Indian J Hematol Blood Transfus (Jan-Mar 2019) 35(1):109–113
https://doi.org/10.1007/s12288-018-0994-1

ORIGINAL ARTICLE

Red Cell Indices and Hemoglobin Profile of Newborn Babies with Both the Sickle Gene and Alpha Thalassaemia in Central India

Dipti Upadhye[1] · Dipty Jain[2] · Anita Nadkarni[1] · Kanjaksha Ghosh[1] · Roshan Colah[1]

Received: 26 March 2018 / Accepted: 21 July 2018 / Published online: 1 August 2018

Abstract This study evaluated the effect of alpha thalassemia on the red cell indices and hemoglobin profiles of normal, sickle heterozygous and sickle homozygous newborn babies in central India where the sickle gene is linked to the Arab-Indian haplotype. 265 newborn babies were analysed with complete blood count and hemoglobin analysis on high performance liquid chromatography (Variant Hb Testing System, BioRad Laboratories, Hercules, CA, USA) using the β-thal short program. The sickle genotypes was confirmed by DNA analysis. The two common alpha gene deletions ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) were detected by multiplex PCR. Among the 102 normal, 106 sickle heterozygous and 57 sickle homozygous newborns, the prevalence of a single alpha gene deletion ($-\alpha/\alpha\alpha$) was 28.3% and that of deletion of 2 alpha genes ($-\alpha/-\alpha$) was 21.5%. In all, 57 normal (55.9%), 35 (33.0%) sickle heterozygous and 41 (71.9%) sickle homozygous newborns had a normal α genotype while $-\alpha/-\alpha$ was seen in 23 (22.5%) normal, 30 (28.3%) sickle heterozygous and 4 (7.0%) sickle homozygous newborns respectively. The presence of associated alpha thalassemia resulted in a reduction in the hemoglobin levels and red cell indices in normal, sickle heterozygous and sickle homozygous newborn babies, MCV and MCH being strong discriminators of alpha thalassemia with two alpha gene deletions in all the three groups. This study also helped us to know the variations in hematological parameters in normal, sickle heterozygous and sickle homozygous newborns with and without associated α thalassemia.

Keywords Newborn screening · Red cell indices · Hemoglobin fractions · Sickle cell anaemia · Alpha thalassemia

Introduction

Alpha thalassemia is the most common monogenic disorder in the world due to a deficiency of alpha globin chain production. It is especially frequent in Mediterranean countries, Southeast Asia, Africa, Middle East and in the Indian Subcontinent [1]. Depending on the number of alpha genes deleted, the severity varies from an asymptomatic condition to a moderate hemolytic anaemia seen in some HbH disease cases. Of the 8 common alpha thalassemia deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-^{SEA}$,$-^{SA}$,$-^{FIL}$,$-^{MED}$,$-^{THA}$, $-(\alpha)^{-20.5)}$, $-\alpha^{3.7}$ is the most common alpha gene defect seen in India [2]. Alpha thalassemia which is present in 1/3rd of sickle cell disease patients, is an important modulator of the disease associated with reduced hemolysis, higher hematocrit (HCT) and lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels [3, 4]. Due to its high incidence and its ameliorating effect in patients with sickle cell disease, early identification through a neonatal screening program is the best way to diagnose this defect at birth. Due to the presence of Hb Barts (γ4), the identification of alpha thalassemia is much easier in newborns than in adults, high performance liquid chromatography (HPLC) and isoelectric focussing (IEF) being the most powerful tools to suspect the presence of alpha thalassemia while screening for any other hemoglobinopathy. Since the burden of both alpha

✉ Roshan Colah
colahrb@gmail.com

[1] National Institute of Immunohaematology (ICMR), 13th Floor, Multistoried Building, K.E.M Hospital Campus, Parel, Mumbai 400012, India

[2] Government Medical College, Nagpur 440003, India

thalassemia and sickle cell disease is high in several regions in India, there was a need to determine the variations in red blood cell indices in normal (AA), sickle heterozygous (AS) and sickle homozygous (SS) newborn babies with different alpha genotypes. We also analysed the effect of alpha thalassemia on different hemoglobins (HbA_2, HbF, HbA and HbS) in normal, sickle heterozygous and sickle homozygous newborn babies.

