Prenatal diagnosis of ß-Thalassaemia by Chorionic Villous Sampling

Samina Tasleem[1], Huma Tasleem[2], Mehfooz Ahmed Siddiqui[3], Malik Muhammad Adil[4], Yasmin Rashid[5]

Rawalpindi General Hospital, Rawalpindi,[1,3], Shifa College of Medicine, Islamabad,[2,4], Ganga Ram Hospital Lahore[5].

Abstract

Objective: To establish intrauterine diagnosis of thalassaemia major in couples with thalassaemia trait by chorionic villous sampling.

Methods: A total of 60 couples with children suffering from transfusion dependent ß-thalassaemia or couples who were known carriers of ß-thalassaemia were included in this study. The standard procedure was followed for the collection of samples which was finally transferred in appropriate medium to Armed Forces Institute of Pathology Rawalpindi for detection of thalassaemia mutation.

Results: After DNA analysis of the submitted samples, no thalassaemia mutation was detected in the foetus in 24 cases. In 8 cases foetus were heterozygote for thalassaemia having a single mutation. In 28 cases, foetus were homozygous for beta-thalassaemia.

Conclusion: Appropriate and extensive screening, accurate detection and counseling of at risk couples, along with antenatal diagnosis is a promising strategy for the reduction of mortality and morbidity from thalassaemia in countries where it is prevalent. Based on these results, it can be concluded that prenatal diagnosis of ß-thalassaemia for prevention can be done using chorionic villous sampling (JPMA 57:528:2007).

Introduction

Thalassaemia was first identified as a clinical entity in 1925 by Thomas Cooley and Pearl Lee.[1] They examined four children from Greece and Italy who were having anaemia along with characteristic facies, splenomegaly, bone deformities with profound erythroblastosis in blood and familial incidence.[2] More than a decade later Wintrobe and colleagues described milder forms of Cooley's anaemia. They noticed that milder manifestations of this disorder were present in both parents of children with classic Cooley's anaemia.[3]

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.[4] According to the chain, which is deficient, several types of thalassaemia have been described. The common types of clinical importance are α, ß and γ thalassaemias.[5]

ß-Thalassaemia is one of the most common single gene disorders worldwide.[6,7] Most of the children affected with this lethal disorder are born in developing countries.[7]

In Pakistan, ß-thalassaemia is one of the commonest inherited disorders. Studies on its carrier frequency have shown an average rate of slightly over 5%.[8] It is estimated that over 4000 thalassaemic children are born in Pakistan every year.[9] In the absence of adequate facilities for diagnosis and treatment, most of the affected children with severe ß-thalassaemia die before the age of 5 years.

ß-thalassaemia is likely to emerge as a serious public health problem and drain on medical resources in many parts of the world including Pakistan. This will become even greater as the incidence of infant and childhood mortality due to infections and malnutrition declines.[6] Prevention of this disease, therefore, forms an essential part of management.

Chorionic villous sampling (CVS) was first started by Hahnemann and Mohr in 1960s using endoscopes.[10] The technique was not taken up, partly because there appeared to be a high rate of complications and failure, and partly because it became clear that amniocentesis was safe and reliable for foetal chromosome analysis.

The successful use of aspiration cannula, passed blindly in the continuing pregnancy, was reported in 1975.[11] This procedure reawakened interest in the west, although work with ultrasound guided forceps was also being done in the USSR.[12] Further development of the methods for chorionic villous sampling followed the demonstration that villi could be used for genetic diagnosis.[13] Blind aspiration was shown to be unreliable[14] and endoscopy was too complicated.[15] Ultrasound guided transcervical sampling became the most widely used technique. It was again Hahnemann in 1984, who made use of transabdominal CVS.[16] Its main advantage was that it can be used from the first trimester to the term whereas transcervical CVS has a limit of 12 weeks. It is not clear whether one method is better than the other but many operators are skilled in both approaches and can, therefore, choose which ever approach seems appropriate.

In this study, the aims and objectives were to establish intrauterine diagnosis of thalassaemia major in couples with thalassaemia trait by chorionic villous sampling.

Patients and Methods

This study was carried out in the department of Gynae and Obstetrics Unit-III of Rawalpindi General Hospital, Rawalpindi. Sixty ladies who fulfilled the inclusion criteria underwent chorionic villous sampling for prenatal diagnosis of ß-thalassaemia. They either reported on their own or were referred from different NGOs looking after thalassaemic children like Thalassaemia Society of Pakistan, Fatmid Blood Transfusion Service and Thalassaemia Welfare Society.

