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Original Article

Role of Prenatal Diagnosis in Thalassaemia Prevention

Nadeem Ikram [1], Shabnum Bashir [2], Saima Khan [3], Rizwana Chaudhry [3]

1. Department of Pathology, Rawalpindi Medical University, Rawalpindi; 2. Punjab Thalassaemia Prevention Programme Sir Ganga Ram Hospital, Lahore ; 3.Department of Gynae/Obs Holy Family Hospital and Rawalpindi Medical University, Rawalpindi

Abstract

Background: To determine the role of chorionic villi examination in the prenatal diagnosis of ß-thalassaemia

Methods: In this descriptive study couples requesting prenatal diagnosis (PND) for ß-thalassaemia were registered for chorionic villous sampling after 10 weeks of gestation. After appropriate counseling placental sample was taken under local analgesics with suction cannula by trans-abdominal approach. The sampling system was then withdrawn under negative pressure .Placental villi were proceeded for further DNA analysis. Each PND was carried out by including the parents DNA, fetal DNA for the mutation, as well as the normal gene, appropriate negative and positive controls and reagent blanks.

Results: Six hundred and twenty females underwent chorionic villi sampling. Most patients (82.25%) , who requested for PND, already had an affected child. Consanguineous marriages were present in 83.20% ..The average reporting time for identification of the mutation was 7 to 10 days. Thalassaemia major was found in 25.48%. In the case of ß- thalassaemia major, majority (98.73) opted for termination of pregnancy. Spontaneous abortion, after the procedure was found in one case .Two cases turned out false negative. The commonest mutations were Fr8-9 (40.85%) and IVS 1-5(25.70%) .

Conclusion: Pre natal diagnosis for ß-thalassaemia by chorionic villi examination is a safe and cost effective procedure.

Key Words: Prenatal diagnosis, Chorionic Villi, ß-thalassaemia

Introduction

ß- thalassaemia is the commonest single gene disorder in Pakistan. Approximately 5% of the population carries ß- thalassaemia trait and each year over 6000 new children are born with ß- thalassaemia major .[1] The cost of treatment of thalassaemia is often beyond the reach of a person with an average income . The best possible solution is the prevention of birth an affected child. [2] Married couples, where both partners are carriers need to be offered prenatal diagnosis (PND). The response of couples to PND is encouraging. [3]

Chorionic villous sample (CVS) is an effective mean of prenatal diagnosis. Ultrasound guided trans-abdominal sampling is the procedure of choice for chorionic villous sampling . The main advantage of trans-abdominal technique is its utilization from first trimester to term. For PND , CVS in first trimester is advantageous, as being of less emotional and physical stress and less obvious pregnancy. If termination is indicated, the contemplation of procedure will be safer in first trimester . Religious scholars, with medical professionals on board, allow it in first trimester.[4]

When compared to the accumulated cost of long term treatment of ß-thalassaemia, cost incurred in chorionic villous sampling and laboratory diagnosing ß-thalassaemia mutations is negligible. [4]Better control of major communicable diseases is leading to a decline in infant morality . As a consequence inherited disorders are becoming a recognized problem. At present the health facilities in developing countries are inadequate to face the challenge of inherited disorders, like thalassaemia . The best approach, to deal these disorders, is preventive programmes including carrier screening and prenatal diagnosis. [5]

The spectrum of mutations in all ethnic groups, studied in Pakistan, is heterogeneous. This heterogeneity appears to be due to geographic location of Pakistan , particularly Northern Pakistan, which has been the gate way for most invasions of Indian subcontinent. Genetic studies utilizing molecular analysis are detrimental, as these can delineate the genetic status. In case of a fetus under analysis it helps in suggesting different options to the parents, after giving due credence to the fetus fate ,i.e., homozygous, heterozygous or normal. Family can be counselled in accordance with it . [5]

Patients and Methods

The couples requesting PND for ß- thalassaemia were registered for chorionic villous sampling after 10 weeks of gestation. Couples were counselled about the

diagnostic procedure, possible outcomes of the test and the chances of misdiagnosis . After appropriate counselling of the couples, placental sample was taken under local analgesics with suction cannula by trans-abdominal approach. Every patient was placed in supine position and placenta was localized by trans-abdominal ultrasonography. Needle was inserted using free hand technique under ultrasound guidance. Needle was advanced at an angle that allowed it to penetrate along the long axis of placenta . Stillet was then removed. Fifty c.c disposable syringe was mounted on holder and the holder was attached to the hub of the needle (Figure 1).The needle tip was removed back and forth inside the placenta applying a continuous suction until an adequate sample had been aspirated (5-10 mg of chorionic villous was needed). The sampling system was then withdrawn under negative pressure .Chorionic villous sample was dissected under a stereo-microscope . Maternal tissue , if any, was carefully separated and placental villi were proceeded for further DNA analysis. The parents' blood samples and CVS were tested for ß-thalassaemia mutations , found in Pakistani population by a Multiplex Amplification Refractory Mutation System (ARMS). Each PND was carried out by including the parents DNA, fetal DNA for the mutation, as well as the normal gene, appropriate negative and positive controls and reagent blanks.