Materials and Methods

Under a targeted newborn screening program for sickle cell disease, a total of 2134 newborns born to sickle heterozygous women at Government Medical College, Nagpur were screened for sickle cell disorders by high performance liquid chromatography (HPLC). Of these, 265 newborns which included 102 normal, 106 sickle heterozygous and 57 sickle homozygous babies delivered at full term were selected for alpha genotyping. The sample selection was based on those which showed a small spike at the start of the chromatogram on HPLC which suggests the possible presence of alpha thalassemia and where the complete blood counts were done (139 babies) along with a few from each group which did not show such a spike but where the complete blood counts were available. Cord blood/Heel prick samples were collected between days 1 and 7 in EDTA vials and analysed. The study was approved by our Institutional Ethics Committee (IEC)-(NIIH/IEC/21-2007). An informed consent was taken from the parents of the newborns. A detailed proforma was filled up to record the gestational age, caste, sex, origin, anthropometric measurements, any clinical presentation at birth, requirement of blood transfusion or any significant family history.

Complete blood count (CBC) was done on an automated cell counter (Sysmex K-1000; Sysmex Corporation, Kobe, Japan). Hemoglobin analysis was done by HPLC on the VARIANT I Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, California, USA) using the β thal short programme [5]. Genomic DNA extraction was done using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). The diagnosis of the newborns was confirmed by Covalent Reverse Dot Blot (CRDB)/Amplified Refractory Mutation System (ARMS) or by restriction enzyme digestion using DdeI [6]. Screening for the common alpha thalassemia deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$) was done by Multiplex PCR [7].

Statistical Analysis

Data analysis was done using Microsoft Excel and GraphPad Prism software (La Jolla, CA, USA). Descriptive statistics including mean and standard deviation were used to describe hematologic and different haemoglobin characteristics of each genotype. The data was analysed using the student *t* test. $P < 0.05$ was considered significant.

Results

The beta globin genotypes of the 265 babies were first confirmed by DNA analysis, and the 102 normal, 106 sickle cell heterozygous and 57 sickle homozygous babies were correctly identified. Alpha genotyping was then done in all the 265 babies. Of the 102 normal babies, 57 had no alpha gene deletion, 22 had one alpha gene deletion and 23 had two alpha gene deletions. Of the 106 sickle heterozygous babies, 35 had a normal alpha genotype, 41 had a single alpha gene deletion and 30 had two alpha gene deletions while in the group of 57 sickle homozygous babies, 41 did not show the presence of these alpha gene deletions, 12 had one alpha gene deletion and only 4 babies had two alpha gene deletions.

Table 1 summarizes the haematological indices in all the three groups of normal, sickle heterozygous and sickle homozygous newborns with and without alpha gene deletions. Among the normal babies with one alpha gene deletion, there was a significant decrease in the MCV ($P < 0.0002$) and the MCH ($P < 0.0001$) levels and in the same group with two alpha gene deletions there was a significant reduction in the Hb, MCV, MCH, MCHC and an increase in the RBC counts and the RDW levels. There was a statistically significant difference in the MCV ($P < 0.0001$) and the MCH ($P < 0.0001$) levels in the sickle heterozygous babies with one alpha gene deletion and the levels further decreased in the sickle heterozygous babies with two alpha gene deletions with a marked increase in the RBC and RDW levels (Table 1). The same trend was observed in sickle homozygous newborns who showed reduced MCV, MCH and MCHC levels with an increase in the RBC counts when they had two alpha gene deletions as compared to a normal alpha genotype. There was no significant difference in the RBC indices between normal, sickle heterozygous and sickle homozygous babies with a normal alpha genotype.

Figure 1 shows the mean and the standard deviation of the Hb, RBC, MCH and MCV levels in the AA, AS and SS babies in the absence and presence of alpha gene deletions. MCV and MCH were the strongest discriminators among the red cell indices to differentiate between babies with a normal alpha genotype, a single alpha gene deletion and two alpha gene deletions in the AA, AS and SS newborns.

