Prenatal Diagnosis of Thalassaemia by Chorionic Villus Sampling: A Clinico- Hematological Study in a Government Medical College of Eastern India

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Abstract: Thalassemia and other hemoglobinopathies are genetic disorders of hemoglobin, which can be prevented by population screening and offering genetic counselling. It is a preventable disease as proved by countries like Italy, Greece and Cyprus by establishing successful national programs resulting in significant reduction in the births of affected children. The main prevention strategies comprise of targeted screening of thalassemic families, extended family screening, screening before marriage and prenatal diagnosis for Thalassemia. Pre-natal diagnoses of single gene disorders and chromosomal abnormalities by CVS is now known and well established procedure. Early diagnosis is important to prevent any possible complications. With this scientific background, the present study is being conducted as a part of State Thalassemia Control Program by Government of West Bengal with an aim to diagnose the genetic status of fetus of carrier parent so that thalassemia can be prevented at that stage only. In the present study, 235 cases of chorionic villus sampling are studied over a period of one year regarding age group wise distribution of mothers, HPLC diagnosis of Mother and father, Timing of chorionic villus sampling (CVS) and complication, Mutant Allele status and Diagnosis by chorionic villus sampling and the results are tabulated. In our present study, CVS show normal genotype in 110 out of 235 cases (46.80%), 70 cases (29.78%) came out to be B thal trait. 5 cases came out to be of E trait genotype. 30 cases (12.76%) came out to have E B thal genotype and 20 cases (8.51%) cases of B thal Major. So it can be concluded that CVS is an acceptable, reliable and safe method of early diagnosis of fetal thalassaemia that helps to prevent birth of the thalassaemia-affected babies with considerable reduction of socio-economic burdens.

Key words: Chorionic villus sampling (CVS), Prenatal diagnosis, Thalassemia.

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I. Introduction

Thalassemia and other hemoglobinopathies are genetic disorders of hemoglobin, which can be prevented by population screening and offering genetic counselling. In India it is estimated that there are over 25 million carriers of this disease¹. Beta-thalassemia (β-thalassemia/B thal), sickle cell anemia, E-beta thalassemia and hemoglobin D Punjab are the common hemoglobinopathies in the world including India. About 4.5% of world population is affected by hemoglobinopathies².

Thalassemia in children, beside physical and emotional suffering, puts a major strain on national resources. The birth incidence calculated for homozygous thalassemics would be 11316 per year in India which are added each year to the existing load of homozygous thalassemics³. Majority of these children become transfusion-dependent and have reduced life expectancy. Till date, the most cost-effective way of prevention of the birth of thalassemia-affected babies is prenatal diagnosis (PND). It is a preventable disease as proved by countries like Italy, Greece and Cyprus. They were amongst the first to establish successful national programs resulting in significant reduction in the births of affected children. The main prevention strategies comprise of targeted screening of thalassemic families, extended family screening, screening before marriage and prenatal diagnosis for thalassemia⁴. Over the last decade, techniques of first trimester fetal tissue sampling have enabled diagnosis of many genetic disorders to be made early in pregnancy thus allowing patients to have the option of a pregnancy termination if the fetus is affected⁵. Prenatal diagnoses of single gene disorders and chromosomal abnormalities by CVS is now known and well established procedure. Early diagnosis is important to prevent any possible complications.
Chorionic Villus Sampling (CVS) is one of such prenatal diagnostic method, in which few villi from the chorion frondosum are collected by trans-cervical (TC-CVS) or trans-abdominal (TA-CVS) route. CVS has the advantage of early termination of pregnancy in a relatively safer way in case the results are abnormal. Again, if the CVS result is normal, it is very much reassuring for the couple. Many studies showing the effectiveness of CVS have been done in other countries. We have not come across a study of notable size on CVS in Eastern India in the literature.

The advantage of procedure is the diagnosis of disease in early pregnancy (7-12 weeks) and thus early termination of pregnancy is associated with lower morbidity and decreased psychological trauma. CVS is a technique for retrieval of fetal cells from developing pregnancy during the first trimester. Transcervical approach, was used initially and was first to be started clinically in Europe and Northern America. In 1984, the alternative trans-abdominal approach was introduced. This technique, offered benefits of lowered risk of infection and higher patient acceptability. To-date all over the world multiple studies have demonstrated high efficacy, safety and acceptability of this procedure, and to be the gold standard for prenatal diagnosis of thalassemia.

