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INCIDENCE OF ALPHA-THALASSEMIA IN TURKEY*

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Abnormal hemoglobins and their combinations have frequently been reported from Turkey[1-8]. A recent survey revealed that the prevalence of β-thalassemia is more than 2% in the general population[9]. In spite of several case reports related to α-thalassemia[3,5] and its combinations with β-thalassemia[3,10] and sickle-cell anemia[1], the knowledge about the incidence of α-thalassemia is slight because of the diagnostic difficulties due to the mildness of the hematologic changes in the heterozygous state of this disorder.

The thalassemias are characterized by discordant rates of synthesis of structurally normal polypeptide chains. Therefore the precise diagnosis of the α-thalassemia trait should depend on globin chain synthesis. But in the neonate, defective alpha chain synthesis leads to the presence of excess gamma chains which form Bart's hemoglobin thus making the recognition of the α-thalassemia trait possible up to 6 months of age. For that reason we studied cord blood from the standpoint of the presence of Bart's hemoglobin and mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCHb). The parents of the newborns with high Bart's hemoglobin ($\geq$ 2%) were also studied from the standpoint of these parameters.

Material and Methods

Heparinized cord blood samples were obtained from 1100 full term babies born in a maternity hospital and the hemoglobins applied within 24 hours to starch gel (both at pH 8.6 tricitric acid and pH 7 phosphate buffer) and agar gel (pH 6.4 citrate-phosphate) electrophoresis by the methods of Smithies[11] and Robinson et al[12], respectively. Erythrocyte indices could also be evaluated in 873 of the specimens. The amounts of Bart's and A_2 hemoglobins were determined by using chromatography on CM-52 cellulose[13] and on diethyl amino ethyl (DEAE)[14] cellulose, respectively, and the percent of ghost cells was determined by counting 500 cells by the acid elution method for fetal hemoglobin[15]. Intraerythrocytic inclusions were sought according to Dacie and Lewis[16].

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Direct measurements of the erythrocyte, hemoglobin, hematocrit and mean corpuscular volume (MCV) were made with the Coulter counter Model "ZF". Mean corpuscular hemoglobin (MCHb) and corpuscular hemoglobin concentration (MCHC) were calculated from the above data.

Results

Trace amounts of Bart's hemoglobin (< 2% by column chromatography) by starch gel electrophoresis at pH 7 were shown by benzidine staining in 795 (72.3%) of the cord blood specimens. In most of these specimens (673, or 84.65%) it was also shown at pH 8.6 (Figure 1). More than trace amounts (2.0 to 8.8% by column chromatography) were found in 15 (1.36%) cord bloods (Figure 2). In the remaining 290 cord bloods not even trace amounts of Bart's hemoglobin were shown, but Hb A_2 was present by starch gel electrophoresis at pH 8.6 in 49 of them; in two cord

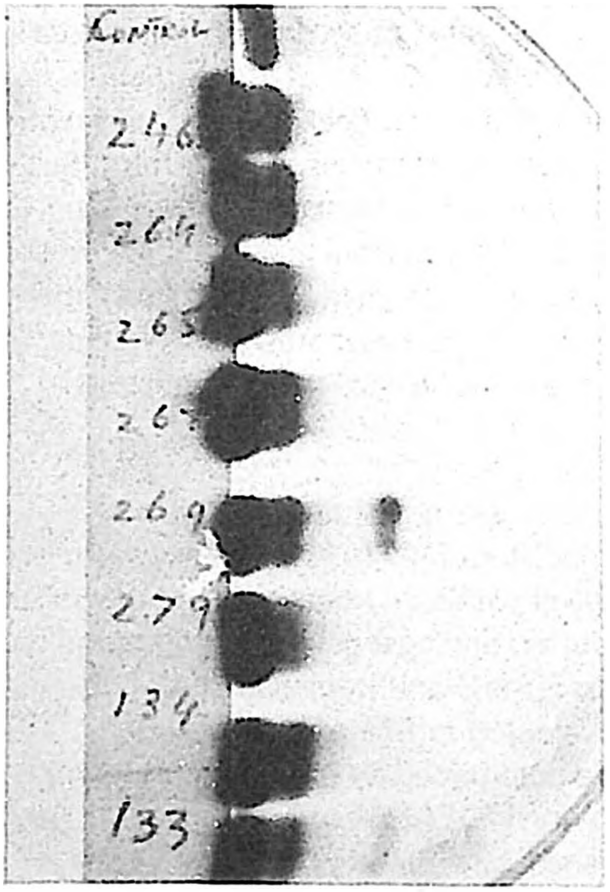

Figure 1

Starch gel electrophoresis at pH 8.6. From top control of Bart's hemoglobin, the second, sixth and ninth show elevated Bart's hemoglobin (2.2, 7.7 and 2.7% respectively). Trace Bart's hemoglobin is seen in the others.

