

THE EFFECT OF α -THALASSEMIA ON CORD BLOOD RED CELL INDICES AND INTERACTION WITH SICKLE CELL GENE

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Background: α -thalassemia is known to be prevalent in the Eastern region of Saudi Arabia. There are no large-scale reports regarding the effect of α -thalassemia on red cell indices of cord blood from Saudi Arabia. Similarly, there are no reports regarding the interaction of α -thalassemia and the sickle cell gene in relation to red cell indices in cord blood. To address these issues, we undertook a study on neonatal cord blood samples.

Materials and Methods: In a prospective study, cord blood samples from 504 neonates from the Qatif area of the Eastern Province of Saudi Arabia were analyzed for complete blood counts (CBC) and cellulose acetate Hb electrophoresis. Hb S was confirmed by citrate agar Hb electrophoresis.

Results: There were 243 case samples with normal Hb electrophoresis (Hb A 27.2 \pm 7% and Hb F 72.6 \pm 7.7%). Their mean Hb (g/dL), RBC ($\times 10^{12}$ /L), Hct (%), MCV (fl), MCH (pg), MCHC (g/dL), RDW-SD (fl) and RDW-CV (%) were 15.05 \pm 1.6, 4.5 \pm 0.5, 47.4 \pm 5.3, 106 \pm 8, 33.6 \pm 2.3, 31.8 \pm 1.7, 69.2 \pm 9.5 and 17.9 \pm 1.7, respectively. There were 136 cases with α -thalassemia trait (α TT), 57 cases with sickle cell trait (SCT) and 50 cases of sickle cell trait with α -thalassemia trait (SCT/ α TT). There were 10 cases of Hb H disease (6 definite), including one with sickle cell disease (SCD) and two with SCT, Hb Bart's 23.9%-43.6%; four probable with Hb Bart's 10.9%-16.1%, and one with SCT. The effect on red cell parameters in Hb H disease were most pronounced. In addition, there were seven cases of SCD, four of whom had coexistent α -thalassemia trait (SCD/ α TT).

Conclusion: The prevalence of α -thalassemia in this cohort of Saudi population was 39.99%. Hb H disease appeared as common as SCD. Sickle cell gene was seen in 23.4% of neonatal samples. α -thalassemia gene significantly reduces MCH, MCV, RDW-SD, Hct, Hb, and increases RBC count in both normal or sickle cell trait neonates. Generally, the variation of red cell parameters is directly proportional to the amount of Hb Bart's in the cord blood. Sickle cell gene in itself produces low MCV, RDW-SD and MCH in cord blood. Further, normal reference values for red cell parameters of cord blood are established.

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Key Words: α -thalassemia, cord blood, red cell indices, Hb Bart's, Hb H disease, sickle cell trait, sickle cell disease.

The α -thalassemia (the most common genetic disorder in humans) occurs widely throughout Africa, the Mediterranean countries, the Middle East and Southeast Asia.^{1,2} It is reported that 28%-60% of the population in the Eastern Province of Saudi Arabia have α -thalassemia.²⁻⁴ The α^0 -thalassemia is thought to be rare in Africa and Middle East, but the deletional forms of α^+ -thalassemias are very common in Middle East and West Africa.^{1,2,5,6} Because of this, it is thought that Hb H disease and Hb Bart's hydrops fetalis are rare in the Middle East and West Africa, as they require the action of α^0 thalassaemia determinant. The nondeletional form of α -thalassemia is

Accepted for publication 9 July 2000. Received 21 September 1999. thought to be the major cause of Hb H disease in Saudi Arabia and Bahrain.⁶⁻⁹

The effect of α -thalassemia on hematological parameters is related to the exact phenotype, which depends on the number of genes affected (deletion or nondeletion mutation). The cord blood red cell indices in α -thalassemia trait have been reported in only five cases from Eastern Saudi Arabia.¹⁰ We studied the red cell parameters in cord blood in various phenotypes of α -thalassemia based on the presence and quantity of Hb Bart's as revealed by Hb electrophoresis. As well, we studied the effect of interaction of α -thalassemia and sickle cell genes on red cell parameters. We also wanted to determine the effect (if any) of sickle cell gene per se on red cell indices in cord blood.