Haemoglobin E is widespread in the north eastern States. In eastern India the prevalence of Hb E trait varies from 3-10 per cent in West Bengal. Both Hb E and Hb S when co-inherited with β-thalassaemia result in a disorder of variable clinical severity. It is also important to identify carriers of δβ-thalassaemia as compound heterozygotes of δβ-thalassaemia and β-thalassaemia can lead to a severe disorder. δβ-thalassaemia carriers are characterized by a modest elevation in Hb F levels (5-20%) with reduced or normal HbA2 levels and hypochromic and microcytic red cells. This phenotype partially overlaps with that of carriers of hereditary persistence of foetal haemoglobin (HPFH) and genotyping of high Hb F determinants is required as these are not infrequent in India. Screening in antenatal clinics is the best way to identify couples at immediate risk of having an affected child. However, experiences in India have shown that only 15-20 per cent of pregnant women come to antenatal clinics in public hospitals in the first trimester of pregnancy when prenatal diagnosis should ideally be done. This emphasizes the need for generating awareness in the population for early registration in antenatal clinics as well as among obstetricians to ask for a screening for β-thalassaemia and other haemoglobinopathies along with other investigations which are done routinely. The other target groups for screening in India include high school children, college or university students, high risk communities and extended family members of affected children. The latter cascade screening approach appears to be a practical way of identifying a larger number of β-thalassaemia carriers in a cost-effective way in India. β-thalassaemia is extremely heterogeneous with more than 200 mutations described worldwide. In India, about 64 mutations have been characterized by studies done at different centres. Six to seven mutations [IVS 1-5 (G>C), 619 bp deletion, IVS 1-1 (G>T), Codon 8/9 (+G), Codons 41/42 (-CTTT),Codon 15 (G>A), Codon 30 (G>C)] are common accounting for 85-95 per cent of mutant alleles. However, regional differences in their frequencies have been noted. The prevalence of IVS 1 -5 (G>C), the most common mutation in India varies from 15-88 per cent in different States. Codon 15 (G>A) is the second most frequent mutation in Maharashtra and Karnataka and Codon 5 (-CT) is the third most common mutation in Gujarat. The -88 (C>T) and the Cap site +1 (A>C) mutations are more common in the northern region.

With this scientific background, the present study is being conducted. This work is being carried out as a part of State Thalassemia Control Program by Government of West Bengal with an aim to diagnose the genetic status of fetus of carrier parent so that thalassemia can be prevented at that stage only.

II. Materials And Methods

Many Thalassemia control units have been established in different district hospitals and medical colleges to cover the maximum number of people to identify Thalassemia carriers by HPLC using Bio Rad Polymerase chain reaction amplification refractory mutation system (PCR-ARMS) technique was used, the beta-Thalassemia mutation was detected by PCR-ARMS. Allele encountered in our study was IVS-1-5 (G→C), Codon 6 (A→T), codon 15 (G→A), FS 41/42 (-CTTT), FS 8-9 (+ G), Codon 30 (G→C), Codon 26 (G>A).

The B thalassemia is inherited in an autosomal recessive manner. At conception, each sib of an affected individual has a 50% chance of being an asymptomatic carrier and 25% chance of being unaffected and not even a carrier. Heterozygotes(carriers) may be slightly anemic but are clinically asymptomatic.
In the present study, 235 cases of chorionic villus sampling are studied over a period of one year regarding 1) Age group wise distribution of Mothers, 2) HPLC diagnosis of Mother and father, 3) Timing of Chorionic villus sampling (CVS) and complication, 4) Mutant Allele status and 5) Diagnosis by chorionic villus sampling and the results are tabulated below.

Following are the limitation of CVS:
1. A small percentage of individuals who are carriers or have a disease diagnosis, may have a mutation that is not identified by this method. The absence of a mutation therefore does not eliminate the possibility of positive carrier status or the diagnosis.
2. The error rate of Taq polymerase for base incorporation is 1 in 6000 bases.
3. Note that benign polymorphism are not reported.
4. This assay is unable to differentiate between cis and trans mutations.