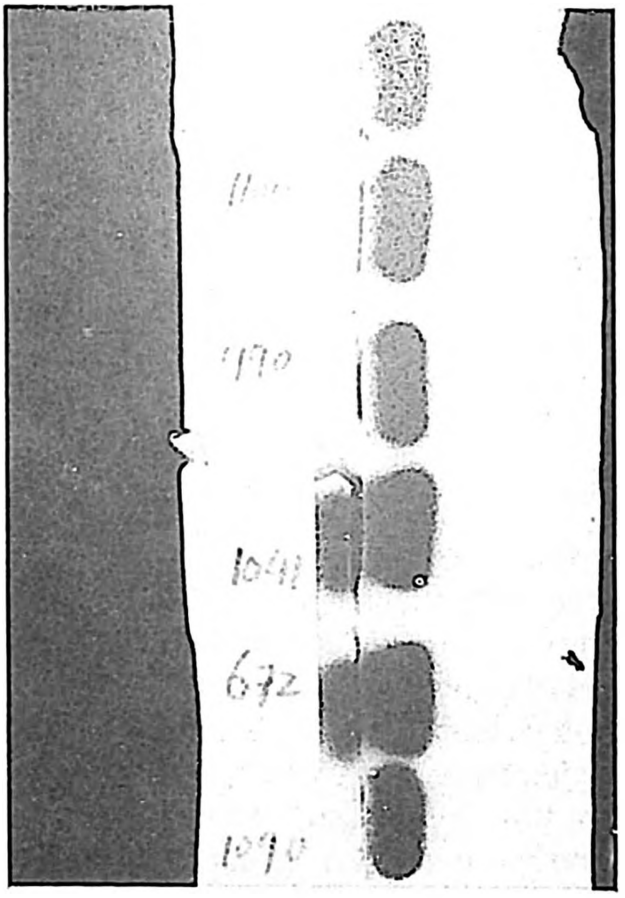

Figure 2

Starch gel electrophoresis at pH 7. Elevated Bart's hemoglobins are seen (4th from top 8.8%, 5th 6.7%).