Table 2 shows the effect of alpha thalassemia on different hemoglobins in all the three groups of AA, AS and SS babies. The concentration of adult hemoglobin (HbA) was found to be higher in the normal and sickle cell trait

Table 1 Hematological indices in normal, sickle heterozygous and sickle homozygous babies with different alpha genotypes

Beta genotype	Alpha genotype	n	RBC (10^{12}/L)	Hb (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)
β^A/β^A	αα/αα	57	5.0 ± 0.74	17.86 ± 2.09	54.92 ± 7.74	110.22 ± 9.88	35.59 ± 3.23	32.35 ± 2.05	18.72 ± 1.97
β^A/β^A	− α/αα	22	5.2 ± 0.9	16.94 ± 2.67	53.42 ± 8.47	101.47 ± 6.05*	32.16 ± 2.27*	31.74 ± 1.85	18.91 ± 1.64
β^A/β^A	− α/− α	23	6.07 ± 0.74*	16.46 ± 1.70*	55.45 ± 6.37	91.49 ± 4.57*	27.37 ± 1.26*	29.94 ± 0.98*	20.12 ± 2.19*
β^A/β^S	αα/αα	35	4.99 ± 0.75	17.64 ± 2.55	55.50 ± 8.56	110.71 ± 7.01	35.46 ± 2.69	32.06 ± 1.87	18.18 ± 1.59
β^A/β^S	− α/αα	41	5.17 ± 0.76	16.24 ± 2.01*	49.79 ± 8.08*	97.92 ± 7.45*	31.09 ± 2.62*	31.77 ± 1.76	18.06 ± 2.12
β^A/β^S	− α/− α	30	5.96 ± 0.81*	16.18 ± 1.80*	53.94 ± 8.01	90.73 ± 7.68*	28.1 ± 1.98*	31.04 ± 1.64*	20.03 ± 2.17*
β^S/β^S	αα/αα	41	5.01 ± 0.53	17.88 ± 2.33	54.91 ± 7.28	109.37 ± 7.75	35.63 ± 2.54	32.62 ± 1.48	19.26 ± 1.97
β^S/β^S	− α/αα	12	5.43 ± 0.42*	17.63 ± 1.39	54.33 ± 4.99	100.06 ± 4.29*	32.5 ± 1.29*	32.53 ± 1.72	18.11 ± 1.62
β^S/β^S	− α/− α	4	5.62 ± 0.60*	14.15 ± 1.16*	48.3 ± 4.61	88.02 ± 0.52*	25.67 ± 0.69*	29.02 ± 0.79*	18.57 ± 1.28

n no. of patients, *RBC* red blood cells, *Hb* hemoglobin, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red cell distribution width

*$P < 0.05$ between red cell indices with and without alpha thalassaemia

newborns with two alpha gene deletions when compared to those showing the absence of alpha gene deletions whereas the concentration of sickle hemoglobin (HbS) was found to be lower in the sickle cell trait newborns with two alpha gene deletions. However, there was no difference in the HbS and HbA levels in SS newborns with alpha gene deletions and a normal alpha genotype and also no statistically significant difference in the red blood cell indices in the AS and the SS group of babies with and without alpha gene deletions when compared to their respective normal groups.

Discussion

Alpha and gamma globin genes play a crucial role in the neonatal period and thus deletions in the alpha globin genes are expected to cause variations in the haematological parameters. IEF and HPLC are the most powerful tools to suspect the presence of alpha thalassemia at birth [8, 9]. However DNA analysis is required for confirmation.

A study in newborns from Thailand where the prevalence of HbE and alpha thalassemia is high had revealed decreasing trends for Hb, MCV and MCH and an increasing trend in the RBC count corresponding to the number of alpha gene deletions when compared to the non-thalassemic group, MCH being the best predictor for alpha thalassemia [8]. A similar study on cord blood samples from the eastern province of Saudi Arabia showed that the presence of alpha thalassemia significantly reduced the MCH, MCV, RDW-SD, HCT and Hb levels and increased the RBC count in both normal and sickle cell trait neonates [10]. Our findings among newborn babies in central India where HbS is very common were similar with a statistically significant reduction in the Hb, MCV, MCH, and MCHC levels and an increase in RBC counts in all the three groups of normal, sickle heterozygous and sickle homozygous newborns with two alpha gene deletions, MCV and MCH both being strong discriminators for alpha thalassemia.

