Brief Reports

Cord Blood Analysis for Prenatal Diagnosis of Thalassemia major and Hemophilia A

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Beta-thalassemia and Hemophilia A are common genetic disorders for which prenatal diagnosis (PND) is an accepted option. Our aim was to evaluate cord blood analysis as a method for PND of these disorders. Cord blood samples at 18-26 weeks gestation from nine mothers with previous thalassemia major child and five families with previous hemophilia A were studied. In the former; HbF, HbA2 and HbF were determined by high performance liquid chromatography (HPLC) and in latter; Factor VIII and IX assays were done by one stage method. In HPLC studies for thalassemia, three out of nine fetuses were affected, five were carriers and one was normal. In hemophilia PND samples, 2 out of five fetuses were affected. Thus, HPLC and factor VIII assay in cord blood are feasible alternatives for PND in β-thalassemia and hemophilia A respectively, especially when DNA analysis is uninformative or there are financial constraints.

Keywords: *Cordocentesis, Factor VllI, HPLC, Linkage analysis, Thalassemia major.*

BETA-THALASSEMIA in its severest form, thalassemia major is transfusion dependent. The common five Indian β-thalassemia mutations are β^0 or severe β^+ and account for 90% of mutations(1). However, molecular diagnosis is not possible in families at-risk especially with non-informative DNA analysis. HbA ($\alpha_2\beta_2$ tetramer) is normal adult hemoglobin. The fetus who is β^0/β^0, or β^0 / severe β^+ or severe β^+/ severe β^+ is likely to have no HbA or marked reduction in HbA synthesis. Normal fetuses have demonstrable low levels of HbA in mid-trimester of pregnancy. This is further reduced or absent in fetus who carries β^0 or severe β^+ on both chromosomes. Quantification of HbA in cord blood is advocated as another option for prenatal diagnosis (PND). Similarly, hemophilia A is common bleeding disorder manifesting in males. The usual method for PND is by linkage analysis in chorionic villi samples. In severe hemophilia families, analysis of Intron 22 and Intron 1 inversion mutations can be tried with approximately 50% results(2). However, in families where linkage is not informative and direct mutational analysis is not possible, cord blood factor VIII assay can be used to detect affected cases. In present communication, we present our experience on cord blood analysis for above two disorders.

Subjects and Methods

The subjects for thalassemia major PND included mothers with previous thalassemia major child attending Pediatrics/Gynecology outpatient clinic in whom DNA analysis detected only one causative mutation. The subjects for cord blood analysis for hemophilia were from families with sporadic hemophilia cases or with previous hemophilia A child where linkage analysis and inversion Intron 1 and 22 were not informative.

Most patients came late in pregnancy and legal limit of MTP (MTP Act, 1971) is 20 weeks. So the respective couples were told that abortion would not be possible even if the fetus were detected affected. Still, they insisted that the tests be done for psychological satisfaction.

Cord blood was obtained at 18-26 weeks gestation in EDTA vials for high performance liquid chromatography (HPLC) diagnosis of thalassemia. The hemolysate was prepared with appropriate dilution and was performed on Variant™ (Biorad) and HbA, HbF and HbA_2 were estimated. The cut-off of HbA used for differentiating affected, carriers and normal fetuses were 0-0.8, 0.8-2.8 and 2.9-7.4 respectivly(3). In all but one case, hemogram was also done on automated counter (Sysmex K 4500).

For factor VIII assay, cord blood at 18-23 weeks gestation was drawn into non-heparinised syringe and collected in sodium citrate. Both Factor VIII and IX assay were performed by established method(4). The normal values taken for F VIII were 25-53% and for F IX 9-11%, respectively(5). Sex of fetus was determined with XY amelogenin gene primers(6) in hemophilia families. To rule out maternal contamination, Apo B molecular marker was tested(7).

Results

Nine cord blood samples from mothers with previous β-thalassemia major child were studied by HPLC. On basis of HbA level, three fetuses were diagnosed affected (HbA_0 <0.2%), 5 were carriers (HbA_0 2-4.6%) and one was likely to have normal β-globin genes (HbA_0 6.8%). The Hb, MCV, MCH, MCHC, HbF and HbA values are listed in *Table I.*

Five cord blood samples (18-23 wks) from male fetuses were analyzed for prenatal

TABLE I–*Hematological Values in Cord Blood Samples Studied for Prenatal Diagnosis of Thalassemia Major.*

