

## Confirmation of the validity of using birth MCV for the diagnosis of alpha thalassemia trait

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### Abstract

Thirty-four blood samples of neonates in Dubai, UAE, with an MCV below 90 fL were checked by high performance liquid chromatography (HPLC) for hemoglobin variants to confirm a previous study carried out in Western Province of Saudi Arabia which showed a very high predictive index of such MCV for alpha ( $\alpha$ -) thalassemia minor (ATM). MCH below 30 pg was an additional factor which supported such a prediction. The Dubai study confirmed the original finding with 100% of such neonates showing Hb Barts band. A control group of 26 neonates with an MCV between 90 and 95 fL showed Hb Barts in only 11 cases (42.3%). Of these, 6 (23.1%) were preterm babies, expected to have higher MCV. Five cases (19.2%) had an MCH below 30 pg, though MCV was 90 or higher. Three of the preterm babies also had MCH below 30. The study confirmed the Saudi results in neonates. It seems very highly probable that a term neonate with MCV below 90 and MCH below 30 has ATM.

### Introduction

The presence of Hb Barts in blood at birth is a finding in  $\alpha$ -thalassemia trait, intermedia (HbH disease) and major (hydrops fetalis).<sup>1,2</sup> Diagnosis of  $\alpha$ -thalassemia major is not a problem and it is incompatible with life<sup>3</sup> as most of the hemoglobin will be Barts. Neonates with  $\alpha$ -thalassemia intermedia have HbF, HbA and Hb Barts, usually more than 15%.<sup>4</sup> This condition is easy to diagnose later in life, even if not investigated at birth, due to the presence of HbH.<sup>5</sup>

The problem lies with  $\alpha$ -thalassemia trait, single or double gene deletion types. Most of these cases have normal HbA<sub>2</sub> levels if checked after the age of six months,<sup>6,7,8</sup> though red cell indices are thalassaemic. This group, which makes up the bulk of gene carriers, will be difficult to confirm by Hb electrophoresis (HBEP) or by HPLC in childhood and adult life. If one can check HBEP or HPLC at birth then

presence of Hb Barts would put the diagnosis on solid grounds. Hb Barts disappears a few weeks after birth.<sup>1</sup>

It is too expensive to perform HPLC or HBEP for all neonates, even in areas where frequency of the  $\alpha$ -thalassemia gene is high. So, if we can predict, with cheap tests, the presence of  $\alpha$ -thalassemia then Hb separation can be performed for the highly probable cases only. This issue was addressed by work carried out in Thailand,<sup>9</sup> and in Jeddah, Saudi Arabia a few years ago.<sup>6</sup> MCV below 90 and MCH below 30 were found to be of very high predictive value in the Jeddah study.

There was a need to repeat the study in another setting, where thalassaemics are of variable ethnic groups, like in Dubai.  $\alpha$ -thalassemia is present in the UAE nationals and residents of Iranian, Arab, Pakistani and Filipino origins in Dubai and it was expected that neonates in such a population would make a good sample for repetition of the study and confirmation of the validity of using the MCV and MCH as predictive parameters in individuals of various ethnic groups.

### Materials and Methods

All full blood counts (FBC) performed for neonates were checked. Counts were performed using a Coulter machine model LH7D1 (Beckman Coulter, Brea, Ca, USA). Thirty-four consecutive neonates, with MCV below 90 fL were checked by HPLC, using Waters 2695 machine (Milford, Ma, USA), and short  $\beta$ -thalassemia program, to look for Hb Barts band.

Blood films of all babies included in the study were checked by the hematopathologist for red cell morphology. FBCs had not been ordered for these babies because of any suspicion of thalassemia. They were random and judged to be necessary by the attending neona-

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tologists for various reasons.

As a control group, 26 neonates, who had MCV between 90 and 95 fL were also checked by HPLC. Again, these were not selected and all consecutive cases with such an MCV level were included into the control group. It took eight months to collect the entire test and control cases because only a minority of neonates in Dubai have blood counts checked.

### Results

All individuals in the test group had MCV below 90 fL (mean 85.35, range 77-88.9). MCH was below 30 in 33/34 patients (97%). One patient (3%) had an MCH of 31.1 pg. Mean MCH was 27.82 and range 25.1-31.1.

Hb Barts was demonstrated in all the 34 cases (100%). It ranged from a trace (less than

Table 1. Results on cord blood of test group.

Hb Barts	MCV		MCH		Total cases
	<90	≥90	<30	≥30	
Positive	34	0	33	1	34
%	100	0	97	3	
Negative	0	NA	0	0	0
	0		0	0	

Table 2. Results on cord blood of test group.