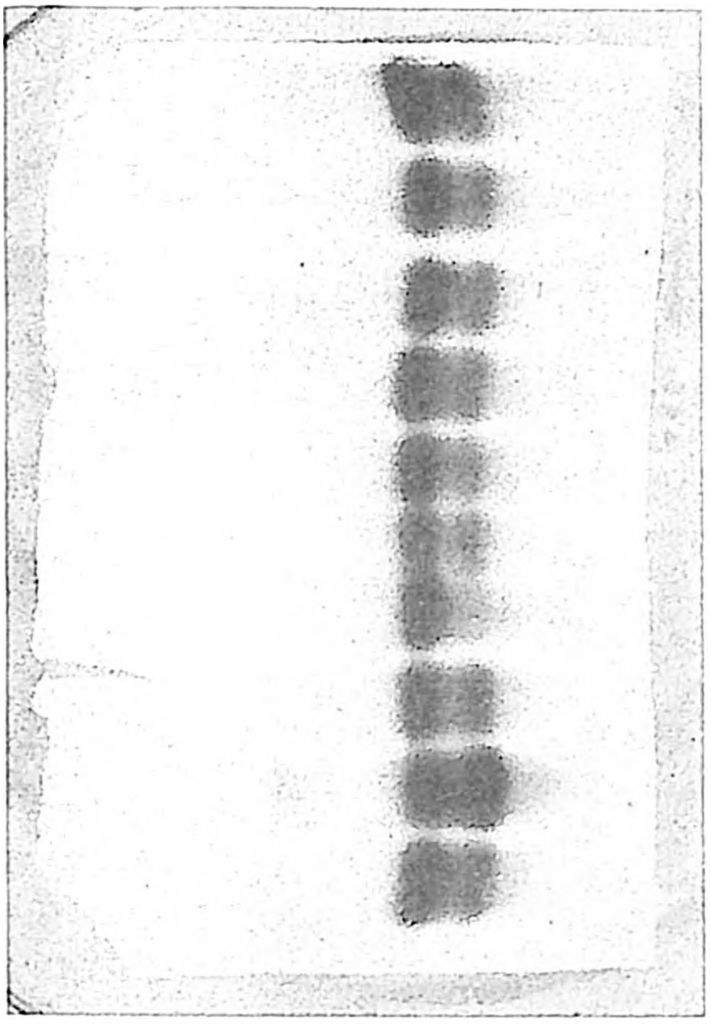

Figure 3

Starch gel electrophoresis at pH 8.6. Elevated Hb A_2 (2.8%) is seen in the second specimen from bottom, trace Hb A_2 in the rest.

blood specimens, both from anencephalic babies, Hb A_2 was found to be markedly elevated (2.8 and 1.3%) (Figure 3). Trace amounts of Hb A_2, by electrophoresis at pH 8.6, were also shown in 10 of the cord blood specimens with trace Bart's hemoglobin; although Hb A_2 was shown in 59 specimens (5.36% of the total number), there was none in the specimens of the 15 with elevated Bart's hemoglobin. No abnormal hemoglobins were seen in any of the 1100 specimens.

Hematological evaluations of 14 of the 15 cord blood specimens with elevated Bart's hemoglobin, 618 with trace Bart's hemoglobin, and 241 without Bart's hemoglobin were available. Although mean values of hemoglobin and hematocrit (Hct) were lower and mean erythrocyte counts of the babies with high Bart's hemoglobin were higher than the mean values of other groups, they were not significantly different from each other ($p > 0.05$ for each) (Table I).

Mean corpuscular volume (MCV) of the babies with elevated Bart's hemoglobin (104 ± 6.91) was found to be significantly lower ($p < 0.001$) than that of babies without (115.6 ± 6.7) or with trace Bart's hemoglobin (114.9 ± 5.9). Although mean corpuscular hemoglobin concentrations of the last groups did not significantly differ ($p > 0.05$), the mean corpuscular hemoglobin of the babies with high Bart's

TABLE I

Hemoglobin Electrophoresis and Hematologic Study Results of 1100 Cord Blood Specimens

	With High Bart's (≥ 2%)	With Trace Bart's (<2%)	No Bart's	Total
Hb Electrophoresis	15 (1.3636%)	795 (72.3%)	290 (26.36%)	1100
Visible A_2	None	10 (1.26%)	49 (16.98%)	59 (5.36%)
Hb (g/dl)	(14)* 15.96 ± 2.2	(618) 16.95 ± 2.7	(241) 16.8 ± 2.45	
	12.3 - 19.3%**	9.3 - 24	9.2 - 25	
Hct (%)	50.8 ± 8.14	53.77 ± 9.49	54.86 ± 12.82	
	35 - 62	29.6 - 78.7	29.6 - 78.2	
RBC ($\times 10^6/\mu l$)	4.905 ± 0.791	4.69 ± 0.799	4.694 ± 0.852	
	3.42 - 5.89	2.69 - 7.167	3.379 - 7.17	
MCV (fl)	104 ± 6.91	114.9 ± 5.9	115.6 ± 6.7	
	92 - 114	101 - 138	100 - 140	
MCHb (pg)	33.05 ± 3.95	36.25 ± 3.36	36.16 ± 3.65	
	26.7 - 42.6	26.8 - 48.2	27.2 - 48.9	
MCHC (%)	31.5 ± 2.67	32.71 ± 3.41	31.12 ± 3.99	
	28.5 - 38.6	26.1 - 39.6	25.5 - 39.0	

* Numbers in parentheses indicate the number of determinations carried out on the cord blood specimens.