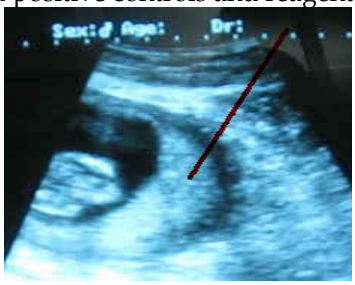

Figure1: Ultrasound guided collection of chorionic villous sample

Results

Most patients (82.25%) , who requested for PND, already had an affected child. Consanguineous marriages were present in 83.20% (Table 1). The average reporting time for identification of the mutation was 7 to 10 days. Thalassaemia major was found in 25.48% (Table 2). The commonest mutations were Fr8-9 (40.85%) and IVS 1-5(25.70%) (Table 3; Figure 2)

Discussion

Gradually the prenatal diagnosis of beta thalassaemia is getting acceptance , after favourable response by religious scholars . [6,7] In a society like Pakistan , where It is not possible for the majority to afford the cost of

Table 1: Prenatal diagnosis – Demographic profile

Characteristic	No(%)
Primigravida	14 (3.87)
2nd pregnancy	94(15.16)
≥ 3rd pregnancy	502(80.97)
Already having an affected child	510(82.25)
Mean maternal age(years) Mean(Range)	28.0 (18-39)
Mean gestational age (weeks)	13.0
Consanguineous marriages	516(83.2)
1st cousin	466(75.16)

Table 2: Prenatal diagnosis - CVS results (n=620)

Parameter	No(%)
Thalassaemia major	158(25.48)
Thalassaemia minor	282 (45.48)
No thalassaemia mutation found	176(28.38)
Inconclusive	4(0.64)
Termination of pregnancy	156/158 (98.73)
Loss of pregnancy	1/620 (0.16)
Refused abortion	2/158 (1.26)
Error rate	2/458(0.43%)

Table 3:CVS sampling-Molecular diagnosis in fetuses with ß- thalassaemia major (n=158)

Mutation	No(%)
Fr 8-9 (+G)	64(40.85)
IVS1-5 (G-C)	41(25.70)
Fr 41-42 (TTCT)	11(6.96)
Cd 15(G -)	06(3.79)
Del619	07(4.43)
Cd 30 (G-C)	06(3.79)
Cd5 (-CT)	06(3.79)
IVS1-1	06(3.79)
Others	11(6.96)

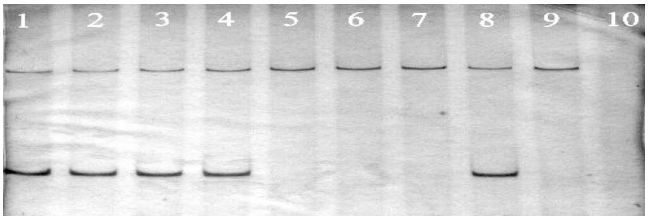

Figure 2: Mutation(1-5):1. Father (Fr 8-9) ;2. Mother (Fr 8-9) ;3. CVS (Fr 8-9) ;4. CVS (Fr 8-9) ;5. -ve ;Normal Gene (6-10): 6. CVS;7. CVS;8. +ve ;9. -ve ;10. Blank

prenatal diagnosis, it is an encouraging respite from Punjab Thalassaemia Prevention Programme (PTPP) to provide free PND services (Collection of chorionic villous sample and mutation analysis). PTPP is catering whole of the province through its field officers. In Rawalpindi it is catering districts of Rawalpindi, Mianwali, Jhelum, Gujrat, Chakwal, Khoshab, Sargodha and Attock. People from Khyber Pukhtonkhwa , Gilgit Baltistan and Azad Jammu and

Kashmir are also taking benefit of this facility. The provision of CVS sampling in first trimester of pregnancy has a better acceptability. Studies revealed that majority of chorionic villi sampling are usually employed in first trimester. [3,4,8-10] In case of homozygous fetus the interruption of pregnancy would be physically and emotionally more acceptable in early gestation. [11] The estimated birth rate of babies born with ß thalassaemia major in Pakistan is around 6000 per year. This means a requirement of approximately 20,000 PND each year . With only a few centres providing PND services the catering number is far away from satisfaction.[3]

Lack of awareness is an important impediment , but mothers' education lead to a significant improvement. [12] It is required to have a prospective identification of couples at risk. [13] Majority of the couples utilizing PND services already have an affected child. To check this trend prospective identification of at risk couples and families is a plausible solution . The extended family screening in cohorts where there is already a case of beta thalassaemia , is the most feasible preventive programme . [14] The Punjab Thalassaemia Prevention Programme is going in the right direction by approaching extended families for thalassaemia screening in all districts of Punjab through field officers and by providing facility of hemoglobin electrophoresis, free of cost.

