

## Detection of Alpha Thalasemia in Cord Blood in Bahawalpur Region

SAEED AHMAD MALIK<sup>1</sup>, MUHAMMAD AURANGZEB SAEED MALIK<sup>2</sup>, SAADIA AURANGZEB MALIK<sup>3</sup>

### ABSTRACT

**Background:** Neonatal screening for abnormal hemoglobins provides a practical approach for the detection of sickling syndromes and other hemoglobin disorders such as  $\alpha$ -thalassemia. Most screening programs utilize various electrophoretic techniques for the detection of abnormal hemoglobins. Electrophoresis on cellulose acetate at pH 8.6 identifies increased amounts of Bart's hemoglobin (Hb) associated with alpha thalassemia.

**Aim:** To detect incidence of Alpha thalasemia in Bahawalpur region so that early measures can be taken in follow up of such patients..

**Methods:** cellulose electrophoresis technique was used on 205 cord samples received from Bahawal Victoria Hospital and were analyzed for presence of Hemoglobin Bart. Out of 205 samples, 2 had positive Bart Hemoglobin.

**Results:** Estimation of Hb, PCV, RBC count, MCV, MCH and MCHC was done on suitable samples. In the present series two cases of alpha thalassaemia were diagnosed, making an overall prevalence of 0.97%. Blood from the umbilical cord (cord blood) is screened for haemoglobinopathies in several neonatal screening programmes

**Conclusion:** Cellulose Electrophoresis technique is reliable for screening for haemoglobinopathies. It enables early detection and reporting of all major haemoglobinopathies. Detection of alpha thalasemia carriers could be helpful for clinicians to think of mild anemia later in life which could be refractory to iron therapy.

**Keywords:** Hemoglobinopathy, Alpha thalasemia, Hb Barts Cellulose electrophoresis

---

### INTRODUCTION

Thalassaemias are a heterogeneous group of inherited disorders, characterized by deficient/absent synthesis of globin part of the Hb molecule resulting into various clinical syndrome<sup>1</sup>. Depending upon the type of chain affected the thalassaemias are classified into alpha, beta, gamma or delta thalassaemias. Alpha Thalassaemia syndromes are characterized by absent or non-functioning of normal  $\alpha$ -globin genes, thus resulting in an imbalance of  $\alpha$ -globin chain synthesis. In majority of cases it occurs as a consequence of gene deletion<sup>2</sup>. In normal individuals alpha chains are synthesized under the control of 4 alpha globin genes, located on chromosome 16<sup>2</sup>. The normal genotype is expressed as ( $\alpha\alpha/\alpha\alpha$ ). The  $\alpha$ -thalassaemia in neonates is characterized by decreased synthesis of chains resulting into accumulation of excessive free gamma chains in the form of tetramers (r4), called Hb Bart's. The level of Hb Bart's in cord blood is directly related to the number of thalassaemia genes deficient in that individual. In a normal neonate, the level of Hb Bart's

is very low (up to 0.2 to 0.5%)<sup>4</sup>. The level of Hb Bart's is below 3% in  $\alpha$ -thalassaemia-2, while in  $\alpha$ -thalassaemia-1, it ranges between 3.5 & 5%. Hb H disease is characterized by Hb Bart's levels of between 20-25%, whereas in Hb Bart's hydrops foetalis syndrome, the concentration of Hb Bart's, is between 80% to 90%<sup>5</sup>. In infants Hb Bart's disappears at the age of six months and for detection of carrier states in adults the available methods are relatively expensive and cumbersome. The estimation of Hb Bart's in cord blood is, therefore, an easy, sensitive, cheap and reliable method for the detection of  $\alpha$ -thalassaemia in early life<sup>6</sup>. The  $\alpha$ -thalassaemia gene has been found to be prevalent throughout the world<sup>3</sup>. It is highest amongst the countries in South East Asia<sup>7</sup>, Mediterranean region<sup>8</sup> and in people of Black ancestry<sup>9</sup>. Alpha thalasemia occurs in individuals of all ethnic backgrounds and is one of the most common genetic diseases worldwide. However, the clinically significant forms occur predominantly among Southeast Asians. If hemoglobin Barts is detected on a newborn screen, the patient is usually referred for further evaluation since detection of hemoglobin Barts can indicate either silent alpha thalassemia carrier (one alpha globin gene deletion), alpha thalassemia trait (two alpha globin gene deletions) or hemoglobin H