** Numbers in black indicate the ranges.

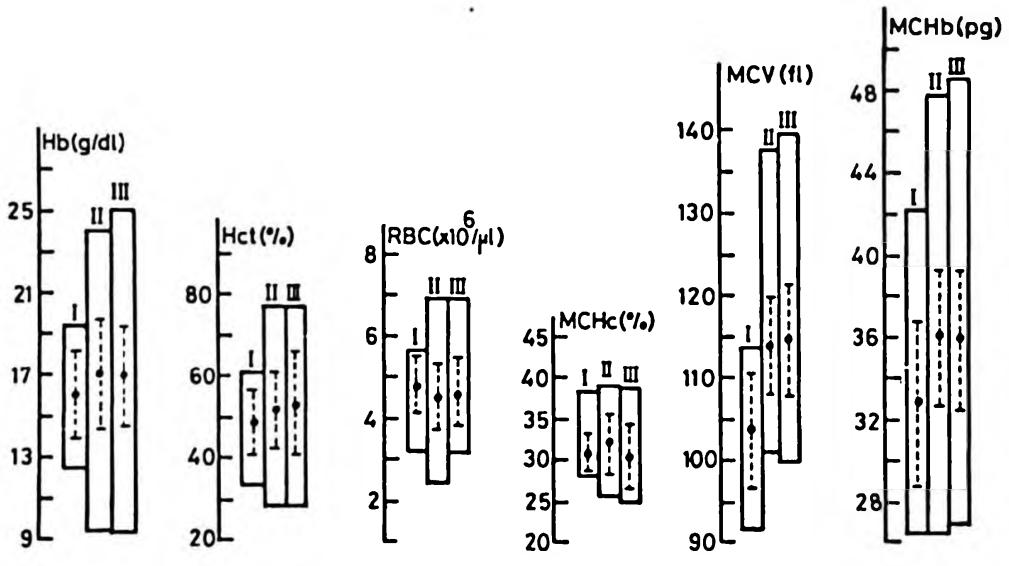

Figure 4

The results of hematological studies of cord blood are illustrated. (Only MCV and MCHb values of babies with high Bart's hemoglobin are found to be significantly decreased.) (I, II, III as in Table II.)

hemoglobin (33.05 ± 3.95 pg) was found to be markedly decreased ($p < 0.01$) when compared to the trace Bart's (36.25 ± 3.36) and no Bart's hemoglobin groups (36.13 ± 3.65) (Table I, Figure 4). The mean percentages of ghost cells in cord blood of these groups were 5.4, 6.3 and 9.6 respectively (Table II). The percentage of ghost cells in the slightly high Bart's group was significantly lower than in the no Bart's group ($p < 0.02$), but the difference between the high and trace Bart's groups was insignificant ($p > 0.05$, Figure 5).

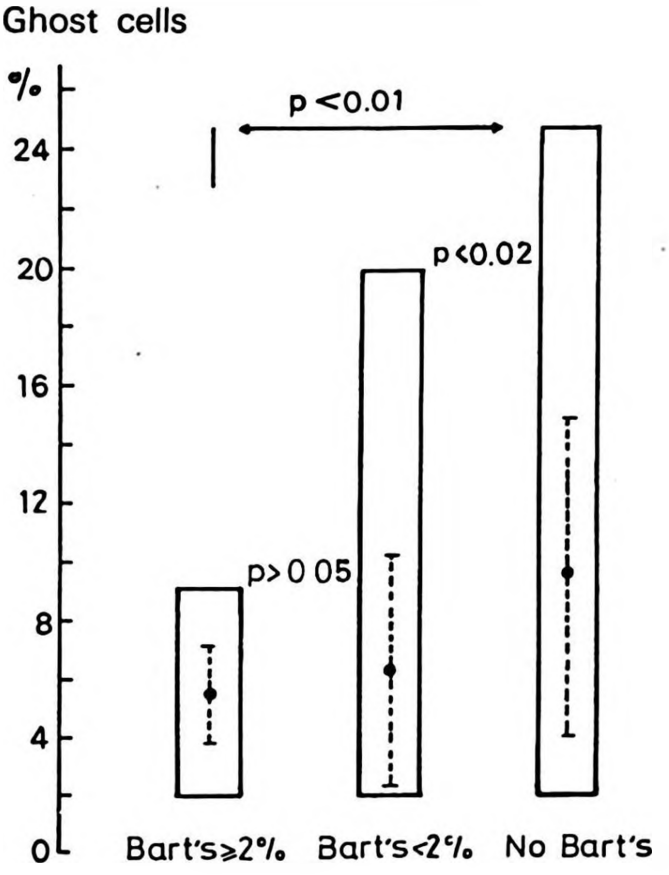

Figure 5

The percentage of ghost cells by acid elution technique was found to be significantly low in patients with high Bart's.

