Open Access Original Article

Decades of Progress: A Seventeen-Year Journey of Prenatal Diagnostic Excellence

Saima Siddiqui, Ghazala Ahmed, Kausar Parveen, Danish Zahid, Sumaira Shaheen, Shariq Ahmed, Eisha Usman, Safia Mehmood Khan, Affaf Sheikh, Muhammad Nadeem, Jawad Hassan, Saira Shan, Saba Shahid, Gul Sufaida, Saqib Ansari, Arshi Naz, Mehwesh Taj, Tasneem Farzana,

National institute of blood disease and bone marrow transplant

Abstract

Objective: To analyze experience of CVS in a single center for different disorders.
Methodology: This retrospective study analyzed 4116 CVS procedures performed at NIBD & BMT (2004–2022) for prenatal diagnosis of β-thalassemia, Down's syndrome, and other genetic disorders. Chorionic villi samples were collected under ultrasound guidance, followed by DNA extraction (QIAamp kit) and ARMS-PCR to detect 15 β-thalassemia mutations. Maternal contamination was ruled out via STR analysis (D13S317, D18S51, D21S11). Karyotyping was performed for chromosomal anomalies. Data included demographics, consanguinity, diagnostic outcomes, and parental decisions on pregnancy termination. Statistical analysis calculated proportions and cumulative prevalence.
Results: A total of 4116 CVS were performed since 2014 till to date with age from 17 to 49 years, Majority 2938 (71.4%) subjects were within age group 25-35 years. Out of 3629 CVS performed for thalassemia, 863(21.5%) were thalassemia major, 1883(45.4%) were carrier and 859 (20.4%) were normal, while 24(0.6%) fetuses revealed unidentified mutations and were advised to undergo advanced testing. Karyotyping for Trisomies of 79 fetuses revealed normal karyotype in 77 while 2 fetuses positive for Trisomy 21. Out of 4116 CVS performed 59% and 14.1% were belong to sindh and KPK respectively. Whereas equal and minor percent of patients came from province of Baluchistan and Punjab. Overall, 866 (21%) fetuses were affected by the disease and 863 (20.9%) parents opted for termination of pregnancy. STR was informative in all the cases except 4/2522(0.15%).
Conclusion: Chorionic Villus Sampling is an effective method for prevention of inherited diseases that is opted comfortably by all patients.

Key words: Beta Thalassemia, Chorionic Villus Sampling (CVS), Consanguineous Marriages, Down's syndrome, Genetic Counseling, Inherited Disorders, Karyotyping, Prenatal Diagnosis, STR Analysis, Trisomy 2.

Address for Correspondence
Dr. Saima Siddiqui;
saimee_80@yahoo.com

Introduction

Prenatal diagnosis plays an important role in the prevention of many hereditary illnesses. There are both invasive and non-invasive procedures available.[1] CVS and amniocentesis are two invasive techniques. CVS is performed between 11 and 12 weeks of pregnancy, and amniocentesis is recommended after 16 weeks. Both approaches make it simple to collect fetal cells for DNA analysis. However, amniocentesis results are delayed, causing excessive stress on families. Furthermore, abortion in the first trimester is generally simple, safe, and permitted in the majority of religions, including Islam.[2,3] As a result, CVS has become the most popular and preferred option. It can be performed either transabdominally or transvaginally. The former path is more accessible and has fewer complications as compared to the other procedure. Noninvasive prenatal testing (NIPT) is another safer option for PND that is available since last decade. However, in this part of the world it's still in infancy.[4]

Because of the culture of consanguineous marriages in Pakistan, numerous rare genetic illnesses, such as beta-thalassemia and haemophilia A, are more common.[5,6] A substantial sum of money is spent on the treatment and management of such disorders, either by the patient or the government. Curative options are only available for a few disorders and are typically highly expensive. On the contrary, supportive care, including frequent transfusions of blood products, increases the risk of developing acute and late transfusion reactions, such as transfusion-transmitted infections and iron overload, which leads to increased morbidity and mortality. Therefore, prevention is the best option for reducing the disease burden in a society. [7]

The prenatal diagnosis of beta thalassemia in Pakistan dates back to 1994. Ahmed *et al,*reported in 2007 that over 2000 CVS had been performed in a single Pakistani facility over a 12-year period.[8] We began prenatal testing

Authorship Contribution: All authors contributed equally to the conception, design, data collection, analysis, and writing of the manuscript. All authors read and approved the final version of the manuscript.

