ISSN: 2320-1983 Print ISSN: 2320-1991 Online Volume 2 Issue 3

Applied Cell Biology

Short Communication

ACB, 2(3), 2013 [121-123]

Carrier detection of â thalassaemia: An emerging role of HPLC

S.Pandey[1]*, R.Saxena[1], R.M.Mishra[2], U.K.Chauhan[3], M.Sharma[4], Sw.Pandey[3]

[1]Dept of Hematology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi - 110 029, (INDIA)

[2]Dept of Environmental Biology, APS University, Rewa - 486003, (INDIA)

[3]Centre for Biotechnology Studies, APS University, Rewa - 486003, (INDIA)

[4]Dept of Hematology, Safdarjung Hospital, Ansari Nagar, New Delhi - 110029, (INDIA)

E-mail: pandeysanjaybt@rediffmail.com

Received: 1st July, 2013 ; Accepted: 30th August, 2013

ABSTRACT

Beta thalassemia is one of the commonest recessive genetic diseases. It results in a severe anemia needing monthly transfusion. Our aim was to evaluate hematological profile of cord blood HPLC for prenatal diagnosis of beta thalassemia. Cord blood samples at 18-24 weeks gestation from 113 mothers suspected for beta thalassemia was studied. Out of them 34 was maternal contamination. HbF& HbA0 were determined by cation exchange high performance liquid chromatography. In HPLC studies for beta thalassemia, 13 fetuses were affected, 32 were carriers and 34 were normal. ! 2013 Trade Science Inc. - INDIA

KEYWORDS

Cord blood;
HPLC;
Beta thalassemia.

INTRODUCTION

Thalassaemia is a heterogeneous group of autosomal ressesive disorders due to mutations causing decreased or absent production of the polypeptide globin chains. The distribution of beta thalassemia gene is not uniform in the Indian subcontinent. The highest frequency of beta thalassemia trait is reported in Gujarat, followed by Sindh, Punjab, Tamil Nadu, South India and Maharashtra (1.9%) so prenatal diagnosis of beta thalassemia is very useful for prevention of affected child birth[1]. The introduction of a new simple HPLC method allowing precise quantification of the various Hb fractions prompted us to evaluate its reliability in prenatal diagnosis of thalassemia and hemoglobinopathies[2]. This method, which utilizes a commercial column designed for hemoglobin fractions measurement, allows much more rapid and precise evaluation of fetal blood samples in the mid trimester of pregnancy[3].

MATERIALS AND METHOD

Total 113 cord blood sample were collected for the study. They were come from *Obstetric & Gynecology* OPD for screening of beta thalassemia in hematology department AIIMS. Blood samples were collected in 2 ml vacutainer containing EDTA as an anticoagulant. Complete blood count and red cell indices were measured by automated Analyzer (SYSMEX K-4500, Kobe Japan). Quantitative assessment of hemoglobin, HbF, HbA0 were performed by HPLC (Bio-Rad-VariantTM Bio Rad, CA, USA)

Short Communication

RESULT AND DISCUSSION

Total 113 cord blood were included in the study out of them 34 were maternal contaminated while 34 (mean age 27.3±3.9) were normal, 32 (mean age 26.7±3.7) were carrier and 13 (mean age 27.7±3) were affected. Hematological features are summarized in TABLE 1. HbF were 100% in affected cord blood and HbA0 level were <.8; however mean Hb (11.5±0.9) and red cell indices were elevated. Beta thalassemia is common in Asian Indians with carrier frequency of 3-7 % (8). Many thalassemia major children are born every year and require regular transfusions to sustain life[4]. HPLC is a sensitive and precise method for detecting thalassemia and abnormal Hbs[5-8]. It has become the preferred method for thalassemia screening because of its speed and reliability. An automatic HPLC system (VARIANT(TM), Bio-Rad) has been developed primarily for the detection of ß-thalassemia disorders such as ß-thalassemia carriers, Hb S and Hb C. However, information is quite limited about using such a system to study the complicated α thalassemia and ß-thalassemia syndromes in Southeast Asia[9]. A recent Indian studyperformed PND for thalassemia major in 58 cases by automated chromatography. Adult hemoglobin (HbA) levels in homozygous beta-thalassemia fetuses varied from 0% to 0.4%. The normal or heterozygous fetuses had beta/alpha ratios of >0.04 and HbA ranging from 2.1% to 10.6%[10]. Other studies on HPLC diagnosis of thalassemia major had been performed in large subjects. The largest study studied 212 cord blood samples at 18-22 weeks gestation and detected 44 affected fetuses[11,12]. We conclude that the Hb level and red cell indices show same level in affected, carrier and normal thalassemia cord blood, a measurement by the HPLC technique proposed should be the reliable method for prenatal diagnosis of thalassemia and related disorders in the mid trimester of pregnancy.

