Original Article

Iran J Ped Hematol Oncol. 2018, Vol 8. No 2, 105-110

Neonatal Screening for Sickle Cell Disease in Southwest Iran

Bijan Keikhaei MD[1,*], Parastoo Moradi-Choghakabodi DVM[2], Fakher Rahim PhD[2], Mohammad Pedram MD[2], Homayoun Yousefi MD[2], Khodamorad Zandian MD[2], Khojasteh Hosseini nejad MD[2], Ashram Samavat MD[3]

1. Research Center for Thalassemia and Hemoglobinopathy, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences ,Ahvaz, Iran.
2. Department of Microbiology, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. Genetics Office, Disease Management Centre, Ministry of Health and Medical Education, Tehran, Iran.
*Corresponding author: Dr. Bijan Keikhaei, MD, Professor of Research Center for Thalassemia and Hemoglobinopathy, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Email: keikhaeib@yahoo.com.

Received: 23 December 2017 **Accepted:** 17 February 2018

Abstract

Background: Early diagnosis and initiation of prophylactic penicillin and pneumococcal vaccine may reduce the risk of sickle cell complications that is a common hemoglobin disorder in Southwest Iran. This study aimed at determining the incidence of Sickle Cell Disease (SCD) and other Hemoglobinopathies in newborn being at risk based on ethnic origin.

Materials and Methods: In this descriptive epidemiologic study, between September 2013 and September 2015, 8363 newborn blood samples were tested in four maternity units from Ahvaz, Khoramshahr, Sosangerd and Dezful. Complete cell count and cellulose acetate electrophoresis at pH 8.4 were performed on each blood sample. Parent's clinical status was also checked for more information. Presence of an abnormal band in the EDTA treated samples were further confirmed by citrate agar gel electrophoresis and automated high performance liquid chromatography (HPLC). Results were analyzed statistically by the One-Way ANOVA analysis.

Results: Among 8363 screened samples, 118 (1.41 %) samples were heterozygous for Hb S, and four (0.047%) for Hb C; none of newborns were Hb SS homozygotes. The incidence of silent and alpha thalassemia minor based on RBC indices was nearly 10%.

Conclusion: Present findings indicated the high quality and considerable impact of conducted screening program starting in 2007 at significantly decreasing the prevalence of SCD among newborns born between 2013 and 2015. The results also showed that the neonatal screening for SCD was not weighed to add as a new program in national health network.

Keywords: Newborn screening, Sickle cell, Sickle cell trait

Introduction

Numerous experimental studies demonstrated that neonatal screening along with timely diagnostic testing, parental training, and comprehensive care, considerably reduce morbidity and mortality rate of sickle cell disease (SCD) in infancy and early childhood (1-4). SCD can cause many acute and chronic complications, including severe infection, periodic episodes of pain, anemia, and chronic organ damage. Moreover, prophylactic penicillin and pneumococcal vaccine considerably reduce the incidence of pneumococcal sepsis (5) provided a strong motivation for the widespread accomplishment of neonatal screening for SCD (6). So far, numerous screening programs for SCD and the other Hemoglobinopathies have been presented in different countries. A few programs used high performance liquid chromatography (HPLC) or cellulose acetate electrophoresis (CAE) as the primary screening method. Most programs retested abnormal screening specimens using a second electrophoretic technique, HPLC, immunologic tests, or DNA-based assays (7, 8). SCD is frequent in Iran, especially in southern regions (9). In Khuzestan province, southwest Iran, SCD is more frequent in Arab ethnicity (10).

Details of statistics on this disease are not available, but based on clinical evidences, the prevalence of sickle trait is reported as 0.2 – 0.5% among some Iranian ethnicities (10-12). Though the extent of disease is low, severity of SCD is high. Considering clinical features, diagnosis age of the disease is extremely variable. So that some patients realize their disease in the elder ages, and refer to other specialists than hematologists due to variation in the symptoms. Despite of being among the first inherited diseases detected, there is little understanding of the prevalence of SCD, the quality and types of care for patient with SCD receive in the Iranian population, especially in southwestern region.

This study intended to detect the incidence of SCD in newborn period and also other Hemoglobinopathies and alpha thalassemia as an extra information based on RBC indices in the newborn in order to evaluate the sickle trait screening program among couples conducted in 2007. Additionally, the results of this confirmatory test can be useful for the national health staff considering management decisions.

Materials and Methods

Population and samples: Between September 2013 and September 2015, this neonatal screening study for sickle cell disease was conducted on 8363 newborns in four cities of Ahvaz, Khoramshahr, Sosangerd and Dezful of the Southwest region in Iran. This study was approved by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (87/10/11/1264), and all parents signed the Informed Consent Document (ICD) prior to registration.

Inclusion criteria: all newborns were included in this study.

Exclusion criteria: Neonates with low Apgar score and all non-healthy newborns were excluded.

Methods: two to three milliliter cord blood was collected and aspirated into a tube. Then, EDTA treated whole blood tube was used for hematological tests. First, complete cell count (CBC) and cellulose acetate electrophoresis at pH 8.4 was performed on each blood sample. Presence of an abnormal band in EDTA treated samples were further confirmed by citrate agar gel electrophoresis and an automated high performance liquid chromatography (HPLC) using a *Bio-Rad* D-10® auto-analyzer (*Bio-Rad Laboratories*, CA, USA)).

Statistical analysis: Statistical analysis was performed using SPSS (version 19). Data were analyzed statistically running One-Way ANOVA. Descriptive analyses were also performed. Overall and ethnicity-based prevalence with 95% CI (confidence interval) was calculated.