Funding Source: none
Conflict of Interest: none
Received: Jan 28, 2025
Accepted: April 14, 2025

in 2005 and have now performed over 4,000 CVS for BTM and other uncommon illnesses.

In this study, we look at the feasibility, safety, and efficacy of CVS in 4116 people. Maternal cell contamination (MCC) was also ruled out by applying STR markers in cases where fetus inherited the maternal mutation in a heterozygous state.

Methodology

This study was conducted at NIBD & BMT, involves human participants and was approved by institutional review board/ Ethic committee NIBD & BMT (NIBD/IRB-265/10-2023). Data from the institutional data base included name, age, CVS indication, gestational age, variant details, heterozygosity, ethnicity, CVS results, and STR information. Additionally, parents' decisions about termination in cases of diseased fetus were also recorded.

CVS procedure: The CVS subject received in-depth counseling regarding the process and any potential negative effects. Genetic counseling of both partners was conducted before the test. During an ultrasound-guided technique, 2-3 ml of chorionic villi was collected in a sterile container after receiving informed written consent and administering local anesthesia. On the same day, fetal DNA was extracted, processed, and analyzed in accordance with the results. Maternal contamination was ruled out by applying short tandem repeats (STR) in all the cases (since 2014) carrying maternal mutations in heterozygous state.

DNA extraction: Manual DNA extraction from CVS Tissue by QIAamp DNA Mini kit(Qiagen).[9]

Molecular analysis: The samples were first screened by the Amplification Refractory Mutation System (ARMS) for 15 β-thalassemia mutations previously reported in Pakistani and Indian subjects.

Amplification Refractory Mutation System (ARMS): The β-globin mutations screened for, and the sequence of the ARMS primers used is presented in Table 1: The primers were in use for prenatal diagnosis of β-thalassaemia at the Perinatal Centre, UCH, London. Most of the primers were used in combination with an upstream (5') primer.

However, for Cd 15 (G-A) and Cap+1 (A-C) a downstream (3') primer was used. Another set of primers was used to amplify an 861 bp fragment of the distal part of the β-globin gene, to serve as an internal control for the PCR. The same set of primers was also used to screen for the 619 bp deletion, which when present results in amplification of a 242 bp fragment instead of the usual 861 bp fragment. Patients with homozygous β-thalassaemia who were positive for a mutation were then tested for the presence of the normal allele to test if they were homozygous for the mutation or not. Primers for the normal sequence were used to identify the normal allele. Subjects with the normal allele were then tested for the presence of other mutations.

PCR conditions for ARMS: The PCR for ARMS was carried out in a 25µl reaction mixture containing 5 pM of each primer, 0.5 units of Taq polymerase (Advanced Biotechnologies, UK), 30 µM of each dNTP (Advanced Biotechnologies, UK), 10 mM Tris HCl (pH 8.3), 50 mM KCL, 1.5 mM MgCl2, 100 mg/ml gelatin (Sigma, UK), and 0.3-0.5 µg of genomic DNA. Primers were synthesized by Oswell UK, and Pharmacia Biotech, UK. Thermal cycling was carried out in an automated DNA thermal cycler (TC-480) from Perkin Elmer, USA. The regimen consisted of 25 cycles each consisting of denaturation at 94°C for 1 minute, primer annealing at 65°C for 1 minute, and DNA extension at 72°C for 1½ minutes. In the final cycle, the extension reaction was prolonged for another 3 minutes.

Electrophoresis of ARMS products: At the end of PCR 2µl of the amplified product was mixed with 5µl of the loading dye (0.25% bromophenol blue in 40% sucrose). Electrophoresis was carried out in a 2% agarose gel at 150 V for 1 hour. The gels were stained in ethidium bromide (250µg/100ml) for 30 minutes and were visualized and Photographed under 302 nm UV light.