In majority of cases diagnosis is positive by mutation analysis, but in a few linkage analysis is required. [3] Uptil now more than 200 mutations have been reported in ß globin chain synthesis . Out of these 25% have been found in heterogeneous subcontinent Indo-Pak population. In a carrier couple, with an autosomal recessive inheritance , there is 25% risk of having a fetus with ß thalassaemia major, 25% chance of normal conception and 50% chance of a fetus with ß thalassaemia minor . Prenatal diagnosis can delineate this status , thereby, helping in reducing the disease burden. [15]. Results of present study, for mutation analysis, are in agreement with other studies in Pakistan which show predilection of Fr8-9 and IVS1-5 mutations .[16-18] Fr 8-9 (+G) is the most common mutation in Northern Pakistan, whereas IVS1-5 (G-C) is the most frequent mutation in Southern Pakistan . [5] Mediterranean region studies revealed IVS 1-1 (G-A) IVS1-110(G-A), IVS1-6 (T-C) as predominant beta thalassaemia mutations. [19] PND is technically feasible in Pakistan by direct mutation analysis in 97% of cases and then in remaining by linkage analysis. Technique has an acceptable error rate . Proportion of cases diagnosed as ß thalassaemia major (25.48 %), in present study, is equal to the expected 25%. To maintain the reliability of PND services a strict protocol of DNA analysis with rigorous standards of quality is required. [3,20] In a study by Old et al , in a sample group of 3254 pregnancies, diagnostic issues were recorded, arising from non-laboratory errors (0.31%) and fifteen 15 due to technical problems associated with the diagnostic techniques. The latter group consisted of eight misdiagnoses by globin chain synthesis (1.55%), five by Southern blot analysis (0.73%) and two by PCR methods (0. 10%). Accuracy of prenatal diagnosis has improved with each development of diagnostic technique, and confirms that prenatal diagnosis of beta-thalassaemia and sickle cell disorders by ARMS-PCR is very accurate and reliable. The overall error rate for prenatal diagnosis by PCR methods in the UK is now 0. 41%. [8] In the present study the error rate is 0.43%, higher than 0.1% by Old et al (2000). [8] It can be ascribed to technical problem or maternal contamination in the CVS. The error rate can be reduced by taking additional steps like duplicate testing or linkage analysis, but this would increase the cost of testing. [3] Chorionic villous sampling with mutation analysis revealed sensitivity and specificity of 98.7% and 100%, respectively. Results of present study are comparable to Ghahramani F et al (2014).[21] Ayub R et al had a sensitivity of 75% and specificity of 91%.[22] Factors leading to misdiagnosis are clerical mistakes and maternal contamination of chorionic villous sampling.[6] Consanguineous marriages, common in Pakistan, have compounded the problem. In present study consanguinity was observed in 84.48% couples. A study from Karachi revealed consanguinity in 59%. It can be ascribed to more urban and literate society in Karachi. [23,24]Screening practices are in vogue , yet in our set up most of the couples , having a child with ß thalassaemia major, give history of screening after the birth of an affected child. Factors identified for the under utilization of CVS sampling , in our set up , include lack of awareness, poor access, delay in seeking advice, non-affordability and religious bias . [12,25-28] Many refuse to utilize the procedure due to inadequate or illogical explanation of the procedure . But if properly counseled then majority opted for the procedure and choose to terminate the pregnancy if fetus is suffering from ß thalassaemia major . Complications associated with the procedure and fetal loss is minimal .This needs to be properly imparted as many refuse to utilize the procedure due to inadequate explanation of the procedure .

A family with a child of ß thalassaemia major, approximately has to spend 20 to 30% of its earning as

the running treatment expenses of that child. For an ideal treatment these expenses can go beyond this. But the cost incurred on CVS and laboratory diagnosis of beta thalassaemia mutations is negligible. [4,5,14,25]

In present study there was one case of fetal loss. Post CVS fetal loss is reported from 1.3 to 3.0% [11,29-32] Procedure related complications in 2.4% cases and 3% couples refused to abort a homozygous fetus. [11] Chorionic villous sampling is an invasive procedure. Non invasive approaches like enrichment of fetal zeta globin containing nucleated red blood cells from maternal blood, are giving positive results. [33-35]

Conclusion

1. PND for ß- thalassaemia, by chorionic villi examination, is a safe and cost effective procedure
2. There is a need to increase utilization of prenatal diagnosis for thalassaemia prevention
3. Strict quality assurance can ensure an acceptably low error rate

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Authorship:[1-3] Conceived the topic of research, designed the study and manuscript writing;[4]provided general supervision to research group and revision of manuscript and proof reading of study