Pregnant ladies at 10-12 weeks of gestation who fulfilled any one of the following criteria were included in the study: couples with children suffering from transfusion dependent ß-thalassaemia; or couples who were known carriers for ß-thalassaemia.

Women with gestation more than twelve weeks, or who refused termination in case the foetus was homozygous for ß-thalassaemia were excluded from the study.

Before going through the procedure, all couples were interviewed in detail particularly about the number of children affected, socioeconomic status of parents, access to the facilities for treatment of thalassaemia, consanguinity and death of an affected child. Subsequently they were counseled about the pattern of inheritance, the chances of having an affected child in current or future pregnancy, procedure of chorionic villous sampling, risk and complications of the procedure and option of termination of pregnancy in case the foetus was detected to be homozygous for ß-thalassaemia.

Before the procedure an informed consent was obtained from both partners. Once they agreed, all of the women included in the study had a detailed ultrasound scanning for gestational age, viability of foetus and placental localization.

The subject was placed in the supine position and placenta was localized by transabdominal ultrasonography (USG) Lower abdomen was prepared with antiseptic solution. The needle was then 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, a 50cc disposable B.D syringe mounted on holder and the holder was then attached to the hub of the needle. The needle tip was moved back and forth inside the placenta applying a continuous suction until an adequate sample had been aspirated (5-10 mg of chorionic villous is needed). The sampling system was then withdrawn under negative pressure. The medium was flushed onto a plastic tissue cutter dish and the content evaluated at a nearby microscope to make certain that enough tissue was collected.

If large amount of maternal blood was present in the sample, the blood was removed promptly to avoid inclusion of villous in blood clots. Blood clots were removed and villi separated from maternal decidua with forceps under a dissecting microscope. The clean villi were transferred in appropriate medium to Armed Forces Institute of Pathology Rawalpindi for detection of thalassaemia mutation.

After the procedure, women were kept under observation for 24 hours. They were monitored for any signs and symptoms as bleeding pervaginum and pain in lower abdomen.

The report of DNA diagnosis was received after four days. In case the foetus was homozygous for ß-thalassaemia mutation, facility for termination of pregnancy soon after the diagnosis was provided.

A record was maintained in all cases to document the possible complications in later months of gestation and also the outcome at birth.

Results

The mean age of the study population was 30.5 ± 5.82 years ranging from 22 to 45 years.

The women included in this study, were married for a period of 10 months to 24 years with the mean of 9.0 ± 5.7 years and median was 8 years. Most of the women included in the study had conceived more than once in the past.

The couples included in the study came from different ethnic groups, 24 were Punjabi, 20 Pathans and 16 were Kashmiris. Partners were first cousins in 32 cases, distant relatives in 16 cases and were unrelated in 12 cases.

Eight couples had two previous affected children, 48 had one previous affected child and 4 couples had conceived for the first time. In the single couple who had not had an affected child in the past, partners were first cousins, had one affected cousin with transfusion dependent thalassaemia major and on screening were found to be carriers for beta-thalassaemia mutation before marriage, therefore opting for prenatal diagnosis and chorionic villous sampling after they conceived.

All women had singleton pregnancy with gestational age ranging from 10-12 weeks. No congenital abnormality was detected. Four women had a spontaneous foetal loss on 3rd day after the chorionic villous sampling. Twelve complained of mild pain in abdomen - especially at the site of abdominal insertion of the CVS needle. Pain was transient and self limiting which did not require medication and settled once the patient was reassured.

Four women had slight PV bleeding following the procedure on first and second day.

After DNA analysis of the submitted samples, no thalassaemia mutation was detected in the foetus in 24 cases. In 8 cases, foetus were heterozygote for thalassaemia having a single mutation. In 28 cases, foetus were homozygous for beta-thalassaemia, which were subjected to termination.