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TABLE 1. CBC and Hb electrophoresis results (mean±1SD) in different conditions in cord blood.

Electrophoresis pattern (no.)	Hb g/dL	RBC x10 ¹² /L	Hct %	MCV fl	MCH pg	MCHC g/dL	RDW SD fl	RDW CV %	Hb A %	Hb F %	Hb S %	Hb Bart's %
Normal	15.1	4.5	47.4	106	33.6	31.8	69.2	17.9	27.2	72.8	-	-
Hb AF (243)	1.65	0.52	5.32	8	2.36	1.7	9.5	1.7	7.1	7.7	-	-
α-thalassemia trait (αTT)	14.3	5.03	46.4	92.5	28.6	30.7	63.4	19.2	28.1	67.8	-	4.1
Hb AF Bart's (136)	1.98	0.68	5.9	9.9	2.9	2.2	8.4	2	8.2	8.3	-	-
Sickle cell trait (SCT)	14.97	4.66	47.1	101.2	32.3	31.9	64.7	17.7	18.5	73.6	7.9	-
Hb AFS (57)	1.47	0.5	5.2	6.2	2.1	1.7	6.3	1.4	3.8	5.4	2.57	-
SCT with αTT	13.88	5.03	45.2	89.3	27.7	31.1	60.2	19.6	18.7	70.1	6.8	4.4
Hb AFS Bart's (50)	1.37	0.57	5.3	8.24	2.2	1.94	6.7	4.2	5.56	5.8	2.02	1.78
Sickle cell disease (SCD)	14.13	4.26	44.8	105.2	33.2	31.7	66.87	17.5	-	89.2	10.8	-
Hb FS (3)	0.1	0.1	3.6	7.8	0.57	2.7	5.8	0.5	-	3.8	3.8	-
SCD with αTT	12.9	4.7	41.1	87.2	27.5	31.8	61.2	20.03	-	83.7	11.87	4.37
Hb FS Bart's (4)	1.6	0.4	9.1	14.8	1.9	2.76	18.5	2.31	-	5.6	5.9	0.8
Hb H disease (9, 3 with SCT)	12.23	5.21	45.8	88.3	24.57	27.99	75.35	24.9	22.75	48.5	6.93	22.36
Hb AF Bart's/S	1.27	1.06	5.57	10.68	3.22	3.98	15.4	3.7	5.56	16.9	1.4	9.66
Hb C trait with αTT	15.7	5.24	49	93.5	30	32	62.3	19.3	19.5	69.9	C-7.8	2.8
Hb AC Bart's (1)												

Materials and Methods

We prospectively analyzed 504 consecutive case samples of cord blood from the Qatif area for CBC and Hb electrophoresis. EDTA cord blood samples were analyzed within 4-12 hours after collection. These samples were refrigerated from the time of collection up to the time of analysis. CBC was performed on Sysmex NE-8000 (Towa Corporation, Japan). Cellulose acetate Hb electrophoresis (pH 8.6) was performed using Helena kits (Helena Laboratories, Beaumont, TX 77704, USA). Samples showing Hb S or any other abnormal Hb on cellulose acetate electrophoresis were subjected to citrate agar Hb electrophoresis (pH 6.0) using Biomidi kits (Parc de la Plaine, 35 Av. Marcel Dassault, F-31500 Toulouse, France). The electrophoresis plates were scanned using the REP (rapid electrophoresis) scanner of Helena Laboratories. The printouts with percentages of various hemoglobins were obtained. The results of CBC and Hb electrophoresis were recorded in Microsoft Excel spreadsheet. The statistical analysis was also carried out using the Excel program. Null hypothesis (Z test) was used as a test of statistical significance.