S.No. Sample code	Gestation (weeks)	Hb (g%)	MCV (fl)	MCH (pg)	MCHC (pg/dL)	HbF (%)	HbA (%)	Status
(1) KR	23	11.6	115	37.8	32.9	95.8	0.2	Affected
(2) RG	18	10.2	117.7	41.1	34.9	98.0	2.0	Carrier
(3) AJ	20	11	126	43.5	34.5	96.7	3.2	Carrier
(4) NR	22	–	–	–	–	76.9	6.8	Normal*
(5) SR	26	13	117.2	39.2	33.4	97.5	2.5	Carrier
(6) KS	24	11.6	138.6	47.2	34.0	95.4	4.6	Carrier
(7) SN	20	12.7	112	37.4	33.3	97.9	0.1	Affected
(8) BW	18	11.7	133	42.4	31.9	97.1	2.1	Carrier
(9) SL	25	11.9	126	43.6	34.6	99.7	0.1	Affected

Note: *D window 15.2 %

diagnosis of hemophilia A. Two of fetuses from sporadic hemophilia families were found to be affected with hemophilia A (level < 1%) and rest three (F VIII 23, 27 and 66%, F IX: 12, 26 and 10% respectively) were detected to be normal. Of normal fetuses, one was from sporadic hemophilia family and other 2 were from families with more than one affected case.

In hemophilia PND cases, tests were repeated after abortion/delivery and no discrepancy was noted in results. In thalassemia PND cases, six out of nine couples came for repeat testing after delivery and results were comparable to antenatal results. Rest did not come for re-testing.

Discussion

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. The management demands enormous commitment and patience on part of parents. Similarly, Hemophilia A is commonest X-linked bleeding disorder manifesting in males. Severe hemophilia (Factor VIII <1%) can have life threatening bleeding manifestations necessitating repeated infusions. However, due to financial constraints, Factor VIII cannot be given for all bleeding episodes in majority of the patients.

Thalassemia and hemophilia account for considerable proportion of disorders coming for genetic counseling(9). Prenatal diagnosis is an accepted option for prevention and control of thalassemia and hemophilia A in at-risk families. DNA diagnosis is usually done on chorionic villus samples at 10-12 weeks gestation in informative families(1,10-12). However, it has certain limitations. There are very few centers for molecular diagnosis and couple has to travel to established center for prenatal sampling and DNA analysis in majority of cases. Moreover, for DNA diagnosis mutations should be detected or the markers should be informative for linkage. Linkage requires sample from the affected hemophilia child, which is not available in all cases. When family comes late in pregnancy and common mutations are not identified in thalassemia, it is not possible to further characterize the unidentified mutation due to time constraints.

Cordocentesis is a safe technique in experienced hand(13). HPLC is sensitive and reliable for quantification of different Hb fractions. In fetuses with β^0/β^+ mutations in homozygous or compound heterozygous state, HbA levels are absent or markedly reduced. In this study, we used cut-off as per Thai study(3) as most mutations there are also β^0 mutations. Of nine patients studied, we could detect three affected fetuses. One fetus had normal β-globin genes but had an unknown Hb (? HbQ India) and is to be retested at 6 months age when HbF levels come down to adult levels.

A recent Indian study by Wadia, *et al.*(14) performed 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%. Other studies on HPLC diagnosis of thalassemia major include that by Rao, *et al.*(15) and Maivacca, *et al.*(16). The largest study reported was by latter group which studied 212 cord blood samples at 18-22 weeks gestation and detected 44 affected fetuses.

We could detect 2 affected cases of hemophilia A by Factor VIII assay in cord blood. There is only one previous report from

Key Message

- Cord blood analysis can be done in selected families at-risk of thalassemia major and hemophilia A.

India(17) in which Factor VIII level was assessed in cord blood for prenatal diagnosis. In centers, where facility for DNA linkage and direct detection of inversion mutation is not available, this option can be tried. Only a few precautions are required for the test. One is collection of samples in non-heparinised tubes and another is estimation of both F VIII and F IX assay to avoid wrong diagnosis due to problem in testing procedure. Third precaution that has to be taken in analysis of all cord blood samples is checking for maternal contamination, as this is likely to influence the results. This can be taken care of by studying for molecular marker like Apo B or by staining of HbF containing cells by Kleihauer-Betke test.

It is thus concluded that cord blood analysis may be an acceptable alternative for prenatal diagnosis of thalas-semia major in cases where, (*a*) DNA diagnostic facilities are not available, (*b*) one or both mutations are unidentified or mole-cular markers for linkage are uninformative. In hemophilia families, cord blood analysis is helpful in cases where (*a*) linkage analysis is not possible (proband unavailable for testing) or molecular markers for linkage are uninformative; and (*b*) inversion (intron 22 and intron 1) mutation is absent or in sporadic hemophilia families. Further larger studies are warranted so that separate cut offs for affected, unaffected and carriers can be established for HPLC with reference to Indian β-thalassemia mutations and cord blood factor VIII and IX levels.

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