leq$ 27 pg was presented as a screening method for alpha thalassemia, but only 25 % of neonates who are positive for this haemoglobinopathy in our population were diagnosed with this level. Different levels of MCH were evaluated for best sensitivity and within a 27, 28, 29 picogram, about 25 %, 34.4 % and 59.4% of cases were detected. After statistical analysis, we concluded that MCH $\leq$ 29 pg is the best level for a screening programme for detection of alpha thalassemia.

Haemoglobin level and MCHC were presented as screening indexes for hereditary spherocytosis. Hb $\leq$ 14 g/dl was acceptable for other populations,[14] but in our study about 56 % of neonates who are positive for hereditary spherocytosis on the osmotic fragility test showed an Hb level higher than 14 g/dl; so we assessed higher levels of haemoglobin, and at a level of 16 about 91.3 % of cases were diagnosed with acceptable sensitivity and specificity. For MCHC, we compared previous data with our results, and found that MCHC > 35 was an acceptable level in our population instead of MCHC > 31 in the Thailand study, detecting 95 % of hereditary spherocytosis anaemia.

DISCUSSION

Total prevalence of haemoglobinopathies in our population as a sample of a southern province of Iran was 12.2 %, although heterogeneous reports are available for prevalence of haemoglobinopathy in Iran. This prevalence was 15 % in the north area, 8-15 % in the south, 8 % in Isfahan and 28 % in Booshehr.[8] The prevalence of haemoglobinopathies is heterogeneous in other countries, such as 1.4 % in Saudi Arabia[9], 0.04 % in United Arab Emirates[10], 2.1 % in Bahrain[11] and 0.4 % in Oman.[13] This heterogeneity showed the influence of several factors such as genetics, environment and migration on the epidemiology of haemoglobinopathies. According to this fact, it is necessary to pay attention to the regional epidemiology of these abnormalities, then

make decisions about the necessity for and cost effectiveness of screening of neonates for haemoglobinopathies. Since the population in the Iran area is at high risk of haemoglobinopathies, all infants should have the opportunity to be tested in the neonatal period by means of a national screening programme.[10] Selection of the best method for screening is the most important point in application of a neonatal screening programme. The effectiveness of any screening programme depends on the test sensitivity, cost effectiveness and availability of the method. Different opinions exist about the adequacy of universal or selective screening of newborns for haemoglobinopathies.[9,14] The costs of the technical process of selective screening are much lower than for universal screening, but low sensitivity is a defect of this cost effective method, so universal screening is recommended in a high prevalence region.[15] The techniques used for neonatal screening must have a high rate of sensitivity and specificity for the identification of newborns with clinically significant haemoglobinopathies, and our goal in this study was the presentation of the best index cut-off points for screening of these disorders with the use of a simple complete cell count test.

In a study by Tritiposm et al. in Thailand, they recommended MCV ≤ 94 fl (which is 2 standard deviations below mean MCV) and MCH ≤ 27 pg (which is a lower limit of MCH for adult age) as acceptable levels of screening for alpha thalassemia[7], but in our population these levels are not appropriate because after haemoglobin electrophoresis we found that we missed 18 % of neonates who had alpha thalassemia. Analysis of our data for detection of the best level for our population and all neonates who were positive for alpha thalassemia were assessed again, and with MCV ≤ 97 fl about 82 % of cases of alpha thalassemia were detected. MCH is another index for the screening of alpha thalassemia, and in our study the threshold level of 27 pg is not adequate for our population, so we concluded that MCH ≤ 29 pg is the best level for a screening programme for the detection of alpha thalassemia.

For hereditary spherocytosis, haemoglobin level ≤ 14 and MCHC ≤ 35 were presented as screening levels[4,6], but this method is not sensitive for our population and we recommend Hb ≤ 16 and MCHC ≤ 35 for the Iranian population in order to optimize the sensitivity of the screening method.

CONCLUSIONS AND RECOMMENDATIONS

The results of the prevalence of anaemia and haemoglobinopathies in this three-year study of newborn screening suggest that Hb, MCV, MCH and MCHC should be indicated in discharge summary, and that universal neonatal screening for haemoglobinopathies should be considered at the national level in our country; it could be integrated into a neonatal screening programme. A successful disease prevention strategy could lead to significant savings in an environment of spiralling healthcare costs and scarcity of blood products, and screening has been shown to be cost effective.

Neonates who were positive for haemoglobinopathies were guided to visit a paediatric haematologist and neonatologist for further follow up in order to reduce late complications of these abnormalities.

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