---

<sup>1</sup>Professor of Hematology, <sup>2,3</sup>Demonstrators Quaid-e-Azam Medical College, Bahawalpur, Pakistan  
Correspondence to Dr. Saadia Aurangzeb Malik Email: saadiazab@hotmail.com

disease (three alpha globin gene deletions). In addition to Hb Barts, low Hb levels and low red cell indices are also found in  $\alpha$ -thalassaemic patients<sup>6</sup>.

So far, screening of  $\alpha$ -thalassaemia gene has not been carried out extensively in Pakistan. Therefore, the present study has been undertaken with a view to find out the overall prevalence in this region of Pakistan. The Presence of  $\alpha$ -thalassaemia has been reported from various parts of the world<sup>5</sup>.

## MATERIALS AND METHODS

For this study, a random collection of cord blood samples from 205 full term infants born in the labor rooms of Bahawal Victoria Hospital was carried out. Two to three ml of free flowing samples was collected from maternal side of the severed umbilical cord in clean, dry heparinized vials. After mixing, the samples were refrigerated at 4°-8°C and the following tests were carried out within 24 hours.

1. Hb estimation, RBC count, PCV and the red cell indices i.e. MCV, MCH and MCHC on coulter counter model Z F6 system with MCV/Hct accessory, coulter haemoglobin and dual diluter III.
2. Hb electrophoresis of haemolysate prepared according to s Lehmann and Huntsman<sup>11</sup>, on:

a) Cellulose Acetate Membrane (CAM) using tris EDTA Borate Buffer PH 8.6-9.17 and

b) Starch Gel using Iris EDTA-Borate buffer system Ph 6,0,0. 8M7.

The specimens which revealed a fast moving haemoglobin band were subjected to confirmation by performing electrophoresis on (a) CAM in phosphate buffer Ph 6.57 and (b) Starch-gel in Phosphate buffer Ph. 7.1, 0.0054.M7. The Hb Barts so identified was quantitated by elution<sup>8</sup>.

## RESULTS

In a total of 205 newborns included in this study, Hb Barts was identified on CAM in two infants Hb Barts identified on CAM as well as on starch gel in the first two infants was also quantitated and found to be 6.2% and 3.02% of the total haemoglobin, respectively. No Hb H disease or Hb Bart's hydrops foetalis syndrome was detected in this study. The hematological statistics of the two infants with Hb Barts as compared to the mean values of infants with normal haemoglobin pattern on electrophoresis (which therefore formed a control group) is given in Table 1.

Table 1: Hematological data of positive cases and control group

| Subject              | Hb gm/dl | RBC count | PCV Gm/dl | MCV fl | MCH pg | MCHC Gm/dl | Hb Barts% of total Hb |
|----------------------|----------|-----------|-----------|--------|--------|------------|-----------------------|
| Control group (mean) | 15.4     | 4.4       | 52        | 118    | 35     | 29.7       | nil                   |
| Infant 1             | 11.6     | 4.08      | 41.5      | 102    | 28.7   | 27.9       | 6.2%                  |
| Infant 2             | 13.0     | 4.00      | 46.1      | 99     | 28.8   | 28.6       | 3.02%                 |