Discussion

ß-thalassaemia is one of the commonest inherited disorders in Pakistan.[17] One out of every twenty individuals carries a gene for ß-thalassaemia. Since population is rapidly increasing in Pakistan, therefore birth of large number of new cases is expected every year. High prevalence among Punjabis are inconsistent with the study done in Department of Genetics, Sanjay Gandhi Post Gratuate Institute of Medical Sciences,India, where high prevalence was found among Sindhi, Punjabis, Gujratis and Bengalis.[18] It is estimated that in our country with a population of 150 millions, five thousand children have the likelihood to be born with homozygous ß -thalassaemia. A large number of these affected children die before diagnosis in the absence of a national program for diagnosis, treatment and prevention of ß -thalassaemia. Among those who are diagnosed, vast majority of children are not treated adequately and have a very poor quality of life and limited survival.[7] Haemoglobin (beta) thalassaemia is an important cause of childhood disease in south Asia.[19]

Standard treatment of ß -thalassaemia includes blood transfusion according to internationally accepted standards to maintain a mean haemoglobin level of 12 gm/dl, and iron chelating therapy with desferrioxamine to control the deleterious effects of progressive iron overload. Such treatment has significantly improved not only the survival but also the quality of life for thalassaemic children. Now more than 90% survival beyond 30 years of age is reported from the European centers.[20] To achieve this target, huge amount of material resources, state of the art expertise of doctors and other health workers looking after thalassaemic children and reliable health delivery system are primary requirements.

Like all other developing countries, the Pakistani, population is growing rapidly, health delivery system is in shambles, poverty is escalating (independent indicators showing absolute poverty rate of over 35%) and resources are limited. This will practically become impossible to provide standard treatment to all the affected children. Therefore the burden of the illness should be reduced and strategies implemented to control birth of new affected children.[21] Dramatic decline in the incidence of ß-thalassaemia in countries with highest incidences in Europe i.e. Cyprus, Greece, Italy and Sardinia, can serve as a sound foundation to develop a reliable and effective strategy for prevention. WHO guidelines on control of haemoglobinopathies provide useful guidelines to develop a national programme to control ß-thalassaemia in our country.[22]

Such programmes involve identification of all individuals carrying a gene for ß -thalassaemia, counseling of these carriers and prenatal diagnosis by chorionic villous sampling in situations where both parents are carriers.[23]

Individuals at risk to be carriers can be identified by simple but specific blood tests. Once identified, they need to

be counseled by experienced persons about the options and risks in future.[24]

Prenatal diagnosis was previously done by multiple methods but now the preferred method is detection of ß-thalassaemia mutation by amplification of foetal DNA - obtained by chorionic villous biopsy.[21] Prenatal diagnosis of ß-thalassaemia by the Reverse Dot Blot (RDB) and Amplification refractory mutation system (ARMS) technique can prevent the birth of an affected child in developing countries in which ß-thalassaemia is quite prevalent.[18] . Since initial reports of prenatal diagnosis in 1994, now this facility is provided to at risk couples at number of centers in the country.[17]

Results of this study show that chorionic villous sampling done at 10-12 weeks of gestation by transabdominal approach is a simple and safe technique. Though, like all other medical and surgical procedures, it requires training and performance of initial procedures under supervision, once learnt, it is an easy, simple to apply and useful technique.

When compared to the cumulated cost of the long-term treatment of ß -thalassaemia, cost incurred on chorionic villous sampling and laboratory diagnosis of ß -thalassaemia mutation is negligible.

In cases where foetus was detected to be homozygous for ß -thalassaemia mutation, termination was done soon after the diagnosis, usually from 13-16th weeks of gestation. Termination of pregnancy was event free in all cases and no unusual complication was observed.

The babies detected to be heterozygous or normal, were all delivered normally, and remained well throughout infancy, pointing towards reliability of the diagnosis. Work has done to develop advanced and accessible protocols for non invasive prenatal diagnosis of genetic disease.[25]

Prenatal diagnosis of ß-thalassaemia in first trimester by chorionic villous sampling is a good tool for early detection of disease. Genetic counseling and prenatal diagnosis can prevent ß -thalassaemia.[26]

Conclusion

Appropriate and extensive screening, accurate detection and counseling of at risk couples, along with antenatal diagnosis is a promising strategy for the reduction of mortality and morbidity from thalassaemia in countries where it is prevalent. However, accomplishment of this goal necessitates detection of all couples at risk. It is important to realize that prevention will be effective only if it is carried out uniformly for the entire population. Based on these results, it can be concluded that prenatal diagnosis of ß-thalassaemia for prevention can be done using chorionic villous sampling.