The studies could be repeated in 9 of the patients, at about 1.5 to 4.5 months of age, and Bart's hemoglobin could not be detected in two of them by any of the methods, including column chromatography; in those cases Bart's hemoglobin had been less than 4% at birth. But it was detected with respective values of 0.7, 0.9, 2, 2.4 and 2.5 up to 3.5 and 4.5 months of age in those babies whose cord blood Bart's hemoglobin had been more than 4% at birth (Table III).

None of the 20 parents of 10 babies with high cord Bart's hemoglobin had any abnormality in hemoglobin electrophoresis at pH 8.6, 7 or 6.4. H-inclusion bodies could only be shown in three fathers (1.4, 2 and 6%), and only one had slightly low hemoglobin (12 g/dl). Low MCV (80 fl) was found in one mother who had anemia (9.6 g/dl). Low hemoglobin was also documented in two additional mothers

TABLE II

Comparison of Percentage of Ghost Cells Between Various Groups by Acid Elution Technique

Groups Studied	Mean	SD*	Range	p Value
Group I**				
Bart's ≥ 2%	5.4	1.7	2 - 9	
				p > 0.05
Group II				
Bart's < 2%	6.3	4.8	2 - 20	
				p < 0.02
Group III				
No Bart's	9.6	5.6	2 - 25	

* SD: Standard Deviation.

** p value for Group I and Group III: $p < 0.01$.

(8.9 and 11.7 g/dl); and Hb A_2 (3.6%) was abnormal in one and low normal (2.2) in the other. Low normal Hb A_2 was detected in two other mothers (2.4, 2.5%) and one father (2.3); but elevated Hb A_2 (> 3.6%) was found in 7 fathers (3.8-4.5%) and 2 mothers (4.3-5.3%) (Table IV).

Discussion

Although the trace Bart's hemoglobin may show the unbalanced synthesis of globin chains in intrauterine life, relatively more γ-chains than α-chains, it should not indicate α-thalassemia. Several authors have reported that many Negro and Caucasian infants show a trace of Bart's hemoglobin ($\leq$ 1.5%) in fresh umbilical cord samples[17,19,22]. However, the presence of a significant amount of Bart's hemoglobin in cord blood has been generally accepted as an indication of some form of α-chain synthesis deficiency, which is identified generally as α-thalassemia[17-20], but Esan[21] believes that it might be a developmental abnormality.

Trace amounts of Bart's hemoglobin were detected in more than 70% of our specimens by starch gel electrophoresis at pH 7 with benzidine stain; which corresponds to the 60% of Vella and Cunningham's results[22]. Since more than 2% Bart's hemoglobin in black newborns has been accepted as an indication of an α-thalassemia syndrome, our data were evaluated accordingly. With this criterion the incidence of α-thalassemia is at least 1.36% in the study group, which, since the population of Ankara has increased 100 times in 50 years with the immigration of people from all over Turkey, is considered to be representative of the entire country. This is fairly

TABLE III

Follow-up Hematological Studies in Babies with High Cord Blood Bart's Hemoglobin

Age in Months	Hb (g/dl)	Hct (%)	MCV (fl)	Inclusion Bodies (%)	Bart's Hemoglobin By Hemoglobin Electrophoresis at: pH:8.6	pH:7	By Column Chromatography (%)
1.5	12	36	60	ND	ND	ND	ND (8.8)•
3.0	11	33	97	A	(−)	(+)	2.5 (8.8)
4.0	9.6	34	113	A	(−)	(+)	2.4 (7.6)
3.0	11.4	35	89	0.4	(−)	(+)	2.0 (6.7)
3.5	9.6	30	90.2	0.2	(−)	(?)	0.9 (5.2)
3.0	8.2	25	83	0.2	(−)	(−)	0.7 (4.0)
2.0	10.0	33	98	ND	ND	ND	ND (2.9)
4.5	10.0	31	93	A	(−)	(−)	A (2.9)
2.0	10.6	32	91	A	(−)	(−)	A (2.0)

ND = Not Done.

A = Absent

• = Numbers in parentheses show the cord blood level.