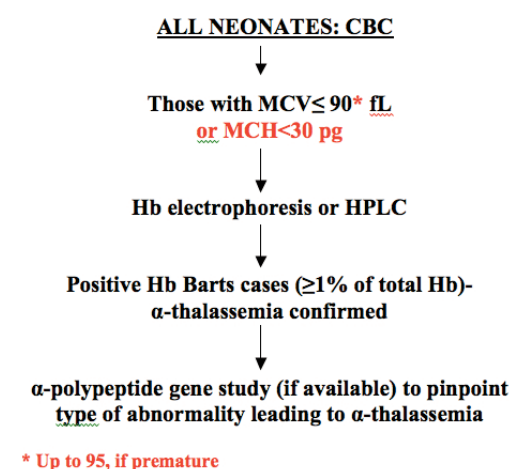
Hb Barts	MCV		MCH		Total cases
	<90	≥90	<30	≥30	
Positive	NA	11	7 (3 preterm)	4 (3 preterm)	11
%		42.3	26.9	15.3	42.3
Negative	NA	15	0	15	15
%		57.6	0	57.6	57.6

1%) to 10.6% (mean 4.65%). The 10.6% figure was obtained in the baby with the lowest MCV (77) and MCH (25.1) (Table 1), who had Hb level of 12.5 and later proved to have Hb H disease. MCV in the control group ranged from 90.1 to 94.8 fL (mean 92.27) and MCH from 28.7 to 31.7 (mean 30.33). Hb Barts was demonstrated in 11 cases (42.3%). It ranged from 2-6% in those cases. Fifteen cases (57.6%) were negative for Hb Barts. Analyzing the 11 Barts-positive cases further showed that 7 of them had MCH below 30 and 6 were preterm (3 of them had MCH below 30). Only one baby (3.8%) out of the 26 controls and positive Barts had MCH above 30 and was full term (Table 2). Blood films of all babies included in the test group showed numerous target cells and microcytosis, but no signs of active hemolysis.

## Discussion

After the work carried out in Jeddah, Saudi Arabia,<sup>6</sup> where the population is basically local with a low expatriate mix, as well as some variation in the origin of the local population itself, it was thought useful to the medical literature to repeat the study at another site to confirm the remarkable results of the original study. Medical literature does mention that  $\alpha$ -thalassemia trait individuals are born with microcytosis.<sup>5,10,11</sup> However, there is no clear demarcation of the level of MCV and MCH where one can expect, with high confidence, to find  $\alpha$ -thalassemia.<sup>3,5,11</sup> The work carried out in Thailand<sup>9</sup> took the MCV cut-off value of 95. In our experience, in both works performed in Jeddah and in Dubai a MCV cut-off value of 95 will give a lot of negative cases and make the need for HPLC/HBEP much higher, thus increasing the cost while one of the objectives of this scheme is to lower costs.

The advantage of using MCV and MCH levels is that these values are easily available from a simple automated blood count (FBC). If one can prove that there are limit levels for these two parameters which carry a high probability of  $\alpha$ -thalassemia trait and HbH disease, then there will be a small proportion of patients for whom one needs to perform Hb separation procedure, by alkaline electrophoresis or HPLC, to demonstrate Hb Barts, and thus prove  $\alpha$ -thalassemia presence. It is confirmed from this study that if MCV is below 90 and/or MCH is below 30 there is a high probability of over 95% that the case is  $\alpha$ -thalassemia trait. MCV may be above 90 if the baby is born premature. In such cases one can extend the MCV level to 95, whether or not



MCV is below 30 pg. It is noteworthy that Hb Barts is not only a diagnostic finding for  $\alpha$ -thalassemia, but it tends to disappear a few weeks after birth.<sup>10</sup> Trying to confirm  $\beta$ -thalassemia trait at a later age, when suspicion is raised by low red cell indices leading to erythrocytosis, hematologists first resort to HBEP or HPLC, looking for low HbA2. However, low HbA2 is not a common finding in this condition.<sup>6</sup> Also, low Hb A2 is found in other conditions, like  $\beta$ -thalassemia. Iron deficiency anemia may also lower HbA2 to below normal level.<sup>12-14</sup> Failing to confirm the condition by this routine test, as in the majority of cases, one can try molecular studies on the  $\alpha$ -polypeptide gene to look for deletions or mutations known to lead to  $\alpha$ -thalassemia trait. These deletions and mutations are so numerous<sup>5,15,16</sup> that, in practice, laboratories only try the common local deletions or mutations, using the proper probes for them, if these are known from previous studies. In a mixed population the task will be difficult, as one can imagine. The cost of these molecular tests is certainly higher than Hb separation studies. In parts of the world where  $\alpha$ -thalassemia gene is of high frequency, the cost will be enormous if one wants to diagnose all carriers. It should be remembered that most countries harboring a high frequency for the gene are also poor in resources.<sup>17</sup> The simple strategy we are recommending, proven by the two studies in two different locations, is valid and is recommended by the authors for WHO and local public health schemes to establish the diagnosis and gene frequency. Further studies are being considered to try to split populations with one, two and three gene deletions/mutations from levels of MCV and MCH caused by them and the HPLC performed as a result of obtaining the low MCV and MCH figures. The simple investigational scheme that we suggest to be followed for the  $\alpha$ -thalassemia screening of neonates is shown in Figure 1.

Figure 1. Investigational scheme for  $\alpha$ -thalassemia screening of neonates.

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