A statistically significant reduction was also seen in the MCV and MCH levels in the normal, AS and SS newborns with a single alpha gene deletion in our group of babies, a finding which differs from the group of Thai neonates [9], however our sample size was small.

A comparative study on the cord blood red cell indices between the Omani and the Saudi neonates also revealed that the presence of Hb Bart's resulted in lower mean Hb, HCT, MCV, MCH, MCHC and HbF levels and increase in mean red cell count and HbA concentration [11]. In our study there was no difference in the HbF levels while the HbA levels were significantly increased in the AA and AS groups with 2 alpha gene deletions while the HbS levels

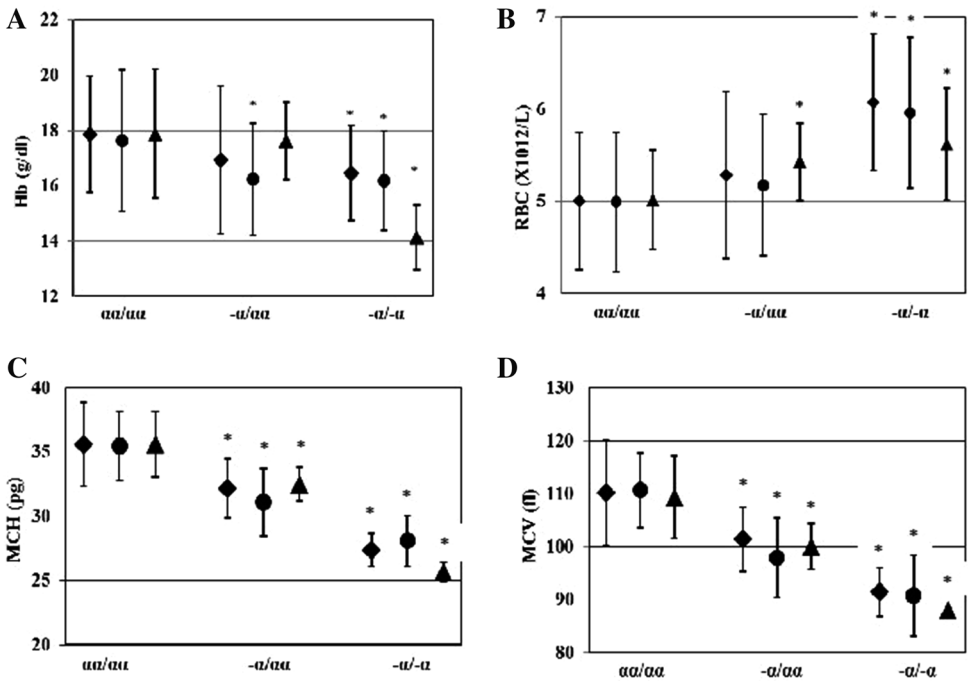

Fig. 1 Distribution of the hematological parameters (mean and standard deviation) in sickle heterozygous, sickle homozygous and normal newborn babies with different alpha genotypes

Table 2 Effect of associated alpha thalassemia on the levels of different hemoglobins in normal, sickle heterozygous and sickle homozygous newborn babies