Results

On the basis of Hb electrophoresis, the cases were divided into the following groups (Table 1): 1) Normal Hb electrophoresis (Hb A and F), 243 case samples. To obtain

normal reference values, those cases that had Hb of less than 12 g/dL in this group were excluded. The normal reference values (mean±1SD) of red cell parameters and Hb electrophoresis for the remaining 235 cases were: Hb 15.18±1.52, RBC 4.52±0.49 x10¹²/L, Hct 47.7±4.97%, MCV 105.98±7.94 fl, MCH 33.68±2.28 pg, MCHC 31.84±1.65 g/dL, RDW-SD 69.34±9.41 fl, RDW-CV 17.89±1.66%, HbA 27.19±7.09%, and Hb F 72.82±7.09%. We did not account for any clinical condition that may have affected the CBC results, as no such information was available; 2) α-thalassemia trait (αTT) (Hb A, F and Bart's), 136 case samples. The cutoff point of Hb Bart's for this group was taken as 10% and below. To see the

TABLE 2. RBC parameters in cord blood (mean±1SD) in α-thalassemia trait with relation to Hb Bart's level.

Level of Hb Bart's (no.)	Hb g/dL	RBC x10 ¹² /L	Hct %	MCV fl	MCH pg	MCHC g/dL	RDW SD fl	RDW CV %
>3% (93)	13.96† 1.95	5.11* 0.71	45.8* 6.2	90.06 8.02	27.39 2.16	30.42* 2.27	62.8* 9.09	19.68 1.97
2.1-3% (19)	14.7*† 2.14	5.07* 0.43	46.9* 1.95	90.93 11.17	31.3* 2.71	31.3*† 2.23	62.2* 6.63	18.82 1.59
<2.1% (24)	15* 1.64	4.69* 0.59	48.1* 5.23	102.97 8.39	32.07* 1.56	31.3† 1.7	66.6* 9.83	17.83 2.01

*OR; †=Z-test not significant, all other values significant P<0.01-0.001.

TABLE 3. CBC and Hb electrophoresis results in Hb H disease (mean±1SD) interaction with Hb S.

Electrophoresis pattern (no.)	Hb g/dL	RBC x10 ¹² /L	Hct %	MCV fl	MCH pg	MCHC g/dL	RDW fl	SDRDW %	CV	Hb A %	HB F %	Hb S %	Hb Bart's %
Hb AF Bart's (6)	12.13	4.81	44.9	93.2	25.08	27.05	81.6	25.33		25.4	46.2	–	22.4
(Hb H disease)	1.07	0.07	2.55	7.19	7.19	1.96	12.39	3.28		4.07	18.5		9.6
Hb AFS Bart's (3)	12.55	6.24	48.5	78.6	23.57	37.5	62.8	24.1		17.5	53.2	6.9	22.3
(Hb H disease/SCT)	1.45	1.1	8.25	1.21	5.27	6.76	9.5	5.1		2.9	2.91	1.14	7.8
Hb AFS Bart's (1)	9.9	4.34	43.2	99.5	22.8	22.9	105.2	31.8		–	51.2	5.2	43.6
(Hb H disease/SCD)													
Hb H disease (5)*	12.54	5.61	47.6	85.9	22.7	26.46	78.24	26.94		23.26	28.38	7.15**	30.12
Hb Bart's >20%	1.21	1.06	6.2	10.3	2.51	2.07	14.07	2.8		7.4	11.36		3.7
Hb H disease (4)	11.73	4.56	42.8	91.35	26.92	29.9	71.75	22.4		22.12	63.56	6.5†	12.67
Hb Bart's 10%-20%	0.97	0.57	2.4	9.55	1.97	3.8	12.37	2.7		2.42	3		1.71

*Case of Hb H disease with SCD is excluded in this group; **mean Hb S of 2 cases with co-existent SCT; †Hb S of a case with co-existent SCT.

correlation between the amount of Hb Bart's and various red cell indices, this group was subdivided into three: those with Hb Bart's <2.1%, those with Hb Bart's 2.1%-3%, and those with Hb Bart's >3% (Table 2); 3) Sick cell trait (SCT) (Hb A, F and S), 57 case samples (Table 1); sickle cell trait with α-thalassemia trait (SCT/αTT), 50 case samples (Table 1); 5) sickle cell disease (SCD), 8 case samples, four cases with coexistent αTT (Hb F, S and Bart's) and one case with Hb H disease (Hb F, S and Hb Bart's of 43.6%); 6) Hb H disease, definite (Hb Bart's of >20%), six case samples, two with coexistent SCT and another one with coexistent SCD; probable (Hb Bart's of 10%-20%), four cases, one with coexistent SCT. The results in nine cases of Hb H disease (excluding one with co-existent SCD) are shown in Table 1. The breakup of cases (including one with SCD) to show effects of interaction with Hb S is given in Table 3; 7) Hb C trait with αTT, one case sample.