Fig.1: Incidence of alpha thalasemia around the world

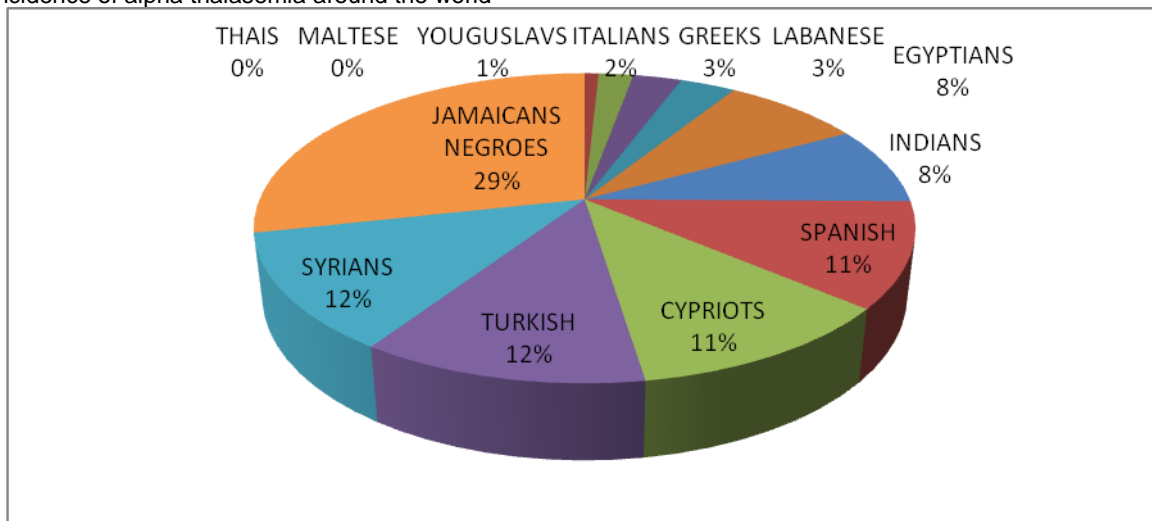
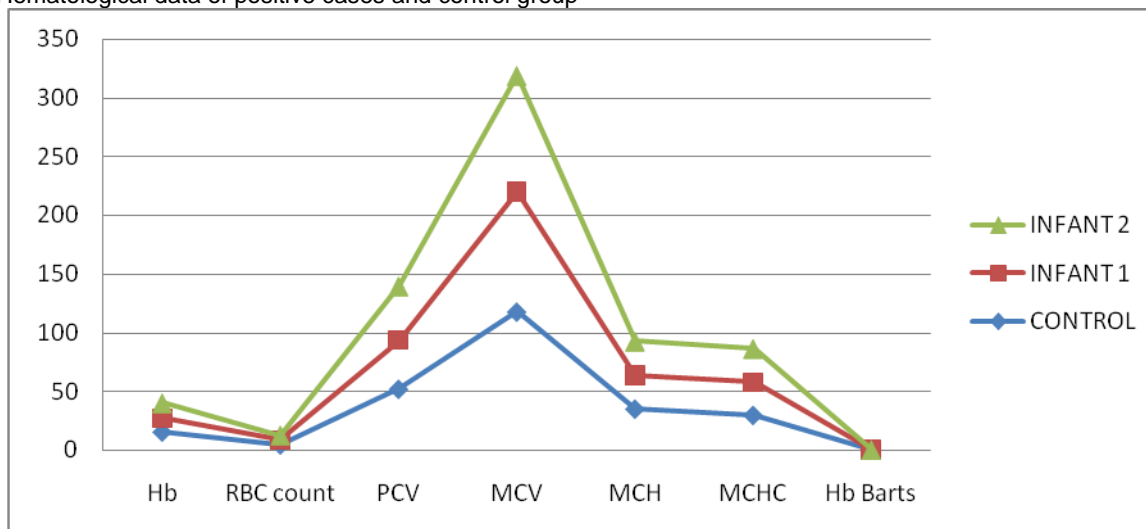