Results

Among 8363 screened samples, 118 (1.4 %) samples were heterozygous for Hb S and four (0.047%) for Hb C particularly among Arabs; none of newborns were Hb SS homozygotes (Table I). Demographic and laboratory data of study group are presented in Table II. The incidence of silent and alpha thalassemia minor based on RBC indices was nearly 10%.

***Table I**: Frequency distribution of hemoglobin variants in various cities.*

Variants	Frequency, n (%)				
	Ahvaz	Khoramshahr	Sosangerd	Dezful	Overall
Hb SS	0%	0%	0%	0%	**0%**
Hb SC	0%	0%	0%	0%	**0%**
Hb Sβ+ thalassemia	0%	0%	0%	0%	**0%**
Hb S heterozygotes variants	26 (0.31 %)	32 (0.383 %)	48 (0.574 %)	12 (0.144 %)	**118 (1.41)**
Hb C	1 (0.0119%)	1 (0.0119%)	2 (0.024%)	0%	**4 (0.0478)**

Table II: Demographic and laboratory data of study group.

Variables	Frequency (%)	Mean ± SD
Gender		----------
Male	4064 (48.6%)	
Female	4299 (51.4%)	
Weight		----------
<2500g	585 (7%)	
>2500g	7778 (93%)	
Ethnicity		----------
Arabs	6452 (77.14%)	
Persians	1694 (20.25%)	
Mixed	56 (0.67%)	
Others	161 (1.92%)	
Mode of delivery		----------
Normal delivery	4766 (57.1%)	
Cesarean Section	3597 (43%)	
Gestational age		----------
Term	7694 (92%)	
Premature	590(7%)	
Post term	79 (1%)	
Apgar score		----------
3	3 (0.035%)	
4	5 (0.06%)	
5	2 (0.023%)	
6	8 (0.095%)	
7	20 (0.23%)	
8	92 (1.1%)	
9	4422 (52.87%)	
10	3811 (45.56%)	
HBS		----------
Yes	118 (1.4%)	
No	8245 (98.58 %)	
MCV		----------
>94	7476 (89.4%)	
≤94	886 (10.6%)	
MCH		----------
>29.5	7527 (90%)	
≤29.5	836 (10%)	
Weight (gr)	----------	3222.34 ± 437.9
WBC	----------	12.76 ± 4.53
RBC	----------	4.35 ± 0.59
Hb	----------	14.7 ± 1.98
HCT	----------	45.34 ± 6.2
MCV	----------	104.4 ± 7.52

Discussion

Khuzestan is an ethnically diverse province of Iran with considerable regional diversity. Ethnic diversity is reflected in the presence of different hemoglobin variants in different ethnic groups, including Arabs, Persians, and others. Most of abnormal variants have low clinical importance in heterozygous state, but when combined with other variants they may lead to severe complications. Neonatal screening is very efficient to recognize the early signs of the disease and minimize morbidity and mortality by early comprehensive care and prophylactic treatment. Nowadays, it's suggested for Hb S screening of all newborns at high risk (14). Screening programs were widely performed in various ways of randomized, double–blind, and placebo-controlled trial reported 84 % reduction in the incidence of pneumococcal sepsis known as the most serious complication of sickle cell anemia among infants during applying the prophylactic oral penicillin (15).

There are various analytical methods to detect thalassemia and hemoglobinopathies such as Hb A2 quantification by ion-exchange column chromatography, alkaline and acid electrophoresis, Hb F quantification by alkali denaturation, and radial immunodiffusion; but none of the mentioned methods can detect multiple hemoglobin fractions in a single step procedure. HPLC has many advantages over the mentioned methods and over the past decades it has evolved as an excellent and powerful diagnostic tool for identification of most of the clinically significant hemoglobin variants especially to beta thalassemia trait owing to its quantitative power and automation. HPLC is sensitive, specific, reproducible, and less time consuming and requires less man power. Hence, it is ideal for a routine clinical laboratory with high work load (16). Accordingly, HPLC method was used in this study.

Present study was carried out on 8363 screened samples obtained from four maternity units in the cities of Ahvaz, Khoramshahr, Sosangerd and Dezful, including different ethnic groups of Arabs, Persians, mixed and others between September 2013 and September 2015, in order to assess the Hemoglobinopathy screening program conducted among couples in 2007. The results showed that none of newborns were Hb SS homozygotes and only four (0.047%) for Hb C, particularly among Arabs. Incidence of sickle trait was higher among Arabs, probably due to the more common rates of consanguineous marriages among them. Incidence of SCD and other hemoglobinopathies in newborn period considerably decreased during the 5 to 8 years after preliminary screening conducted in 2007.

Williams et al., performed also a study to pilot a newborn screening program for SCD in USA using a novel partnership method and showed that universal newborn screening program is feasible when partnered with an established newborn screening laboratory (18). Tubman et al., conducted a descriptive epidemiologic feasibility study on 2,785 consecutive newborns for newborn screening using a South-South partnership and define the incidence of SCD, and reported a high incidence of the disease and the need for newborn screening as an important public health initiative toward improving child health (19).

Conclusion

Present findings indicated the high quality and considerable impact of conducted screening program in 2007 at significantly decreasing the prevalence of hemoglobinopathies and SCD among newborns born between 2013 and 2015.

Acknowledgement

This research was the result of a collaborative project between Industrial

relation office, Deputy of research affair, Ahvaz Jundishapur University of Medical Sciences and Novartis Oncology Company with reference number of 87/10/11/1264.

Conflicts of interest

The authors declare no conflict of interest.

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