The prevalence of α-thalassemia in this cohort of Saudi population is 39.99%. The sickle cell gene is seen in 23.4%. In α-thalassemia trait, there is a statistically significant reduction of MCH, MCV, RDW-SD, MCHC, and Hb (in descending order of significance), as compared to those with normal Hb electrophoresis (P<0.001-0.0001). It increases RBC count and RDW-CV significantly (P<0.001). These changes in red cell parameters are again apparent in cases with SCT/αTT when compared with cases of SCT alone. The changes in red cell parameters are directly proportional to levels of Hb Bart's in cord blood, especially between those with Hb Bart's of >3% and those with Hb Bart's of <3% (Table 2). MCH, MCV, RDW-SD, Hb, and MCHC (in descending order of significance) are reduced in SCT/αTT as compared to SCT alone (P<0.01-0.0001), while RBC count is significantly higher in SCT/αTT (P<0.001).

In SCD/αTT, MCH and MCV are significantly reduced as compared to cases with SCD alone (P<0.001). RBC count is increased in SCD/αTT as compared to SCD alone (P<0.0001). In one case of Hb C trait, MCV,

RDW-SD and MCH are reduced and RBC count and RDW-CV are increased as compared to normals or those with SCT. In Hb H disease, the lowest MCH, MCV, MCHC and Hb along with highest RBC count and RDW-CV are seen in this study (Table 1). Low MCH, MCV and MCHC are seen in SCD with coexistent Hb H disease.

Comparing SCT with normals (Table 1), it is apparent that the sickle cell gene in itself reduces MCV, RDW-SD and MCH significantly (P<0.001). This is again evident from the fact that lower RDW-SD, MCH and MCV (in order of statistical significance) are seen in SCT/αTT as compared to cases with αTT alone (Table 1). Paradoxically, MCHC is significantly higher in SCT/αTT than in αTT alone. Also in Hb H disease with sickle cell trait, MCH, MCV and RDW-SD are much lower than in pure Hb H disease, suggesting again that the sickle cell gene in itself produces low MCV, MCH and RDW-SD (Table 3). Again, MCHC is higher in Hb H disease/SCT than in pure Hb H disease (P<0.01). Hb F is significantly low in αTT and in SCT/αTT as compared to normals and SCT respectively (P<0.001). Hb S is significantly higher in sickle trait than in SCT/αTT (P<0.01). The β-thalassemias cannot be diagnosed on the basis of cord blood Hb electrophoresis. However, as β-thalassemias are uncommon in the Qatif area, we do not expect any significant interference in the overall results of our study.

Discussion

The most simple and practical way to diagnose α-thalassemia in the newborn is by the detection and quantitation of Hb Bart's.^{11,12} However, failure to demonstrate Hb Bart's does not exclude all cases with mild -α/αα interaction. The prevalence of α-thalassemia in our study was slightly higher than what has been reported earlier from the same area.³ The most important reason for this may be that only cases with Hb Bart's of >2% were taken into account in the previous

study. In fact our own estimate may be an underestimation, as we did not involve sensitive techniques like globin chain synthesis or other molecular techniques. We believe that ours is the first large-scale report regarding the effect of α -thalassemia on cord blood RBC parameters from Saudi Arabia. We found that MCH, followed by MCV and RDW, are the best discriminators between normal and α TT, or between SCT and SCT/ α TT. A similar observation has been recorded by Higgs et al.¹

We have noticed that interpretation of RDW in cord blood depends on the parameter of its expression. This discrepancy between RDW-SD and RDW-CV is difficult to explain but may be due to the poor relationship between RDW-SD (measured at 20% relative height of RBC histogram by Sysmex NE 8000) and MCV in cord blood.

