The Turkish Journal of Pediatrics 2023; 65: 959-963
https://doi.org/10.24953/turkjped.2023.17

Original Article

Newborn screening for sickle cell anemia in Antalya, Türkiye

Zeynep Öztürk[1], O. Alphan Küpesiz[1], Gülsün Karasu[2], Vedat Uygun[3], Nihal Oygür[4], M. Akif Yeşilipek[3]

[1]Department of Pediatric Hematology, Akdeniz University Faculty of Medicine, Antalya; [2]Department of Pediatric Bone Marrow Transplantation Unit, Medical Park Göztepe Hospital, İstanbul; [3]Department of Pediatric Bone Marrow Transplantation Unit, Medical Park Antalya Hospital, İstinye University Faculty of Medicine, Antalya; [4]Division of Neonatology, Department of Pediatrics, Akdeniz University Faculty of Medicine, Antalya, Türkiye.

ABSTRACT

Background. In a screening study conducted on adults, the prevalence of sickle cell traits in Antalya was found to be 0.24%. Since no screening studies have been conducted in the neonatal period in our region, the exact incidence has not been determined. In this study, we aim to report our experience of neonatal screening for sickle cell disease in Antalya, Türkiye.

Methods. During a 14-month period, 2562 heel prick blood samples, taken on filter paper from Akdeniz University Hospital, Antalya Education and Research Hospital and Antalya Atatürk State Hospital and four other healthcare centers, were studied using the high pressure liquid chromatography method. Blood samples were studied using the 'Sickle Cell Short Program' test method on a Bio Rad Variant device.

Results. In the study, no patients with sickle cell disease were identified. Four newborns who were sickle cell carriers (0.15%) and two newborns who were Hemoglobin D carriers (0.08 %), were found.

Conclusion. Considering the efficiency and cost calculations made as a result of the data obtained from our study, it was concluded that sickle cell screening would not be effective in newborns. It seems more effective and economical to screen the children of parents, who are found to be at risk for Hemoglobin S carriage as a result of premarital tests.

Key words: newborn screening, sickle cell, hemoglobinopathy, Türkiye.

Sickle cell disease (SCD) is a complex inherited red blood cell disorder with a high worldwide prevalence. SCD are common among people from Africa, Mediterranean countries, Türkiye, Arabian Peninsula, Indian subcontinent, and the United States.[1] Most neonates with SCD are healthy in the early period after birth and they become symptomatic during infancy or childhood with a decrease in fetal hemoglobin (HbF) level and an increase in hemoglobin S (HbS).[2] Delay in the diagnosis of the disease can cause complications that may affect the patient's life. An effective neonatal screening program for SCD will reduce morbidity by providing early diagnosis and treatment of these life-threatening complications of the disease, such as acute splenic sequestration crisis and bacterial sepsis.[3-5] Newborn screening for SCD is being carried out in some countries to prevent morbidity associated with this disease.[6]

In a screening study, including adults, it was established that in Antalya, HbS had the highest prevalence (0.24%) after thalassemia traits (10.2%).[7] We thought that the prevalence of newborns with SCD might be higher than reported. In Türkiye, there is no nationwide

✉ M. Akif Yesilipek
yesilipek@gmail.com

Received 24th January 2023, revised 10th April 2023, 18th April 2023, accepted 12th June 2023.

This work was presented at the XXIV National Biochemistry Congress in Konya, Türkiye on 25th-28th September 2012.

newborn screening program for SCD. Newborn screening programs in our country are being conducted for phenylketonuria, congenital hypothyroidism, biotinidase deficiency, congenital adrenal hyperplasia, cystic fibrosis and spinal muscular atrophy. In this study, we aimed to identify the prevalence of SCD and HbS carriage among the newborns in Antalya.

