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Pre-Natal Diagnosis of beta – Thalassemia by Chorionic Villous Sampling

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Abstract

Background: To promote the utility of Chorionic Villous Sampling (CVS) for pre-natal diagnosis of beta thalassemia in at risk population and to highlight its demographic area distribution and effect of consanguinity.

Method: This Cross Sectional Study was conducted in the Department of Gyne/Obstet-I, Holy Family Hospital, Rawalpindi, Pakistan, from June 1997 to June 2009. All 137 couples having either a previously affected conceptus/child with thalassemia or having strong family history of thalassemia, were recruited for the test. Samples collected by standard procedure were sent to Armed Forces Institute of Pathology, Rawalpindi for thalassemia detection.

Results: In 12 yrs 137 mothers underwent CVS. Mean gestational age at the time of procedure was 12.6 weeks. Consanguineous marriage was seen in 119 cases. Largest group was 'Punjabis'. 126 couples had a previously affected child with homozygous beta thalassemia and 11 had heterozygous beta thalassemia in their children. 95 cases had a family history of the disease. After DNA analysis, 30 cases (22%) were diagnosed to have homozygous beta thalassemia while 77(56%) were having heterozygous beta thalassemia. Report was normal in remaining 30 cases (22%). Early spontaneous pregnancy loss was seen in only 02 cases.

Conclusion: Thalassemia is one of the commonly inherited crippling disorders. Its familial transmission must be checked by effective public awareness programme, pre marital screening, genetic counseling and pre-natal diagnosis by CVS.

Key Words: Chorionic Villous Sampling, Pre-Natal Diagnosis, beta- Thalassemia.

Introduction

Thalassaemias are a heterogeneous group of disorders in which the production of normal haemoglobin is partly or completely suppressed because of diminished synthesis of one or more globin chains. According to the chain, which is deficient, several types like α, ß and γ thalassaemias have been described [1].Thalassaemia was first identified as a clinical entity in 1925 by Thomas Cooley and Pearl Lee [2] and more than a decade later, Wintrobe and colleagues described milder form of Cooley's anemia in both parents of the children with classic Cooley's anaemia [3].

ß thalassemia is the commonest autosomal recessive single gene disorder of haemoglobin synthesis. In Pakistan it is one of the most frequent inherited disorders, with carrier rate of 5.4%[4]. Worldwide carrier frequency is 1.4-7.96 %[5]. Many social problems like preference to marry within same ethnic group/consanguineous marriages have contributed to its increased incidence.

Chorionic Villous Sampling (CVS) was first introduced as a rapid means of pre natal diagnosis in early pregnancy in1980s[6]. For prenatal diagnosis, first trimester CVS is advantageous over second trimester amniocentesis[7] because of less emotional and physical stress in early pregnancy in couples at risk, a less obvious pregnancy and therefore more privacy. Moreover, if termination of pregnancy is indicated, it can be done in such cases at a safer time. The first report of successful CVS[8] for diagnosis of thalassemia was by Old et al. In Pakistan, prenatal diagnoses for ß-thalassemia was introduced in 1994, Since then, the use of this service has increased from 26 in 1994 to 381 in 2006. Over 97% of the couples who requested prenatal diagnosis already had an affected child [9]. To date, all over the world, multiple studies [3,4,8,10] have demonstrated high efficiency, safety and acceptability of the procedure suggesting that first trimester CVS should be the gold standard for prenatal diagnosis. Objective of our study was to promote the utility of the procedure and to give demographic area distribution of the disease with effect of consanguinity.

Subjects and Methods

All cases of CVS, done in our deptt for prenatal dignosis (PND) of ß thalassemia from June1997 – June2009 were studied retrospectively. Cases of PND for other chromosomal anomalies were not included. Couples were selected for the test after checking red cell indices and haemoglobin electrophoresis. After appropriate counseling of the

couple and a written consent, placental biopsy was taken under local analgesia with suction cannula by transabdominal approach. Specimen was sent in 0.9% Normal Saline to Armed Forces Institute of Pathology (AFIP), where analysis of the sample was done by ARMS (Amplification Refractory Mutation System) method. This method is a modified variant of PCR method. Data was collected on pre designed proforma and was entered and analyzed through SPSS version 10. For Quantitative variables (age, gestational age), means and standard deviation (S.D) were calculated and for Qualitative variables (demography, consanguinity, thalassemia), frequency and percentages were presented.

