

## RED CELL INDICES AND ALPHA-THALASSEMIA

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### ABSTRACT

This study was performed to verify whether red cell indices can be used to identify carriers of alpha+-thalassemia ( $\alpha^+$ -thal). The hemoglobin concentration (Hb), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were compared among Black newborns with normal and  $\alpha^+$ -thal genotypes, inferred by the presence and measurement of Hb Bart's in cord blood (5  $\alpha^+$ -thal postulated homozygotes, 31  $\alpha^+$ -thal heterozygotes and 252 in which Hb Bart's was not detected). In addition, the red cell indices obtained from two groups of clinically normal Black adults who had their genotype determined by DNA analysis (10  $\alpha^+$ -thal heterozygotes and 33 with normal genotypes) were also analysed. The MCV and MCH values obtained from the  $\alpha^+$ -thal homozygotes were lower than those from the heterozygotes, and both were lower than those of the control group. However, the distribution of these parameters presented considerable overlap among groups, showing that they cannot be used alone as indicators of  $\alpha^+$ -thal.

### INTRODUCTION

The  $\alpha$ -thalassemia syndromes comprise a heterogenous group of disorders of  $\alpha$ -globin chain synthesis and represent one of the most frequent hereditary diseases in man (Bunn and Forget, 1986). In Blacks the prevalent form of  $\alpha$ -thal is  $\alpha^+$ -thal, which is due to a deletion of one of the two genes of the haploid genome (Davis *et al.*, 1979; Dozy *et al.*, 1979; Embury *et al.*, 1980; Higgs *et al.*, 1980; Higgs *et al.*, 1981). DNA analysis is the only way to ultimately confirm the existence of the  $\alpha$ -thal deletion (Higgs *et al.*, 1989). An alternative technique for detection of  $\alpha$ -thal, although less sensitive, is the measurement of globin chain synthesis *in vitro* (Weatherall and Clegg, 1981). However, the complexity and elevated cost of both procedures limit their use to research

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laboratories. Another more simple and less expensive technique for  $\alpha$ -thal detection is the identification and determination of Hb Bart's levels during the neonatal period (Ohene-Frempong *et al.*, 1980; Lie-Injo *et al.*, 1982; Rousseau *et al.*, 1985). This procedure allows the detection of homozygotes for  $\alpha^+$ -thal ( $\alpha^+$ -thal/ $\alpha^+$ -thal), but it is unreliable for the identification of all of the  $\alpha^+$ -thal heterozygotes (Higgs *et al.*, 1982). In addition to the above methods, it has been suggested that the mean cell volume (MCV) and mean cell hemoglobin (MCH) may be used to screen for  $\alpha$ -thal syndromes in newborn infants (Schmaier *et al.*, 1973).

In order to determine the predictive value of MCV and MCH to detect  $\alpha$ -thal, we performed a comparison of the MCV and MCH values between  $\alpha^+$ -thal heterozygotes,  $\alpha^+$ -thal homozygotes and individuals with normal  $\alpha$  genes, identified either by DNA analysis in adults or Hb Bart's measurement among newborns.

## MATERIAL AND METHODS

The identification of  $\alpha$ -thal in term newborns from Black women was carried out by detection and measurement of Hb Bart's in cord blood. The cord blood samples were collected at the Maternity of Campinas and at the University of Campinas Hospital, in tubes containing heparin (15 UI/ml of blood). Hb Bart's was detected by cellulose acetate electrophoresis with Tris-EDTA-boric acid (pH 8.6) and sodium phosphate (pH 6.5) buffers. The percentages were determined by elution after phosphate buffer electrophoresis as previously described (Sonati and Costa, 1990). From the results of this procedure the newborns could be assigned to one of three groups: 1) those with no Hb Bart's detectable in the cord blood, which indicates a normal  $\alpha$ -globin genotype ( $\alpha\alpha/\alpha\alpha$ ) and some of the  $\alpha^+$ -thal heterozygotes ( $-\alpha/\alpha\alpha$ ); 2) newborn babies with between 1.5 to 3.8% of Hb Bart's, suggesting  $\alpha^+$ -thal heterozygotes only; and 3) those with Hb Bart's between 5.9 to 9.0%, which would include the  $\alpha^+$ -thal homozygotes ( $-\alpha/-\alpha$ ). For this study we considered the first group as a control, in spite of the fact that the inclusion of some  $\alpha^+$ -thal heterozygotes may have contributed to reduce their mean MCV and MCH values.