STRs Analysis for maternal contamination. Those CVS samples showing one Beta chain mutation (Beta Thalassemia Minor) resembling the mother's mutation were also tested for STR by Amplification Refractory Mutation System (ARMS) PCR. DNA was extracted with QIAGEN extraction kit. The primers used for different STR markers included D13S317, D18S51, and D21S11. These loci are internationally recognized as the standard for human identification. PCR amplification was performed and product separated on Polyacrylamide Gel Electrophoresis (PAGE)

Karyotyping: In order to detect the chromosomal anomalies in the fetus, karyotyping of the chorionic villi

was performed. Initially it is important to separate all the maternal deciduae from the Chorionic villi sample by performing STR analysis. The CVS was then cultured by using various mechanical as well as enzymatic methods. Both short term and long-term cultures were proceeded. The cultures were continuously monitored for colony formation under the inverted microscope. Once the cultures were confluent with dividing cells, they were harvested as per the standard cytogenetics protocol. After harvesting, slides were prepared and were preceded for Giemsa banding to prepare a fetal karyotype.[10,11]

Statistical analysis: Proportion of each diagnostic category relative to the total sample size was showed as percent. Adjusted percentage considering only valid data points were represent by valid percent. cumulative prevalence was represented by cumulative percent.

Results

A total of 4116 CVS procedures were performed from 2004 until 2022. The age of subjects undergoing CVS was 17 to 49 years, with a majority of 2938 (71.4%) within the age group of 25–35 years. A huge portion of population 2703 (65%) who came for CVS had first-degree consanguineous marriage. Which was followed by third-degree 929 (22%) and second-degree consanguineous marriage 484 (11%)(Figure 1)

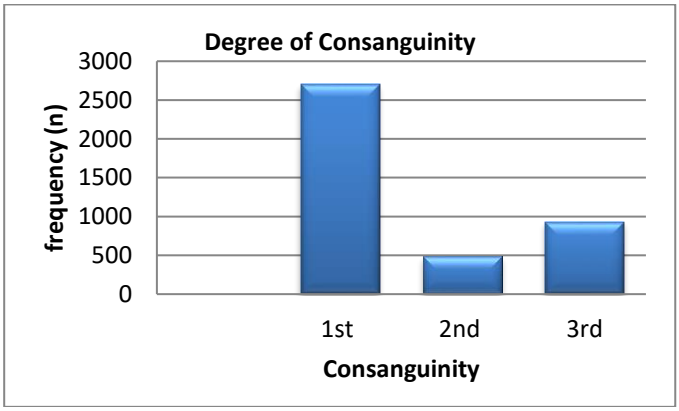

Figure 1: Degree of consanguinity.

Out of 4116, 3996 (97%) CVS were performed for beta thalassaemia major, of which, 863 (21%) fetuses were thalassemia major, 1883 (45.4%) were carriers, and 859 (20.4%) were normal, while 24 (0.6%) fetuses revealed unidentified mutations and were advised to undergo sequencing of the beta-globin gene. On 93 fetuses, karyotyping for trisomies and CAH was carried out. Of these, 79 were checked for trisomies and the remaining was checked for other rare disorders.

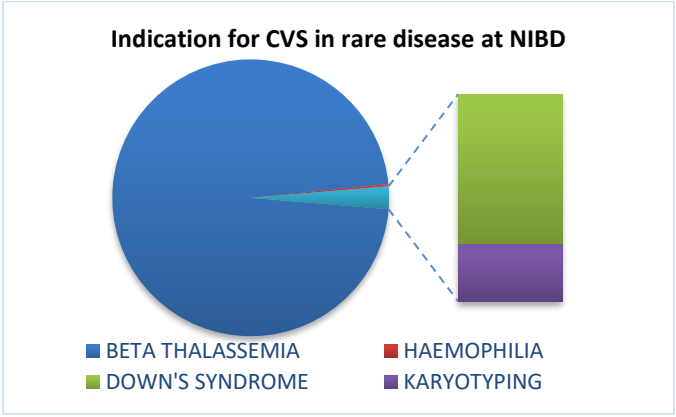

Figure 2. Indication for CVS in Rare disease.

A normal karyotype was found in 77 of them, whereas trisomy 21 was found in 2 fetuses Moreover, 11 CVS were also done for haemophilia. Among them, 4 were normal and carrier of hemophilia. Moreover, 14 CVS was found to be the carrier of other rare genetic disorders, whereas one fetus was affected with DMD (Figure 02; Table 01). Data of 426 patients were not available as only CVS procedure was performed. The regional distribution is shown in Figure 3that out of 4116 CVS, 59% were belong to sindh region whereas 14.1% patients were come from the Khyber Pakhtunkhwa. .