Results

In a 12-year period 137 mothers underwent CVS. Mothers were between the ages of 18-35 yrs. Mean maternal age was 27.4 yrs. Primigravida were only 04, mothers in their 2nd pregnancy were 21 and 112 were in ≥ 3rd pregnancy.

Consanguineous marriage was observed in 119 cases; out of these 105 were 1st cousin and 14 couples were relatives, while 18 couples were not related.

126 couples had a previously affected child with homozygous beta thalassemia and 11 had heterozygous beta thalassemia in their children. 95 cases had a family history of the disease but in 42 couples there was no such history.

CVS was attempted between the gestational ages of 07-23 weeks; mean gestational age at the time of procedure was 12.6 weeks.

After DNA analysis, 30 cases (22%) were diagnosed having homozygous beta thalassemia while 77(56%) were having heterozygous beta thalassemia. Report was normal in remaining 30 cases (22%). This disease distribution and consanguinity according to demographic area are shown in Table-1.

Early spontaneous pregnancy loss was seen in only 02 cases. All couples having fetus with homozygous beta thalassemia opted for termination of pregnancy except for one couple. In later months of pregnancy, premature rupture of membranes was seen in 04 cases.

Discussion

Pregnant women of all ages should be offered screening and invasive diagnostic testing for chromosomal abnormalities before 20 weeks' gestation. Diagnostic options include CVS in the first trimester and amniocentesis in the second trimester [11]. Introduction of first trimester screening programs has led to a decrease in the utilization of CVS, particularly among women 35 years or older [12]. However as patient gets instant results in 60.6% of cases; this appears to increase the use of CVS [13].

Over 200 mutations so far have been reported in β globin chain synthesis [14-16]. Out of these, 25% have been found in heterogenous Indian population [17,18]. Most of the children affected with this lethal disorder are born in developing countries [19,20].

In Pakistan there are 40,000 registered transfusion dependent thalassemic patients [4]. CVS remains the gold standard for PND of β thalassemia. Procedure is simple and safe. All our CVS were successful and done in first attempt except 05 cases, which required interval attempt. No failure was seen. All mothers who underwent CVS were already having an affected child with thalasemia mutation and a strong family history of the disease was seen in 95% couples. Major groups studied were Punjabi and Pathan families, as referrals to our hospital are mainly from Punjab and NWFP. In both groups thalassemia frequency was very high. Such result was also seen in other studies [4].

A carrier couple is at 25% risk of having a fetus with Cooleys anemia and at another 50 % risk of having a carrier child. In Punjabis, thalassemia was seen in 76% of our cases while 24 % were normal. In Pathan families this disease was in 82% cases. In both major groups studied, consanguinity was also high (86%, 88% resp.). The high prevalence of thalassemia in these areas is clearly explainable by the high degree of consanguinity, providing us a point for a breach in familial transmission of the disease. Countries like Greece, Italy, Iran, Iraq, Saudi Arabia and United Arab Emirates, where thalassemia mutation has almost come to an end, have achieved this goal by carefully designed health programme whereby inter-cousin marriages are discouraged. In our country cultural values are very religiously followed and there is very strong trend of marriage. within family for generation after generation. This practice is leading to an ever growing risk of increased transmission of, not only this autosomal recessive trait, but also of many other familial diseases. Affected families and country could be relieved of this avoidable burden only by effective public awareness programme, pre marital screening, genetic counseling and pre-natal diagnosis. In at risk population, inter cousin marriages should be discouraged . If both partners are carriers of the mutation they must be offered CVS in first trimester of pregnancy, so that a timely termination of pregnancy

with homozygous mutation could minimize the management load of this expensive disease.

TABLE-1 Demograhic Area Distribution of beta-Thalassemia and Consanguinity

Demographic Area Distribution	No.	Haemoglobinopathy n (%) *Homozygous		*Heterozygous		Normal		Consanguinity n (%)	
Punjabi	100	26	(26)	50	(50)	24	(24)	86	(86)
Pathan	34	03	(08)	25	(74)	06	(18)	30	(88)
Hindko	02	01	(50)	01	(50)	--		02	(100)
Afghan	01	--		01	(100)	--		01	(100)

*Homozygous beta Thalassemia
*Heterozygous beta Thalassemia

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