Material and Methods

Approval for the study was obtained from the Antalya Provincial Health Directorate. This study was conducted over a 14-month period (in 2004-2005 (as a master's thesis) and 2007-2008 (as a project supported by the Turkish Society of Hematology) at Akdeniz University Hospital. During the study period, the heel prick blood of 2652 newborn babies, including those born in Akdeniz University Hospital, Antalya Education and Research Hospital, Antalya Atatürk State Hospital, and babies, and those who applied to four health centers (Health centers no. 6, 7, 11, and Maternal Child Health and Family Planning Center no. 1), determined by the Antalya Provincial Health Directorate, were collected. Ethical approval was obtained before the start of the study. Before the blood samples were taken, informed consent was obtained from the parents of the newborns. Ninety of the samples were excluded from the study due to improper storage conditions or incompliance with the age range. The results of 2562 newborns were evaluated. These newborns consisted of 1062 males and 1035 females, yet the genders of 465 newborns were not specified. Newborns, who were born preterm or had received red blood cell transfusions, were not included.

Heel prick samples of the newborns were taken onto Guthrie filter paper (Sergio Bianchi). Infants within the first month were included in the study. In general, blood samples were taken just before hospital discharge and usually on day 2 of life. Birth date, sample date, gender, mother's name, address and telephone number information were written on Guthrie paper. The blood samples were left to dry for at least four hours and the samples were stored at 4°C before and after the study. The blood samples were studied within the next two weeks. High performance liquid chromatography (HPLC) was performed using the Bio-Rad VARIANT™ instrument. Experiments were carried out in accordance with the kit procedure (Variant Sickle Cell Short Program Pack, Bio-Rad Laboratories, Milan, Italy). This program is specifically designed to provide a qualitative result for hemoglobins A, F, S, C, D, and E in neonates. No limit value was reported for the hemoglobin variants in the testing procedure. Regardless of the abnormal hemoglobin percentage, all patients showing abnormal hemoglobin were recalled for HPLC study. The families of all babies with hemoglobin variants were requested to be tested in the following months and HPLC analysis was performed using 'thalassemia short program' to confirm the accuracy of the diagnosis. Genetic counseling was offered to the families.

Results

In this study, 2562 babies were screened. As a result of the screening, no homozygous sickle cell patients were detected. HbS carriers were found in four (0.15%) neonates. In addition, Hemoglobin D (HbD) carriage was detected in two (0.08%) cases. Apart from HbS and HbD, no other abnormal hemoglobin was found during our study. The HPLC results and hematological parameters of these four HbS carriers are shown in Table I. The HbS percentage of seven cases was found to be in the range of 0.1-0.3% and when the control electrophoresis of these cases was studied, the results were found to be false positive. In our study, the HbS value in a one-day-old baby who was an HbS carrier was found to be 1.9%. No intermediate value between 0.3% and 1.9% was found in all cases. HbS percentages of all other HbS positive cases were found to be above 2%. The hemoglobin variants of the three families were studied again to confirm the diagnosis, but the family of the fourth baby (HbS 4) refused a retest.

Table I. Hematological parameters of newborns with HbS.

HbS carriers	Age	RBC ($x10^6$mL)	Hb (g/dL)	MCV (fL)	MCH (pg/cell)	MCHC (g/dL)	HbF (%)	HbA (%)	HbS (%)
HbS 1	7 days	5.06	17.90	100.10	35.30	35.30	61.2	6.6	4.7
	2 months	3.06	9.50	87.10	31.00	35.60	70.4	16.2	12.5
HbS 2	5 days	4.90	17.50	89.70	35.80	39.90	68.4	3.3	2.7
HbS 3	2 days	4.86	17.20	104.70	35.40	33.80	68.9	5.7	5.2
	4 months	3.76	9.90	76	26.40	34.70	17	42.7	37.2
HbS 4	1 day	NK	NK	NK	NK	NK	55.9	2.6	1.9

Hb: hemoglobin, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, MCV: mean corpuscular volume, NK: not known, RBC: red blood cell.

Table II. Hemoglobin variants of family members.

Newborns	Subjects	Hemoglobin percentages (%)			
		HbA	HbA_2	HbF	HbS
HbS 1	Mother with HbS	53.00	4.10	0.50	42.40
	Sibling with HbS	51.70	2.20	2.20	38.10
	Sibling with SCD	3.10	4.60	4.60	76.20
HbS 2	Father with S/β-thalassemia	11.7	6.9	6.9	74.8
HbS 3	Mother with HbS	43.8	4.40	4.40	41.80
	HbS carier sibling	54.8	4.5	4.5	40.40

Hb: hemoglobin, SCD: sickle cell disease

Hemoglobin variants of the family members are shown in Table II. When the family of the first baby, who was a carrier of HbS, was contacted, it was learned that they already had two more children, one of whom had homozygous sickle cell and the other was a carrier. It was determined that the family had this baby (HbS 1) with the prenatal diagnosis method. When the family of the second HbS carrier baby (HbS 2) was reached, it was learned that his father had Sβ thalassemia. Although these two families were knowledgeable about sickle cell anemia, it was found that the family of the third carrier baby (HbS 3) was not aware of such a disease. As a result of the HPLC analysis of her family, it was seen that her mother and sibling were carriers of HbS. The family was informed about the disease.