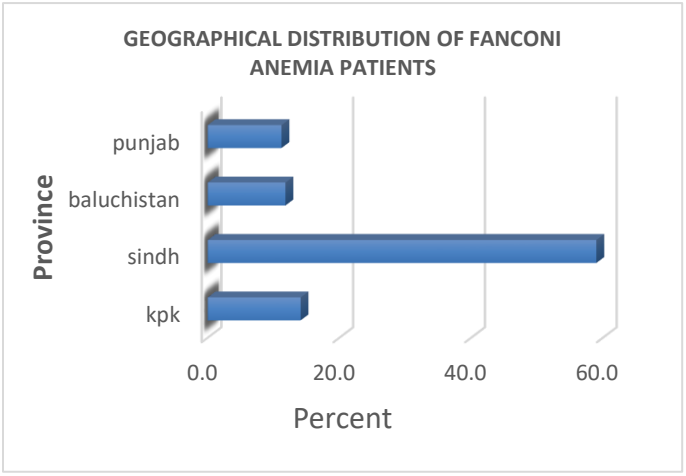

Figure 3. Regional Patient distribution for CVS testing.

Additionally, Balochi and Punjabi constitute the 11.8% and 11.2% of total sample population. Overall 866 (21%) fetus were affected by the disease, 863(20.9%) parents opted for termination of pregnancy, while 3/4116(0.07%) patients refused. STR was applied in 2522/4116 cases and it was informative in all except 4 cases (0.15%).

Table I: Outcome of CVS.				
Diagnosis	N	%	Valid %	Cumulative %
Beta thalassemia				
Major	863	21.0	21.0	21.0
Normal	859	20.4	20.4	41.4
Minor	1883	45.4	45.4	86.8
Unidentified mutation	24	0.6	0.6	87.4
Down's syndrome				
Evidence of trisomy 21	2	0.0	0.0	87.4
No evidence of trisomy 21	77	1.0	1.0	88.4
Other rare disorder				
Hemophilia carrier and normal	4	1.0	1.0	89.4
Carrier of other rare disorders	14	0.3	0.3	89.7
DMD	1	0.0	0.0	89.7
Only Procedure	426	10.3	10.3	100
Total	4116	100.0	100.0	

Discussion

Over a period of 17 years 4116 CVS procedures were performed. Beta thalassemia was the main indication followed by Down's syndrome and other rare disorders. TOP was opted was opted by 97% families in case fetus was affected by the disease. Consanguinity was seen in 65% patients. This is the largest study on prenatal diagnosis from Pakistan in which CVS of 4116 subjects during 17 years was performed. Ahmed et al reported 2174 CVS for beta thalassemia in 12 years.[12] Moatter et al reported 383 CVS cases of beta thalassemia since 2003-2007.[13] CVS of 1869 subjects were performed thru Punjab thalassemia prevention program during 5 years span. Consanguinity rate was very high among all the ethnic groups in our study. Reason is custom of intermarriages in our part of the world that should be discouraged and families should be counseled.[5] Baig et al has highlighted 81% consanguinity in Southern Punjab.[14] Fetuses analyzed for beta thalassemia followed pattern of autosomal recessive inheritance with 50% being carriers for the disease and quarter normal and rest affected. Genetic mutation identification was not performed because of unavailability of resources. Secondly, Karyotyping was helpful in ruling out trisomies to Downs in 79 patients in which majority subjects had normal male or female. Furthermore, 41 CVS procedures were performed for hemophilia and other rare disorders including DMD, fibrinogen deficiency, cystic fibrosis, SCID etc. Among all 4116 CVS performed, fetus was affected in 21% cases. Majority families with affected fetus opted for termination leading to refusal rate of only 0.07 %. As per Bashir et al 0.85% couples in their study refused for TOP. [15]

STR was applied to rule out maternal contamination in more than half of the patients that was informative in almost 99% patients. Patients with non-informative STR were advised for resampling.

Strength of the study was large sample size involving more than 4000 of subjects from Pakistan. CVS performed not only for beta thalassemia but also for other disorders. Single centre study; Sindhi and Balochs were majority patients. Not encompassing every ethnicity. CVS provides a reliable method of diagnosing thalassemia and other genetic disorders prior to birth, allowing parents to make informed decisions about their pregnancy and approach timely medical interventions if necessary.[16,17] This innovation has helped to reduce disease burden by permitting earlier detection and care.[18] This study opens a new window for utilizing CVS for prenatal testing for rare disorders other than Beta thalassemia. [19] CffDNA in future can replace CVS in near future once its expertise is developed in our country. [20]

Conclusion

In current scenario Chorionic Villus Sampling is an effective method for prevention in our country that is opted comfortably by majority of the families. In future with the development of expertise it may be replaced by non-invasive-Cff-DNA.