Studies from various racial groups have shown that there is a broad correlation between the quantity of Hb Bart's and α -thalassemia genotype.^{1,2,6,7} The most appropriate and widely accepted values of Hb Bart's in different phenotypes of α -thalassemia are: mild α -thalassemia trait (3 functional genes) 0%-2%, severe α -thalassemia trait (2 functional genes) 2%-8%, Hb H disease (1 functional gene) 10%-40%, Hb Bart's hydrops fetalis (no functional gene) ~80%.¹ We used densitometry for the quantitation of various Hb bands, which may not be as accurate as elution technique. Therefore, we divided our cases of α TT into three groups: those who had Hb Bart's >3% (presumably having 2 gene deletion), those who had Hb Bart's of <2.1% (presumably having 1 gene deletion), and an intermediate group where Hb Bart's was between 2.1%-3% (presuming this group might have 1 or 2 gene deletion). The statistical analysis showed that there is marked difference in RBC parameters (MCH, MCV, MCHC, RDW-CV and Hb) between those who have Hb Bart's of >3% and those with Hb Bart's of <3%. Only MCV and RDW-CV were significantly different in those who have Hb Bart's of 2.1%-3% and those with Hb Bart's of <2.1%. Therefore, we believe that those cases where Hb Bart's is >3% (<10%) on densitometry generally have 2 gene deletion/dysfunction and those with Hb Bart's of <3% most likely have only a single gene deletion. A similar observation has been reported in the literature after performing DNA studies.¹³

It is widely accepted that Hb Bart's of >10% is seen in Hb H disease.¹ However, Hb Bart's of up to 16% has been reported in a few severe α -thalassemia trait cord blood samples from Eastern Saudi Arabia.¹⁰ Therefore, we divided our cases of Hb H disease into those whose Hb Bart's was >20% (definite cases of Hb H disease) and those whose Hb Bart's was 10%-20% (probably Hb H disease). Both groups had low Hb (statistically insignificant from each other), but there were

statistically significant differences in MCH and RDW-CV between the two groups. It was not possible to exclude severe nondeletional α TT as a cause of high Hb Bart's in our cases of "probable Hb H disease."

From our study, it is apparent that at least one in 85 children is born with Hb H disease in the Qatif area. This observation appears contrary to the previous reports suggesting that Hb H disease is uncommon in Saudi Arabia.⁴ We have recently published hematological parameters in 99 Saudi cases of Hb H disease from Dammam region.¹⁴ It appears that Hb H disease is underdiagnosed, as these patients may be asymptomatic and do not report for medical attention, as is the case in SCD. Another important finding in this study was that sickle cell gene in itself reduces MCV, RDW-SD and MCH. This is very clear when we compare the results between SCT and those with normal Hb electrophoresis. One might presume that low MCV, MCH and RDW-SD in our cases of SCT might be due to the coexistence of mild α -thalassemia ($-\alpha/\alpha\alpha$) which did not show any Hb Bart's on electrophoresis. But this looks unlikely, as we do not expect that α -thalassemia affects preferentially those who have sickle cell gene. Rather, it would affect those with normal electrophoresis in the same way and there would be no change in overall results. Further, the fact that sickle cell gene by itself affects red cell parameters is supported by findings of low MCH, MCV and RDW in SCT/ α TT than in pure α -TT ($P<0.01$). Similar results are seen in Hb H disease with SCT when compared with pure Hb H disease. We also found that MCHC is elevated in conditions associated with sickle cell gene. We believe that this effect of sickle cell gene on red cell indices needs confirmation by more sensitive techniques. These findings, if proven, will have clinical significance, especially in relation to the role of low MCV and high MCHC in producing vaso-occlusive crisis in SCD, and the beneficial role of hydroxyurea in SCD in causing macrocytosis.¹⁵⁻¹⁶

Higher Hb S in SCT than in SCT/ α TT confirms the known fact that α chains preferentially bind to β^A rather than β^S .¹⁷ Similarly, low Hb F in α TT, SCT/ α TT and Hb H disease with Hb Bart's of >20% than in normals, and SCT

and Hb H disease with Hb Bart's of 10%-20%, respectively, suggest that α chains bind less efficiently to γ chains than β^A or β^S chains.

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