TABLE 1 : Hematological profile of affected, carrier & normal cord blood

Type	HbF	HbA0	WBC Ths/µl	RBC millions/µl	HGB g/dl	HCT %	MCV Fl	MCH Pg	MCHC g/dl	PLT Ths/µl
Affected	100±2.2	0.5±0.7	15.71±19.10	2.7±0.2	11.5±0.9	35.5±2.3	127.4±7.9	41.5±2.3	32.5±1.1	203.4±38.5
Carrier	96.9±3.6	2±0.4	12.46±11.32	2.7±0.3	11.37±1.27	35.6±3.9	130.9±8.9	41.62±3.33	31.9±1.4	216.1±63.6
Normal	94.3±2.8	5±1.7	15.3±19.7	2.5±0.6	10.81±2.60	33.9±7.6	131.5±10.4	42.14±3.70	32±2.1	178.3±59.9

REFERENCES

[1] S.S.Ambekar, M.A.Phadke, G.D.Mokashi, M.P.Bankar, V.A.Khedkar, V.Venkat, D.G.Basutkar; Pattern of hemoglobinopathies in Western Maharashtra. Indian Pediatr., **38**, 530-534 **(2001)**.

[2] A.Mosca, A.Carpinelli, R.Maiavacca, et al.; A new HPLC method for simultaneous determination of HbA2 and HbF evaluated in haematological practice. J.Autom.Chem., **11**, 273-9 **(1989)**.

[3] Rita Malavacca, Silvana Tedeschi, Andrea Mosca, Sara Calmi, Patrizia Do Leonardis, Clara Todaro, Guglielmo Zuliani, et al.; Nonradioactive quantification of low concentrations of hemoglobin A by HPLC for midtrimester prenatal diagnosis of J3-thalassemia, Clin.Chem., **38/9**, 1906-1908 **(1992)**.

[4] D.R.Higgs, S.L.Thein, W.G.Wood; Distribution and population genetics of the thalassemias. In D.J.Weatherall, J.B.Clegg, (Eds); The thalassemia syndromes, 4th Edition, Oxford: Blackwell Science, 237-284 **(2001)**.

[5] J.B.Wilson, M.E.Headlee, T.H.J.Huisman; A new high performance liquid chromatographic procedure for the separation and quantitation of various hemoglobin variants in adults and newborn babies. J.Lab.Clin.Med., **102**, 174-185 **(1983)**.

[6] B.B.Rogers, R.A.Wessels, C.N.Ou, G.J.Buffone; High performance liquid chromatography in the diagnosis of hemoglobinopathies and thalassemias. Am.J.Clin.Pathol., **84**, 671-674 **(1985)**.

[7] G.B.Tan, T.C.Aw, R.A.Dunstan, S.H.Lee; Evaluation of high performance liquid chromatography for routine estimation of haemoglobin A_2 and F. J.Clin.Pathol., **46**, 852-856 **(1993)**.

[8] F.Lorey, G.Cunningham, F.Shafer, B.Lubin, E.Vichinsky; Universal screening for hemoglobinopathies using high-performance liquid chromatography: Clinical results of 2.2 million screens. Eur.J.Hum.Genet., **2**, 262-271 **(1994)**.

[9] S.Fucharoen, P.Winichagoon; Hemoglobinopathies in Southeast Asia: Molecular biology and clinical medicine. Hemoglobin., **21**, 299-319 **(1997)**.

[10] M.R.Wadia, S.F.Phanasgaokar, A.H.Nadkarni, R.R.Surve, A.C.Gorakshakar, R.B.Colah, et al.; Usefulness of automated chromatography for rapid fetal blood analysis for second trimester prenatal

diagnosis of beta-thalassemia. Prenat.Diagn., **22**, 153-157 **(2002)**.

[11] V.B.Rao, P.G.Natrajan, C.P.Lulla, S.B.Bandodkar; Rapid mid-trimester prenatal diagnosis of beta-thalassaemia and other haemoglobinopathies using a non-radioactive anion exchange HPLC technique—an Indian experience. Prenat.Diagn., **17**, 725-731 **(1997)**.

[12] R.Maiavacca, S.Tedeschi, A.Mosca, S.Calmi, P.De Leonardis, C.Todaro, et al.; Nonradioactive quantification of low concentrations of hemoglobin A by HPLC for midtrimester prenatal diagnosis of beta-thalassemia. Clin.Chem., **38**, 1906-1908 **(1992)**.