References

1. Endilcioglu I, et al. Prenatal diagnosis of β-thalassemia and other hemoglobinopathies in southwestern Turkey. Hemoglobin. 2011;35(1):47-55. https://doi.org/10.3109/03630269.2010.544607
2. Afzal M, et al. Noninvasive prenatal testing of beta-thalassemia for common Pakistani mutations: a comparative study using cell-free fetal DNA from maternal plasma and chorionic villus sampling. Hematology. 2022;27(1):353-359. https://doi.org/10.1080/16078454.2022.2045052
3. Hedayat KM, et al. Therapeutic abortion in Islam: contemporary views of Muslim Shiite scholars and effect of recent Iranian legislation. J Med Ethics. 2006;32(11):652-657. https://doi.org/10.1136/jme.2005.015289
4. Li DZ, et al. Invasive prenatal diagnosis of fetal thalassemia. Best Pract Res Clin Obstet Gynaecol. 2017;39:41-52. https://doi.org/10.1016/j.bpobgyn.2016.10.011
5. Aslamkhan M. CULTURAL CONSANGUINITY AS CAUSE OF β-THALASSEMIA PREVALENCE IN POPULATION. medRxiv. 2023:2023.06.01.23290856. https://doi.org/10.1101/2023.06.01.23290856
6. Nouri N. Consanguineous marriages in the genetic counseling centers of Isfahan and the ethical issues of clinical consultations. J Med Ethics Hist Med. 2017;10.

7. Origa R. Beta-thalassemia. Genet Med. 2021;23(4):613-626.
8. Ansari SH. Molecular epidemiology of β-thalassemia in Pakistan: far reaching implications. Blood. 2011;118(21):5309. https://doi.org/10.1182/blood.V118.21.5309.5309
9. Chomczynski P. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. Biotechniques. 1993;15(3):532-4, 536.
10. Arsham MS, et al. The AGT cytogenetics laboratory manual. 4th ed. Hoboken: John Wiley & Sons; 2017. https://doi.org/10.1002/9781119061199
11. Schreck RR, Distèche CM. Chromosome banding techniques. Curr Protoc Hum Genet. 1994;4.2.1-4.2.36. https://doi.org/10.1002/0471142905.hg0402s00
12. Ahmed Kiani R, Ghani A, Usman M, Anwar M, Khurshid M. Epidemiology of Transfusion Transmitted Infection among Patients with β-Thalassaemia Major in Pakistan. J Blood Transfus. 2016;2016:8135649. https://doi.org/10.1155/2016/8135649
13. Moatter T, Aban M, Tabassum S, Pervez S. Prenatal screening for β-thalassemia major reveals new and rare mutations in the Pakistani population. Int J Hematol. 2012;95:394-398. https://doi.org/10.1007/s12185-012-1036-7
14. Azhar Baig HM, et al. Genetic analysis of consanguineous Pakistani families with congenital stationary night blindness. Ophthalmic Res. 2022;65(1):104-110. https://doi.org/10.1159/000520895
15. Bashir S. Reproductive Behavior in Pakistan: Incorporating Men and Couples to Understand Change Over Time [dissertation]. Bowling Green: Bowling Green State University; 2017.
16. Filoche S, et al. Implementing non-invasive prenatal testing into publicly funded antenatal screening services for Down syndrome and other conditions in Aotearoa New Zealand. BMC Pregnancy Childbirth. 2017;17:1-6. https://doi.org/10.1186/s12884-017-1535-x
17. Siddiqui A, et al. Assessing health-related quality of life, morbidity, and survival status for individuals with Down syndrome in Pakistan (DS-Pak): Protocol for a web-based collaborative registry. JMIR Res Protoc. 2021;10(6):e24901. https://doi.org/10.2196/24901
18. Williams J 3rd. Utilization of noninvasive prenatal testing: impact on referrals for diagnostic testing. Am J Obstet Gynecol. 2015;213(1):102.e1-102.e6. https://doi.org/10.1016/j.ajog.2015.04.005
19. Ahmed S. Genetic haemoglobin disorders in Pakistan. Natl J Health Sci. 2017;2(3):95-99. https://doi.org/10.21089/njhs.23.0095
20. Chakravorty S, Dick MC. Antenatal screening for haemoglobinopathies: current status, barriers and ethics. Br J Haematol. 2019;187(4):431-440. https://doi.org/10.1111/bjh.16188