Discussion

The main objective of the newborn screening program is to improve outcomes in SCD through early treatment and care. It has been emphasized that knowledge of the diagnosis and parent education will enable the implementation, in the early intervention period, of treatment approaches which change the course and severity of the disease and follow-up of possible complications and therefore, will become life-saving.[8] It will be beneficial to screen newborns for sickle cell anemia in regions where hemoglobinopathies are common, in order to prevent lethal complications of SCD in childhood. Although the benefits of screening for hemoglobinopathies are well known, the most important limiting factor for widespread use is the cost of screening. A sickle cell screening program, including systematic screening of all newborns, particularly in areas where the disease incidence is 0.5 per 1000 or higher, has been proposed.[9] In a screening study on adults, the incidence of HbS was found to be 0.24% in Antalya.[7] Since some of the patients with SCD had died due to intervention problems in the early stages of life, it was thought that this rate may be higher in the neonatal period. However, consequently, we found a carrier rate

that was much lower than we had expected. No SCD patients were found in our study. On the other hand, it has been demonstrated that at a prevalence and incidence of 16 sickle cell traits per 1000 there is no significant identification cost difference between universal and targeted screening programs.[10] In our study, the HbS carrier rate was 1.5/1000 and was 10 times less than the stated rate. In this case, it does not seem very beneficial to perform universal newborn screening for sickle cell anemia in our region. Hemoglobinopathy screening programs in Türkiye are aimed at preventing hemoglobinopathies and are based on premarital screening and prenatal diagnosis. The Hemoglobinopathy Control Program has been implemented in all 81 provinces of the country under the name "Pre-Marital Hemoglobinopathy Screening Program".[11] A premarital hemoglobinopathy test is mandatory and free of charge under this program. A 90.0% reduction has been recorded in the number of affected newborns with hemoglobinopathy as a result of the educational and prevention programs in the previous 10 years.[12] No cases of homozygous sickle cell anemia were found in our study. It can be thought that this situation is a result of social education about hemoglobinopathies and widespread prenatal diagnosis. The reason for her unawareness of sickle cell carriage by the mother of our third case, was that at the time of her marriage, only 33 provinces in our country had mandatory hemoglobinopathy screening programs and their marriage was held in another city, that did not offer this. Since 2019, premarital hemoglobinopathy screening has been carried out throughout the country.[11] However, sickle cell patients continue to be born in our region despite premarital screening tests. This is mainly because families refer to a range of personal, cultural, social and religious beliefs when making decisions. Health professionals have to be sensitive to and respect these beliefs.

Our study has some limitations. The main limitation of our study was that it was conducted only in health institutions in the center of Antalya. The data obtained from this study cannot give a general idea about the frequency of sickle cell disease in Türkiye. A nationwide study is needed to determine whether neonatal hemoglobinopathy screening is suitable for our country. On the other hand, the fact that this study was conducted in a region where hemoglobinopathies are common makes its data valuable. One of the limitations of our study is the time and duration of the study. Although there were no homozygous HbS patients in our study, it is known that children with sickle cell disease have been born in our region in the following years. Therefore, longer-term studies with a larger population are needed.

Newborn screening is an important public health measure to enable early detection of certain diseases for which early treatment is both possible and beneficial. We planned this study with the consideration that it could be necessary to screen newborns for sickle cell anemia due to the high incidence of hemoglobinopathy in our country. However, we did not find any homozygous sickle cell patients and we obtained a lower rate of HbS carriage than the rate found in the study on adults. Consequently, considering the efficiency and cost calculations, we concluded that newborn screening for sickle cell is not necessary in Antalya. We think that it will be more effective and economical to prevent the disease with premarital screening tests and to screen the children of parents who are found to be at risk for HbS carriage as a result of premarital tests.