Fig.2: Hematological data of positive cases and control group



## DISCUSSION

The incidence of alpha thalassaemia varies from 0% to 21% in various races<sup>5,9-12</sup>. In the present study, cord blood samples from 205 newborns were investigated for alpha thalassaemia. Two positive cases were identified giving a prevalence of 0.97% in Bahawalpur region. The prevalence of alpha thalassaemia varies from 1% to over 80% in world  $\alpha$ -thalassaemia gene with full spectrum of clinical presentations is widely distributed in South East Asia<sup>7</sup>. The populations residing in Mediterranean region, have been found suffering from both classical pattern and non deletion type of disease<sup>4</sup>. In Blacks, it has been found as mild  $\alpha$ -thalassaemia but frequency is up to 27%. In this group Hb H-disease is rare and Hb Bart's hydrops foetalis is not on record<sup>9</sup>. In Asia, the area starting from western border of Burma and extending up to Turkey presents a pattern of  $\alpha$ -thalassaemia similar to that found in Blacks. The carriers of  $\alpha$ -thalassaemia are common, but Hb H-disease is rare and Hb Bart's hydrops foetalis syndrome is not found. In some areas of southern India 50% of the population is carrying alpha-thalassaemia representing areas of focal concentrations of this gene<sup>10</sup>. 50% of people residing in the eastern coast of Saudi Arabia also possess  $\alpha$ -thalassaemia gene<sup>11</sup>.  $\alpha$ -thalassaemia has been studied to a very small extent in Indo-Pak subcontinent. Chouhan et al<sup>12</sup> studied  $\alpha$ -thalassaemia in India and pointed out a prevalence of 2.05%. Vora and colleagues<sup>13</sup> found the prevalence in Indian people as 0.45% only. In another study in Indian state of Orissa a frequency of 0.29% was recorded<sup>6</sup>. Few studies have been conducted in Pakistan on this problem. One study conducted in

Pakistan revealed a prevalence of 0.94% in population in and around Lahore<sup>14</sup>. Another study conducted at AFIP, Rawalpindi, 2.40% of population of northern Pakistan has been found to be carrying  $\alpha$ -thalassaemia gene. No case of Hb H disease or Hb Bart's hydrops foetalis syndrome was detected during this study. The absence of Hb H disease and Hb Bart's hydrops foetalis in our population is probably indicative of the presence of a genotype different from that of classical  $\alpha$ -thalassaemia found in South East Asia and Mediterranean region. In the latter case the 2 alpha genes are deleted from the same chromosome ( $-\alpha$   $-\alpha$ ) i.e. cis position, whereas Blacks have been found to be carrying  $\alpha$ -thalassaemia-1 gene deletion in transposition ( $-\alpha$   $/-\alpha$ )<sup>9</sup>. In India, gene mapping studies in  $\alpha$ -thalassaemia-1 revealed two genes deletion in transposition ( $-\alpha$   $/-\alpha$ )<sup>6</sup>. On the basis of the rarity of Hb H disease and absence of Hb Bart's hydrops foetalis syndrome and its similarities to  $\alpha$ -thalassaemia presentation and Indians, it is likely that our population also carries similar genotype i.e., ( $-\alpha$   $/-\alpha$ ). This needs confirmation by conducting gene mapping studies in our population. This study has revealed that alpha-thalassaemia trait is present 0.97% of the population of this area of Pakistan. It is further concluded that severe forms of  $\alpha$ -thalassaemia i.e., Hb H-disease and Bart's hydrops foetalis syndrome have not been detected in this study. In the present study, cord blood samples from 205 newborns were investigated for alpha thalassaemia. Two positive cases were identified giving a prevalence of 0.97%. The hematological indices which show a definite variation in the positive cases as compared to the control group are: haemoglobin levels, MCV and MCH all three indices

being low in the affected infants<sup>6,15,16</sup>. Therefore babies with a low MCV associated with a low MCH at birth should be investigated further for alpha thalassaemia by Hb electrophoresis, to detect presence of Hb Barts. Hb Bart's screening of fresh umbilical cord blood is an effective method to evaluate globin chain imbalance. This strategy could be utilized to screen populations for the incidence of  $\alpha$ -thalassaemia.