In addition, we compared the red cell indices between Black adults with normal  $\alpha$ -globin genotypes (29 males and 4 females) and  $\alpha^+$ -thal heterozygotes (9 males and 1 female), identified by DNA analysis. The blood samples were collected at the Blood Bank of the University of Campinas Hospital, in tubes containing EDTA (1.5 mg/ml of blood). The DNA analysis was carried out by Southern-blot, using Bam HI and Bgl II endonucleases in the digestion and an  $\alpha$ -probe in the hybridization techniques (Costa *et al.*, 1989; Sonati *et al.*, 1991).

In all the cases studied, the red cell indices were determined electronically (Coulter Counter Ssr), and statistically compared, using the Student's *t* test.

## RESULTS

The mean values for Hb, MCV and MCH for the Black babies are shown in Table I. The values of Hb did not differ significantly among the three groups, although the lowest values were found among the  $\alpha^+$ -thal homozygotes. The MCV and MCH values were statistically different among groups. They were lower for the homozygotes than for the heterozygotes, and both were lower than those of the control group. However, the distribution of the MCV (Figure 1) and MCH values showed considerable overlap among the three groups.

Table I - Statistical parameters and t test for comparison of the Hb, MCV and MCH values obtained from the three groups of black newborns.

	Group 1* (n-252)	Group 2* (n-31)	Group 3* (n-5)	t Test
	$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$	( $\alpha=0.05$ )
Hb (g/dl)	15.03 $\pm$ 1.82	14.66 $\pm$ 1.64	13.80 $\pm$ 0.82	group 1 x group 2 - ns group 2 x group 3 - ns group 1 x group 3 - ns
MCV (fl)	106.86 $\pm$ 7.01	100.87 $\pm$ 6.45	92.60 $\pm$ 7.99	group 1 x group 2 - P < 0.001 group 2 x group 3 - P < 0.05 group 1 x group 3 - P < 0.001
MCH (pg)	34.18 $\pm$ 2.55	31.73 $\pm$ 1.98	28.64 $\pm$ 2.90	group 1 x group 2 - P < 0.001 group 2 x group 3 - P < 0.05 group 1 x group 3 - P < 0.001

\*Group 1 - Hb Bart's not detected; Group 2 - 1.5 - 3.8% of Hb Bart's; Group 3 - 5.9 - 9.0% of Hb Bart's; ns - not significant.

The results for the adult controls and heterozygous individuals identified by DNA analysis are given in Table II. They are in agreement with those observed among the newborns: there was a statistically significant difference between the two groups in MCV and MCH values, but not for Hb. The individual distribution of the MCV values is depicted in Figure 2.

The serum iron of all the adults was in the normal range.

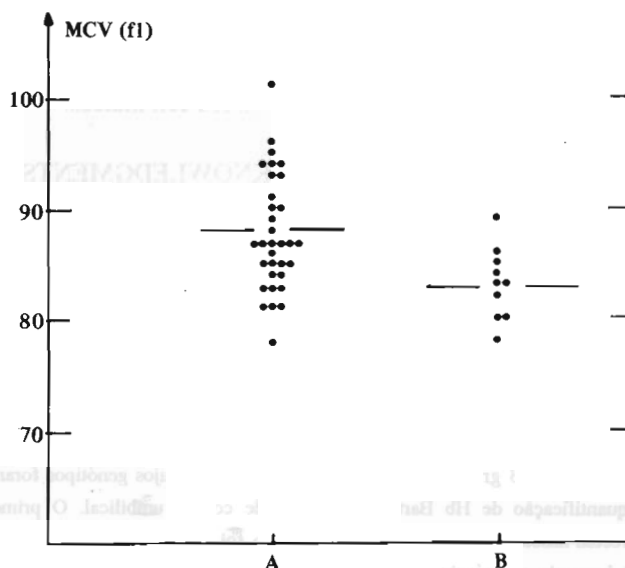


Figure 2 - MCV values from black adults. A: normal genotype ( $\alpha/\alpha$ ). B:  $\alpha^+$ -thal heterozygotes ( $-\alpha/\alpha$ ).