Acknowledgement

The authors would like to thank all members of the Pediatric Hematology Department of Akdeniz University Hospital. The authors would like to give special thanks to health institutions and nurses who contributed to the collection of blood samples, and Biologist Pınar Hos who contributed to the laboratory studies.

Ethical approval

The study was approved by the local Ethics Committee of the Akdeniz University. An administrational approval was received for the study from Antalya Provincial Health Directorate with report number B104ISM4070005/22776 and date 15.10.2004. Another ethnics approval was obtained from Akdeniz University Faculty of Medicine Ethics Committee with report number 264-272 and date 11.04.2006.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AY; data collection: ZO and NO; analysis and interpretation of results: ZO, AK, GK, VU; draft manuscript preparation: Z.O and AY. All authors reviewed the results and approved the final version of the manuscript.

Source of funding

This study was supported by the Scientific Research Projects Coordination Unit of Akdeniz University and was carried out as a master's thesis (Project number: 2004.02.0122.004) by Z. Ozturk and presented to Akdeniz University Health Sciences Institute. This study was also supported by the Turkish Society of Hematology (Project number: 06/F).

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Nietert PJ, Silverstein MD, Abboud MR. Sickle cell anaemia: epidemiology and cost of illness. Pharmacoeconomics 2002; 20: 357-366. https://doi.org/10.2165/00019053-200220060-00001
2. Pass KA, Lane PA, Fernhoff PM, et al. US newborn screening system guidelines II: follow-up of children, diagnosis, management, and evaluation. Statement of the Council of Regional Networks for Genetic Services (CORN). J Pediatr 2000; 137(4 Suppl): S1-S46. https://doi.org/10.1067/mpd.2000.109437
3. Gaston MH, Verter JI, Woods G, et al. Prophylaxis with oral penicillin in children with sickle cell anemia. A randomized trial. N Engl J Med 1986; 314: 1593-1599. https://doi.org/10.1056/NEJM198606193142501
4. Sobota A, Sabharwal V, Fonebi G, Steinberg M. How we prevent and manage infection in sickle cell disease. Br J Haematol 2015; 170: 757-767. https://doi.org/10.1111/bjh.13526
5. Rankine-Mullings AE, Owusu-Ofori S. Prophylactic antibiotics for preventing pneumococcal infection in children with sickle cell disease. Cochrane Database Syst Rev 2017; 10: CD003427. https://doi.org/10.1002/14651858.CD003427.pub4
6. Daniel Y, Elion J, Allaf B, et al. Newborn screening for sickle cell disease in Europe. Int J Neonatal Screen 2019; 5: 15. https://doi.org/10.3390/ijns5010015
7. Bircan I, Sişli S, Güven A, et al. Hemoglobinopathies in the district of Antalya, Turkey. Pediatr Hematol Oncol 1993; 10: 289-291. https://doi.org/10.3109/08880019309029499
8. Olney RS. Preventing morbidity and mortality from sickle cell disease. A public health perspective. Am J Prev Med 1999; 16: 116-121. https://doi.org/10.1016/s0749-3797(98)00140-8
9. Tshilolo L, Kafando E, Sawadogo M, et al. Neonatal screening and clinical care programmes for sickle cell disorders in sub-Saharan Africa: lessons from pilot studies. Public Health 2008; 122: 933-941. https://doi.org/10.1016/j.puhe.2007.12.005
10. Davies SC, Cronin E, Gill M, Greengross P, Hickman M, Normand C. Screening for sickle cell disease and thalassaemia: a systematic review with supplementary research. Health Technol Assess 2000; 4: 1-99.
11. Canatan D, Vives Corrons JL, Piacentini G, et al. Immigration and screening programs for hemoglobinopathies in Italy, Spain and Turkey. Acta Biomed 2021; 92: e2021410. https://doi.org/10.23750/abm.v92i4.11965
12. Canatan D, Kose MR, Ustundag M, Haznedaroglu D, Ozbas S. Hemoglobinopathy control program in Turkey. Community Genet 2006; 9: 124-126. https://doi.org/10.1159/000091493