## REFERENCES

1. Weatherall, Di. and Clegg, J.B. The thalassaemia syndromes. 3rd ed. Oxford, Blackwell, 1981
2. Pootrakul, S., Wasi, P. and Na-Nakorn, S. Studies of haemoglobin Bart's (Hb-gamma-4) in Thailand; the incidence and the mechanism of occurrence in cord blood. *Ann. Hum. Genet.*, 1967; 31: 149
3. Nigga, D. R. and Weatherall, D.G. Alpha thalassaemia, in current topics in haematology. Edited by Piomelli, S. and Yachrin, S. New York, Lippincott, 1983, p. 37.
4. Kanavakis, B., Tzotzos, S., Liapaki, A., Metaxotou-Mavrounati, A. and Kattamis, C. Frequency of thalassaemia in Greece. *Am. J. Hematol.*, 1986; 22: 225.
5. Lie-Injo, L.B., Solai, A., Herrera, A.R., Nicolaides, L., Kan, Y.W., Wan, W.P. and Hasan, K. Hb Bart's level in cord blood and deletions of alpha globin genes. *Blood*, 1982; 59:370.
6. Kulozik, A.E., Kar, B.C., Serjeant, G.R., Serjeant, B.E. and Weatherall, D.J. The molecular basis of CC - thalassaemia in India. Interaction with the sickle cell gene. *Blood*, 1988; 71:467.
7. Wasi, P., Na-Nakorn, S. and Pootrakul, S. The cc - thalassaemias. *Clin. Haematol.*, 1974; 3: 383.
8. Ashiotis, T. H., Zachariadis, Z., Sofroniadou, K., Loukopoulos, D. and Stamatoyannopoulos, G. Thalassaemia in Cyprus. *Br. Med. J.*, 1973; 2:38.
9. Dozy, A.M., Kan, Y.W., Embury, G.H., Mentzer, W.C., Wang, W.C., Lubin, B., Davis, J.R. and Koenig, H.M. Alpha globin gene organization in blacks precludes the severe form of alpha thalassaemia. *Nature*, 1979; 280:605
10. Wasi, P. Haemoglobinopathies including thalassaemia. Part I. Tropical Asia. *Clin. Haematol.*, 1981; 10:707.
11. Pembrey, M.E., Weatherall, D.J., Clegg, J.B., Bunch, C. and Perrine, R.P. Haemoglobin Bart's in Saudi Arabia. *Br. J. Haematol.*, 1975; 29:221.
12. Chouhan, D.M., Sharma, R.S. and Parekh, J.G. Alpha-thalassaemia in India. *J. Indian Med. Assoc.*, 1970; 54:364.
13. Vora, S., Iyer, P.D., Bhagat, M.P., Patel, J.C. and Mehta, B.C. Incidence of hemoglobin Bart's in cord blood samples. *Indian J. Med. Sci.*, 1975; 29:205.
14. Khan, L.R. and Hayee, A.A. Study of haemoglobin Bart's in cord blood. *JPM.*, 1986; 36:285.
15. Pornpatkul, M., Wasi, P. and Na-Nakorn, S. Haemoglobin parameters in obligatory alpha-thalassaemia. *J. Med. Ass. Thai.*, 1969; 52: 801.
16. Todd, D., Lai, M.C.S., Braga, C.A. and Soo, H.N. Alpha-thalassaemia in Chinese: Cord blood studies. *Br. J. Haematol.*, 1969; 16: 551.
17. Hegde, U.M., White, J.M., Hait, G.H. and Marsh, G.W. Diagnosis of alpha thalassaemia from Coulter Counter 'S' indices. *J. Clin. Pathol.*, 1977; 30 : 884.
18. Lie-Injo, L.E., Solai, A., Herrera, A.R., Nicolaides, L., Khan, Y.W., Wan, W.P. and Hasan, K. Hb Bart's level in cord blood and deletions of alpha globin genes. *Blood*, 1982; 59 : 370.
19. Weatherall, D.J., Pressley, L., Clegg, J.B., Weatherall, D.J., Higgs, S., Carey, P. and Serjeant, G.R. Detection of alpha thalassaemia in Negro infants. *Br. J. Haematol.*, 1980; 46 : 39.
20. Smith, M.B. and Cauchi, M.N. Incidence of alpha thalassaemia in Australian population of Mediterranean origin. *Med. J. Austr.*, 1978; 2:357.
21. Pootrakul, S.N., Wasi, P., Pornpatkul, M. and Na-Nakorn, S. Incidence of alpha-thalassaemia in Bangkok. *J. Med. Ass. Thai.*, 1970; 53: 250.
22. Higgs, D.R., Pressley, L., Clegg, J.B., Weatherall, D.J. and Serjeant, G.R. Alpha thalassaemia in black populations. *Johns Hopkins Med. J.*, 1980; 146: 300.
23. Chouhan, D.M., Sharma, R. S. and Parekh, J.G. Alpha-thalassaemia in India. *J. Indian Med. Assoc.*, 1970; 54: 364.