procedure is not reliable for the detection of all the  $\alpha^+$ -thal heterozygotes, although it is very useful for the identification of the  $\alpha^+$ -thal homozygotes. The measurement of the  $\alpha/\beta$ -globin chain synthesis ratio may be employed for  $\alpha$ -thal detection, but it is costly, and for carriers of  $\alpha^+$ -thal it does not provide a clear distinction of the genotype (Schmaier *et al.*, 1973; Higgs *et al.*, 1989). DNA restriction endonuclease mapping, which directly determines the genotype, is the most reliable method for the detection of  $\alpha$ -thal syndromes and can be performed at any age. However, as for the globin chain synthesis, it is only available in a few research laboratories.

Schmaier *et al.* (1973) studied the Hb Bart's levels among 200 black neonates and concluded that the red cell indices could be used effectively to screen  $\alpha$ -thal. But these findings were not confirmed by other reports (Higgs *et al.*, 1981; Johnson *et al.*, 1982; Fei *et al.*, 1989). Detection of  $\alpha$ -thal carriers during the newborn period is important because it alerts to the possibility of microcytic anemia unresponsive to iron therapy and allows the early recognition and characterization of the interaction of  $\alpha$ -thal and other hemoglobinopathies, like sickle cell disease. Our results show that the MCV and MCH averages are significantly different from both the normal  $\alpha$ -genotype newborns and adults, when compared to  $\alpha^+$ -thal heterozygotes ( $-\alpha/\alpha$ ) or homozygotes ( $-\alpha/-\alpha$ ). However, there was a considerable overlap among the three groups. This overlap is so pronounced that, for example, the interval of all  $\alpha$ -thal MCV values includes about 50%

of the MCV values from the control group. Thus, although MCV or MCH averages are statistically different among the groups, they are not reliable to be used as a good screen for  $\alpha$ -thalassemia. In conclusion, our data suggest, as also previously reported (Higgs *et al.*, 1981; Johnson *et al.*, 1982 and Fei *et al.*, 1989) that it is very difficult to classify any particular individual, based solely on red cell indices.

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## RESUMO

O presente trabalho teve o objetivo de verificar se a concentração de Hb e os índices hematimétricos VCM e HCM podem ser empregados na identificação de indivíduos portadores da talassemia  $\alpha^+$ . Assim, foram comparados 3 grupos de recém-nascidos negróides cujos genótipos foram deduzidos através da detecção e quantificação de Hb Bart's em sangue de cordão umbilical. O primeiro grupo era composto de 252 recém-nascidos nos quais a Hb Bart's não foi detectada, o segundo por 31 prováveis heterozigotos para talassemia  $\alpha^+$  (níveis de Hb Bart's entre 1,5 - 3,8%); e o terceiro grupo por 5 prováveis homozigotos para talassemia  $\alpha^+$  (Hb Bart's entre 5,9 - 9,0%). Foram comparados ainda os dados obtidos em adultos negros (doadores de sangue) cujos genótipos foram diretamente determinados por análise de DNA com enzimas de restrição (33 com genótipo normal e 10 heterozigotos para talassemia  $\alpha^+$ ). Os resultados mostraram que a concentração de Hb não se correlaciona com a presença de talassemia  $\alpha^+$ , não diferindo significativamente entre os grupos analisados. Já com relação ao VCM e HCM, as médias foram significativamente diferentes, permitindo a distinção entre os grupos. No entanto, a distribuição individual dos valores mostrou considerável sobreposição, comprovando que estes índices não são capazes de identificar casos individuais, o que não os recomenda, de forma isolada, como indicadores da talassemia  $\alpha^+$ .

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