### III. Results

#### Table 1: Age group wise distribution of Mothers

<table>
<thead>
<tr>
<th>Age group (yrs)</th>
<th>18-30 yrs</th>
<th>&gt;30-40 yrs</th>
<th>&gt;40 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers (n=235)</td>
<td>215 (91.4%)</td>
<td>20 (8.51%)</td>
<td>nil</td>
</tr>
</tbody>
</table>

#### Table 2: HPLC diagnosis of Mother and father

<table>
<thead>
<tr>
<th>HPLC diagnosis</th>
<th>Mother (n=235)</th>
<th>Father (n=235)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thal B trait</td>
<td>200 (85.10%)</td>
<td>190 (80.85%)</td>
</tr>
<tr>
<td>E trait</td>
<td>35 (14.89%)</td>
<td>30 (12.76%)</td>
</tr>
<tr>
<td>Others</td>
<td>nil</td>
<td>10-EB trait, 5-E disease</td>
</tr>
</tbody>
</table>

#### Table 3: Timing of Chorionic villus sampling (CVS) and complication

<table>
<thead>
<tr>
<th>CVS time</th>
<th>No. of cases</th>
<th>Complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12 wk</td>
<td>05 (2.12%)</td>
<td>Nil</td>
</tr>
<tr>
<td>12-14 Wk</td>
<td>210 (89.36%)</td>
<td>Nil</td>
</tr>
<tr>
<td>&gt;14 Wk</td>
<td>20 (8.51%)</td>
<td>Nil</td>
</tr>
</tbody>
</table>

#### Table 4: Mutant Allele status

<table>
<thead>
<tr>
<th>Mutant Allele status</th>
<th>No. of cases (n=235)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous</td>
<td>30 (12.76%)</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>80 (34.04%)</td>
</tr>
<tr>
<td>Compound heterozygous</td>
<td>15 (6.38%)</td>
</tr>
<tr>
<td>Absent</td>
<td>110 (46.80%)</td>
</tr>
</tbody>
</table>

#### Table 5: Diagnosis by chorionic villus sampling.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>110 (46.80%)</td>
</tr>
<tr>
<td>B trait</td>
<td>70 (29.78%)</td>
</tr>
<tr>
<td>E trait</td>
<td>05 (2.12%)</td>
</tr>
<tr>
<td>E B thal</td>
<td>30 (12.76%)</td>
</tr>
<tr>
<td>B thal major</td>
<td>20 (8.51%)</td>
</tr>
</tbody>
</table>

### IV. Discussion

Standard management of Beta thalassemia includes blood transfusion and iron chelating therapy (to control the deleterious effects of progressive iron overload). Bone marrow transplant from HLA-identical siblings in patients who have no evidence of iron overload results in disease-free survival and has significantly improved not only the survival but also the quality of life for thalassemia patients. However, in developing countries where thalassemia is prevalent and health care resources are limited, these forms of management are usually not easily affordable. So prevention is the most effective and least expensive means of dealing with this problem. The present study of CVS clearly supports the above notion. Correct prenatal diagnosis can make the parent to take decision regarding continuation of pregnancy/abortion without any health hazard to the mother.

As per table 1, in our study, we get 91.4% mothers (215 out of 235) are in the age group of 18-30 yrs. Only 8.51% mothers are in the age group of >30-40 yrs.

Table 2 elaborates the HPLC diagnosis of the parent. Out of total 235 mothers (n=235), 200 mothers are beta thal trait (85.10%) and 14.89% are E trait as we found in our study. In case of male, 80.85% persons are Thal B trait, 12.76% are E trait. 10 cases are EB trait and 5 cases are having Hb E disease.

Table 3 elaborates the timing of chorionic villus sampling in mothers with occurrence of complication. Most CVS was performed in 12-14 weeks gestational time (210 out of 235 cases). In 20 cases CVS was
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performed after 14wk of gestational period(8.51% cases) In 5 cases CVS was performed below 12 wk of gestational age(2.12% cases). No complication was encountered in any of the cases. In several studies the sampling success rate of CVS varied between 97-100%, which increased with advanced gestation. In one study the procedure failed to obtain an adequate sample in 0.3% cases. In our study, the sampling success rate was 100% as in all cases chorionic tissue was collected.

Table 4 elaborates the mutant allele status in chorionic villus samples. Here, it is observed from the above data set and result that IVS1-5(G-C) heterozygous are the most dominant genotypes in the Kolkata extended city area of Eastern India based state named West Bengal. HBB is located at chromosome 11p15.4. The assay test for mutation is PCR and sequencing. Assay is against common asian mutation.619 bp deletion and mutant nomenclature is IVS1-5(G>C). In the present study,30 cases(12.76%) come out to be homozygous. 80 cases are heterozygous(34.04%). 15 cases appears to be compound heterozygous(6.38%). Absence of mutant allele is seen in 110 cases(46.80%). This mutation leads to formation of premature stop codon in the protein sequence as a result of splice site alteration. The resultant protein is truncated and unable to function effectively.