TABLE IV

Hematological Studies of Parents

Patient No.		Hb (g/dl)	Hct (%)	MCV (fl)	Inclusion Bodies (%)	Hb A_2 (%)	Remarks
1	Father	14.1	47.0	102	1.4	4.5	
	Mother	13.4	44.0	93	0	5.3	
2	Father	14.1	43.0	89	0	3.8	OF * slightly decreased
	Mother	12.0	36.0	87	0		OF slightly decreased
3	Father	12.0	38.0	96	6	3.4	OF decreased
	Mother	14.5	46.0	104	0	2.5	
4	Father	15.8	48.0	93	0	2.3	OF decreased
	Mother	9.6	29.0	80	0	4.3	OF normal
5	Father	14.4	44.0	91	–	3.8	
	Mother	8.9	27.0	86	–	2.2	
6	Father	15.2	46.0	91	0	4.0	
	Mother	12.8	38.0	94	0	3.1	
7	Father	14.0	46.0	90	2	4.5	
	Mother	13.0	39.0	97	0	3.6	
8	Father	15.0	44.9	85	–	3.6	
	Mother	12.0	41.8	95	–	3.0	
9	Father	17.5	51.0	92	0	3.8	
	Mother	14.3	41.0	91	0	2.4	
10	Father	14.9	48.0	97	0	4.4	
	Mother	11.7	37.0	90	0	3.6	

* OF = Osmotic fragility.

close to the figures of Dozy et al (1.9%) concerning the incidence of α-thalassemia in American Negroes with α-$thal_2$ homozygosity by endonuclease gene mapping[23]. $\alpha^0\alpha/\alpha\alpha$ genotype was shown in 27.5% of the cord bloods in the same study. This type of thalassemia is the most common form in Saudia Arabia[24].

MCV and MCHb of the babies with elevated Bart's hemoglobin were markedly lower than those of the rest of the babies (Table I, Figure 4) as in the findings of Friedman et al[19] and Schmaier et al[25]. By the acid elution technique the Hb A-containing cells were fewer in these babies than in the others (Table II, Figure 5). Bart's hemoglobin disappeared by 4 months of age in those babies who had had less than 4% of it in cord blood samples, and it was found to be markedly decreased in all the samples. Mild anemia (Hb < 11 g/dl) was observed in at least 6

of them (Table III). It was interesting that Hb A_2 could not be seen in any of the cord blood samples with more than 2% Bart's hemoglobin, but in 16.9% of the samples with no Bart's hemoglobin. A suggestive evidence of α-thalassemia by brilliant cresyl blue inclusions (> 1%) was shown only in 3 fathers among the 16 parents on whom it could be carried out. The elevated Hb A_2 in the cord blood could not be Hb C since it was not seen in agar gel (pH 6.45) electrophoresis and its incidence was very high for Hb E in Turkey. Nor was Hb E detected in the parents of children with high Hb A_2. The presence of elevated Hb A_2 in two babies, both with anencephaly, suggests that Hb A_2 needs to be evaluated in more babies with the same malformation.

In spite of several reports about families with Hb H disease[3,5,26] and at least a 1.36% incidence of α-thalassemia in Turkey, as shown by this study, so far no cases of Bart's hemoglobin hydrops fetalis syndrome have been reported from Turkey, which is quite puzzling. Altay et al[26] recently showed that hemoglobin H disease in Turkey was more frequently due to a combination of dysfunctional α-genes than to gene deletion. This finding could explain the rarity of Bart's hemoglobin hydrops fetalis syndrome in Turkey.

Summary

Starch gel (both at pH 8.6 and pH 7) and agar gel (pH 6.45) hemoglobin electrophoresis were carried out on the cord blood of 1100 full term babies. Trace amounts (not measurable by column chromatography) of Bart's hemoglobin were shown in 795 (72.3%) of the specimens; there was more than a trace (2 to 8.8%) in 15 (1.36%) of the babies. Trace amounts of Hb A_2 were also shown by starch gel electrophoresis in 10 cord bloods with trace Bart's hemoglobin. In two cases with anencephaly Hb A_2 was found to be elevated (2.8 and 1.3% respectively).

Although cord hemoglobin levels were found to be insignificantly decreased, the MCV and MCHb values of the babies with elevated Hb Bart's were found to be significantly lower than those of the babies without Hb Bart's or with trace amounts. The follow-up studies and the hematological evaluation of their parents are presented in detail.

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