Beta genotype	Alpha genotype	n	HbA_2 (%)	HbF (%)	HbA (%)	HbS (%)
β^A/β^A	αα/αα	57	0.29 ± 0.31	82.91 ± 12.07	20.50 ± 9.19	–
β^A/β^A	− α/αα	22	0.22 ± 0.29	81.18 ± 11.76	20.55 ± 9.40	–
β^A/β^A	− α/− α	23	0.28 ± 0.31	74.37 ± 12.2	29.6 ± 7.88*	–
β^A/β^S	αα/αα	35	0.32 ± 0.50	82.75 ± 8.85	11.13 ± 4.62	8.36 ± 3.69
β^A/β^S	− α/αα	41	0.31 ± 0.42	84.11 ± 10.74	12.1 ± 4.95	6.38 ± 1.87*
β^A/β^S	− α/− α	30	0.31 ± 0.45	83.93 ± 11.92	16.12 ± 6.25*	6.40 ± 2.08*
β^S/β^S	αα/αα	41	0.24 ± 0.5	89.6 ± 9.64	0.28 ± 0.14	15.4 ± 4.16
β^S/β^S	− α/αα	12	0.16 ± 0.14	88.78 ± 8.39	0.28 ± 0.16	14.05 ± 4.62
β^S/β^S	− α/− α	4	0.2 ± 0.23	87.08 ± 8.31	0.25 ± 0.05	13.25 ± 2.8

n no. of patients, *HbA2* hemoglobin A2, *HbF* fetal hemoglobin, *HbA* adult hemoglobin, *HbS* sickle hemoglobin

*$P < 0.05$ between various hemoglobins with and without alpha thalassaemia

were decreased in the AS group of babies with one or two alpha gene deletions when compared to babies with a normal alpha genotype (Table 2). A recent study from South East Asia which analysed the HbA, HbA2, HbE and HbF levels in babies from birth up to 1 year of age also showed higher HbA levels in cord bloods of babies with alpha thalassemia and slightly lower HbF levels, however the number of newborns in this series was only 15 which included 10 babies with a normal alpha and beta genotype and 5 who had a normal beta genotype but different alpha gene deletions [12].

This clearly indicates that alpha thalassemia largely affects the RBC indices and the hemoglobin profile at the newborn level. Therefore in laboratories where there is no facility for IEF or HPLC to pick up cases with alpha thalassemia at birth, MCV and MCH values could still be used to suspect the presence of alpha thalassemia in newborns [13–15]. MCV ≤ 95 fl and MCH ≤ 30 pg yielded 100% sensitivity to identify 2 α-globin gene deletions in Southeast Asian newborns [16]. In our population too, majority of normal, sickle heterozygous and sickle homozygous newborns with associated deletion of 2 α genes showed a similar reduction in MCV and MCH levels, however those with a single α gene deletion had higher MCV and MCH levels. Only one sickle heterozygous baby had shown MCV > 100 and MCH > 30 in our 3 groups of babies with 2 alpha gene deletions. Thus, in our babies taking MCV ≤ 100 and MCH ≤ 30, all except one baby with 2 alpha gene deletions would have been picked up giving an accuracy of 98.2%.

In India, where the load of sickle cell disease is high and alpha thalassemia is also prevalent and it is one of the major ameliorating factors for the severity of the disease, studying the alpha gene defects in our population is important. One of the limitations of our study is the small sample size and some bias in sample selection. However, this study helps to provide the ranges of hematological values in normal, sickle heterozygous and sickle homozygous newborns with and without associated alpha thalassemia.

Acknowledgement This work was supported by the Department of Biotechnology, Government of India.

Author's Contribution RC designed the study, DU did the experimental work, analysed the data and prepared the first draft of the manuscript, AN supervised the laboratory work and data analysis, DJ and YT recruited the newborns and did the clinical evaluation, RC finalized the manuscript and KG reviewed the manuscript.

Compliance with Ethical Standards

Conflict of interest All the authors declare that there is no conflict of interest.

Ethical Approval This article does not include any studies with animals performed by any of the authors.

Human and Animal Rights All procedures performed in this study involving human participants were in accordance with the ethical standards of the Institutes and/or National Research Committee.

Informed Consent Informed consent was taken from all the parents of the newborn babies.