However there are limitations to this test also. A very small percentage of individuals, who are carriers or have a disease diagnosis, may have a mutation that is not identified by this method. Absence of a mutation, therefore does not eliminate the possibility of positive carrier state or the diagnosis. For carrier testing, it is important to first document the presence of a gene mutation in an affected family member, with subsequent targeted mutation analysis of the same mutation in suspected carriers. Furthermore the error rate of the Taq polymerase for base incorporation is 1 in 6000 bases.

In our present study, CVS study shows normal genotype in 110 out of 235 cases(46.80%). 70 cases(29.78%) came out to be beta thal trait. 5 cases came out to be of e trait genotype. 30 cases(12.76%) came out to have E B thal genotype and 20 cases(8.51%) cases of B thal Major(table 5). In one study, result of CVS showed that among these 28 cases, were diagnosed as thalassemia major and 9 as thalassemia minor. None of the patients reported for any complication after getting CVS done. In another study done for intrauterine diagnosis of thalassemia major by CVS in 60 couples with thalassemia trait, 28(47%) were homozygous for beta-thalassemia, 8(13%) thalassemia minor and 24(40%) cases were normal. In the same study 4(2%) out of 60 women had a spontaneous fetal loss after the chorionic villous sampling. In another study done in Iran on outcome of CVS in 300 women, 18% fetuses turned out to be of thalassemia major and rate of spontaneous abortion was 1.4%.

In another study done on geographic distribution and safety of CVS, out of 223 cases in which CVS was done 43% were Thalassemia minor, 38% thalassemia major and 19% were normal and rate of pregnancy loss after CVS was 2%.

Another study done in Multan on DNA analysis of post CVS samples revealed that 12(20%) out of sixty fetuses studied were homozygous for thalassemia and all the couples opted for termination of pregnancy. Post CVS fetal loss has been reported from 1.3-3.0 %. In the above mentioned study fetal loss was not observed in any case after the procedure, the findings comparable with our results.

Inconclusive results may be a problem after CVS. One study reported that 3.4% of CVS had inconclusive result. However, in our study, none of the patient followed up had inconclusive results. (Table 5).

A Chinese study reported that 12% of the fetuses had thalassemia major after CVS. Conversely, an Iranian study also demonstrated 18% of fetuses were diagnosed to have beta thalassemia major. Similarly, in our study, 8.51 % of the fetuses were diagnosed to have thalassemia major, consistent with the other Asian studies (Table 5).

In this study, we also initiated antenatal screening and counselling as well as a genetic counselling program mainly in the districts of Kolkata (WB). Colah et al reported that antenatal screening is acceptable in India; however, awareness generation is still a primary requisite. Several programs, with the aim of preventing homozygous b thalassemia, based on carrier screening and counselling of couples at marriage; preconception or early pregnancy, are operating in several at-risk populations in Mediterranean areas. Until gene therapy becomes a reality, the only approaches to the control of hemoglobinopathies are prevention and avoidance. Cao et al. reported reduction of the birth rate of thalassaemia major from 1:250 live births to 1:4,000 in Turkey after execution of comprehensive genetic preventive programme based on voluntary screening and non-directive counselling. Our study also show that prenatal diagnosis by CVS in motivated parent can add appreciable momentum to thalassemia control program in our country.

V. Conclusions

Thalassaemia is a prevalent condition in the population we studied. Obstetricians have to play important role by generating awareness among the general population as well as screening of antenatal population for thalassaemia besides other routine antenatal investigations. CVS is an acceptable, reliable and safe method of...
early diagnosis of fetal thalassemia that helps to prevent birth of the thalassemia-affected babies with considerable reduction of socio-economic burdens. Thalassemia is a genetic disorder, if we really want to eradicate it from our society where about 10% of the population is thalassemia carrier this routine screening is the quintessential way which is cost effective and can be performed within a viable time limit.

Limitation of the Study

Though the State Thalassemia Control Programme is going in the right direction but still quite a few obstacles have to be overcome since there are many social obduracy and village women are still obsessed with prejudices and social stigma. Regionally there are sporadic religious obstacle which is yet to be subverted. Despite having a healthy daughter many families opt for a male child and avid for it resulting sometimes in toll to the health security, a new thalassemia male child is added. They refuse PND in the hope of begetting a male child. Another limitation in this study was that pregnant women present for antenatal care at varied gestational age and uniformity for screening at specific gestational age could not be maintained.

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