References

1. Cooley TB, Lee P. A series of cases of splenomegaly in children with anemia and peculiar bone changes. Trans Am Pediatr Soc 1925; 37:29-30.
2. Cooley TB, Lee P: Erythroblastic anaemia. Am. J. Dis. Child. 1932; 43:705.
3. Kapoor C. Prevalence of malarial parasite, HIV, Hepatitis B and Hepatitis C virus in multitransfused thalassaemia patients. (Thesis) Lahore. University of the Punjab, 1997.
4. George R. Honig. Nelson Text Book of Paediatrics. 15th ed. Philadelphia, W.B. Saunders Co, 1996; sec 419.9, pp 1401.
5. Weatheral DJ, Clegg JB. The Thalassaemia Syndromes. 3rd ed. Oxford: Blackwell Science 1981.
6. Weatherall D.J. Disorders of the synthesis or function of haemoglobin. Oxford Text Book of Medicine, 3rd ed. 1996; sec. 22.4.7, pp 235.
7. Weatherall DJ, Clegg JB. Thalassaemia - a global public health problem. Nat Med 1996; 2: 847-9.
8. Khatak M.F., Saleem M. Prevalence of heterozygous β-thalassaemia in Northren areas of Pakistan. JPMA 1992; 42:32-34.
9. Saleem M, Ahmed S, Khatak MF. An overview of thalassaemia in Pakistan. Proceedings of 4th International Conference. Peshawar: Pakistan Association of Pathologists, 1996:76.
10. Hahnemann N, Mohr J. Genetic diagnosis in the embryo by means of biopsy from extra-embryonic membranes. Bulletin of European Society of Human Genetics 2 1968: 23-9.
11. Fetal sex prediction by sex chromatin of chorionic villi cells during early pregnancy. Chin Med J 1975: 117-26.
12. Kazy Z, Rozovsky I S, Bakharev V A. Chorion biopsy in early pregnancy: a method of early prenatal diagnosis for inherited disorders. Prenatal Diagnosis 2 1982: 39-45.
13. Williamson R, Eskdale J, Coleman D V, Niazi M, Loeffler F E, Modell BM. Direct gene analysis of chorionic villi: a possible technique for the first trimester diagnosis of haemoglobinopathies. Lancet 1981: 2: 1125-7.
14. Horwell D H, Loeffler F E, Coleman D V. Assessment of a transcervical aspiration technique for chorionic villus biopsy in the first trimester of pregnancy. Br J Obstet Gynaecol 1983: 90: 196-8.
15. Gosden JR, Mitchell AR, Gosden CM, Rodeck CH, Morsman JM. Direct vision chorion biopsy and chromosome specific DNA probes for determination of fetal sex in first trimester prenatal diagnosis. Lancet 1982: 2: 1416-9.
16. Smidt-Jensen S, Hahnemann N. Transabdominal fine needle biopsy from chorionic villi in the first trimester. Prenatal Diagn 1984; 4: 163-9.
17. Ahmed S, Saleem M, Rashid Y. Prenatal diagnosis of thalassaemia in Pakistan: First case report. Pak. J. Path 1994; 5.
18. Agarwal S, Gupta A, Gupta UR, Sarwai S,Phadke S, Agarwal S S. Prenatal diagnosis in Beta thalassaemia : An Indian experience, Fetal diagnosis and therapy . 2003; 18, 328-32.
19. Fucharoen S, Winichagoon P. Clinical and haematological aspects of haemoglobin E beta thalassaemia. Curr Opin Hematol. 2000; 7: 106-12.
20. WHO 1989. Report of the VIth WHO Working Group on the Feasibility Study, on Heriditary Disease Community Control Programmes (Hereditary Anaemias), Cagliari, Sardinia.
21. Rashid Y, Ahmed S. Prenatal diagnosis of Beta thalassaemia. Mother & child. 1996; 34.
22. WHO 1993. Report of a joint WHO/TIF Meeting on the Prevention and control of Haemoglobinopathies, April 3-4, Nicosia, Cyprus.
23. WHO 1987. Report of the Vth WHO Working Group on the Feasibility Study on Hereditary Disease Community Control Programmers, October 24-25, Herakleion, Crete.
24. Mason G.C. Prenatal diagnosis of β-thalassaemia. Abstracts of the First International Seminar on Recent Advances in Medical Sciences. PMA Lahore 1996.
25. Galbiatli S, Restagno G, Foglieni B, Bonalumi S, Travi M, Piga A et al. Different approaches for non invasive prenatal diagnosis of genetic diseases based on PNA-Mediated enriched PCR. Ann NY Acad Sci 2006; 1075:137-143
26. Baig SM, Azhar A, Hassan H, Baig JM, Kiyani A, Hameed U et al. Spectrum of beta-thalassemia mutations in various regions of Punjab and Islamabad, Pakistan: establishment of prenatal diagnosis. Haematologica. 2006; 91: ELT02.