References

1. Harteveld CL, Higgs DR (2010) α-thalassaemia. Orphanet J Rare Dis 5:13
2. Nadkarni A, Phanasgaonkar S, Colah R, Mohanty D, Ghosh K (2008) Prevalence and molecular characterization of α-thalassemia syndromes among Indians. Genet Test 12(2):177–180
3. Embury SH, Dozy AM, Miller J, Davis JR, Kleman KM, Preisler H, Vichinsky E, Lande WN, Lubin BH, Kan YW, Mentzer WC (1982) Concurrent sickle-cell anemia and alpha thalassemia: effect on severity of anemia. N Engl J Med 306(5):270–274
4. Steinberg MH, Rosenstock W, Coleman MB, Adams JG, Platica O, Cedeno M, Rieder RF, Wilson JT, Milner P, West S (1984) Effects of thalassemia and microcytosis on the hematologic and vasoocclusive severity of sickle cell anemia. Blood 63(6):1353–1360
5. Bain BJ, Lewis SM, Bates I (2006) Basic hematological techniques. In: Lewis SM, Bain BJ, Bates I (eds) Dacie and Lewis practical hematology, 10th edn. Churchill Livingstone, Philadelphia, p 25
6. Colah RB, Gorakshakar AC, Lu CY, Nadkarni AH, Desai SN, Pawar AR, Lulla CP, Krishnamoorthy R, Mohanty D (1997) Application of covalent reverse dot blot hybridisation for rapid prenatal diagnosis of the common Indian thalassemia syndromes. Ind J Hematol Blood Transf 15(1):10–13
7. Tan AS, Quah TC, Low PS, Chong SS (2001) A rapid and reliable 7 deletion multiplex polymerase chain reaction assay for α thalassemia. Blood 98(1):250–251
8. Tritipsombut J, Sanchaisuriya K, Fucharoen S, Fucharoen G, Siriratmanawong N, Pinmuang-ngam C, Sanchaisuriya P (2008) Hemoglobin profiles and hematologic features of thalassemic newborns: application to screening of alpha-thalassemia 1 and hemoglobin E. Arch Pathol Lab Med 132(11):1739–1745
9. Charoenkwan P, Taweephol R, Sirichotiyakul SI, Tantiprabha Sae-Tung WR, Suanta S (2010) Cord blood screening for α-thalassemia and hemoglobin variants by isoelectric focusing in northern Thai neonates: correlation with genotypes and hematologic parameters. Blood Cell Mol Dis 45(1):53–57
10. Quadri MI, Islam SI, Nasserullah Z (2000) The effect of α-thalassemia on cord blood red cell indices and interaction with sickle cell gene. Ann Saudi Med 20(5–6):367–370
11. Al Kindi S, Al Zadjali S, Al Madhani A, Daar S, Al Haddabi H, Al Abri Q, Gravell D, Berbar T, Pravin S, Pathare A, Krishnamoorthy R (2010) Forecasting hemoglobinopathy burden through neonatal screening in Omani neonates. Hemoglobin 34(2):135–144
12. Wong P, Weerakul P, Sritippayawan S (2016) Hemoglobin analysis in the first year of life. Mediterr J Hematol Infect Dis 8(1):e2016012
13. Tanphaichitr VS, Pung-amritt P, Puchaiwatananon O, Winichagoon P, Fucharoen S, Suvatte V, Wasi P (1987) Studies of hemoglobin Bart's and deletion of alpha-globin genes from cord blood in Thailand. Birth Defects Orig Artic Ser 23(5A):15–21
14. Kyriacou K, Kyrri A, Kalogirou E, Vasiliades P, Angastiniotis M, Ioannou PA, Kleanthous M (2000) Hb Bart's levels in cord blood and alpha-thalassemia mutations in cyprus. Hemoglobin 24(3):171–180
15. Rugless MJ, Fisher CA, Stephens AD, Amos RJ, Mohammed T, Old JM (2006) Hb Bart's in cord blood: an accurate indicator of alpha-thalassemia. Hemoglobin 30(1):57–62
16. Uaprasert N, Settapiboon R, Amornsiriwat S, Sarnthammakul P, Thanapat T, Rojnuckarin P, Sutcharitchan P (2014) Diagnostic utility of isoelectric focusing and high performance liquid chromatography in neonatal cord blood screening for thalassemia and non-sickling haemoglobinopathies. Clin Chim Acta 427(1):23–26