Original Article

MATERNAL CELL CONTAMINATION IN CHORIONIC VILLOUS SAMPLES FOR THE PRENATAL DIAGNOSIS OF THALASSAEMIA

Shawana Kamran[1], Suhaib Ahmed[2], Kamran Nazir Ahmad[3], Asad Mahmood Abbasi[4], Aniqa Bano[4], Farah Bano[5]

[1]Shifa International Hospital Islamabad, Pakistan
[2]Islamic International Medical College, Rawalpindi, Pakistan
[3]Pakistan Air Force Hospital Islamabad, Pakistan
[4]Armed Forces Institute of Pathology Rawalpindi, Pakistan
[5]Combined Military Hospital Rawalpindi, Pakistan

ABSTRACT

Objective: To assess the frequency of maternal cell contamination (MCC) in chorionic villous samples (CVS) for prenatal diagnosis of thalassaemia.

Materials & Methods: This descriptive study was carried out at the Department of Hematology, Armed Forces Institute of Pathology (AFIP) Rawalpindi over a period of one and a half year from July 2008 to January 2010. Seventy CVS having β thalassaemia trait were selected by non-probability purposive sampling. The CVS was cleaned by microdissection under stereo zoom microscope at 16X magnification. DNA was extracted by phenol chloroform method after proteinase-K enzyme digestion. Parent's β-thalassaemia mutations were tested by amplification refractory mutation system (ARMS). The presence of MCC in the CVS was tested by short tandem repeats (STR) analysis at D3S1358, D5S818, D8S1179 and D21S11 loci.

Results: None of the seventy samples showed any molecular evidence of MCC.

Conclusion: Meticulous microdissection of CVS can almost completely rule out errors in prenatal diagnosis of β thalassaemia due to MCC.

Key Words: Maternal contamination, Chorionic Villous Sample, Prenatal diagnosis, Short Tandem Repeats, Polymerase Chain Reaction.

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INTRODUCTION

Chorionic villous sampling (CVS) is a useful outdoor procedure in which small piece of placental villous is obtained for DNA analysis. Many single gene disorders like thalassaemia can be diagnosed prenatally by direct mutation analysis of the CVS obtained under ultrasound guidance. CVS is a safe and reliable prenatal diagnostic technique and is one of the options available to pregnant women who require prenatal diagnosis [1]. However, the presence of maternal cell contamination (MCC) in CVS poses a serious preanalytical risk for error in prenatal diagnosis. Best practice is to perform MCC studies on all prenatal samples. MCC in the fetal samples can be minimized by meticulous dissection of CVS to pick only fetal tissue for further DNA extraction [2]. MCC in CVS is traditionally detected by analysis of Short Tandem Repeats (STR). It is one of the most polymorphic markers made up of tandem repeats of sequences ranging from 2-6 base pairs. The STR shows wide and uniform distribution throughout the genome. They have high level of relatively stable polymorphism and are easy to detect by polymerase chain reaction (PCR) based techniques. PCR based techniques are highly sensitive but can provide false positive results in case of prenatal diagnosis due to amplification of contaminating maternal DNA that may be present in the fetal samples. Currently established PCR based diagnostic tests may require a separate test to exclude MCC especially when fetal mutation is the same as mother's [3].

Clinical practice for prenatal diagnosis of thalassaemia was introduced in Pakistan in 1984. Armed Forces Institute of Pathology (AFIP) is the major diagnostic center for performing this test. The presence of MCC in CVS received at AFIP is mainly excluded by careful dissection of the sample under microscope. This study was planned to find molecular evidence of MCC after microdissection of the CVS samples.

MATERIAL AND METHODS

Seventy samples having prenatal diagnosis of β thalassaemia trait were tested for MCC using

Correspondence: Dr. Kamran Nazir Ahmad, Classified Pathologist, Department of Pathology, Pakistan Air Force Hospital Islamabad, Pakistan

Email: **saadzaid1@hotmail.com**

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STR analysis. On the receipt of CVS specimen in the laboratory microdissection was done with the help of dissecting microscope to remove the maternal decidual tissue. Dissected chorionic villi were digested with proteinase-K at 37˚C overnight. DNA extraction was done by phenol chloroform method. The CVS specimen were tested for the parent's mutation for β thalassaemia. When fetal genotype was similar to that of mother's genotype, STR analysis was done to rule out MCC. Four STR markers including D3S1358, D5S818, D8S1179 and D21S11 were employed in succession using the following primers:

D3S1358-F 5'-ACT GCA GTC CAA TCT GGG T
D3S1358-R 5'-ATG AAA TCA ACA GAG GCT TG
D5S818-F 5'-GGG TGA TTT TCC TCT TTG GT
D5S818-R 5'TGA TTC CAA TCA TAG CCA CA
D8S1179-F 5'-TT GTA TTT CAT GTG TAC ATT CG
D8S1179-R 5'-CGT AGC TAT AAT TAG TTC ATT TTCA
D21S11-F 5'-GTG AGT CAA TTC CCC AAG
D21S11-R 5'-GTT GTA TTA GTC AAT GTT CTC C

The PCR amplified products of STR alleles were run on 6% polyacrylamide gels. The gels were stained in silver nitrate. Quantitative analysis of STR alleles was done by using densitometry. Data was analyzed using SPSS version 11.0.

RESULTS

STR analysis was done on 70 chorionic villous samples. Age of the mothers ranged between 18 to 35 years. Mean age was 26 ± 2 years. The results of informativeness of the four STR markers are shown in Table-1. D21S11 marker was the most informative marker with overall success rate in 90 % of the samples (63 out of 70 samples) whereas D5S818 had the lowest frequency of informativeness i.e. 80 % (56 out of 70 samples). The results of polyacrylamide gel electrophoresis in the samples tested are shown in Figure 1-4. On the whole, none of the CVS showed any molecular evidence of MCC.

Table-1: Informativeness of various STR markers.

STR Markers	Informativeness %
D3S1358	82.00 %
D5S818	80.00 %
D8S1179	86.00 %
D21S11	90.00 %

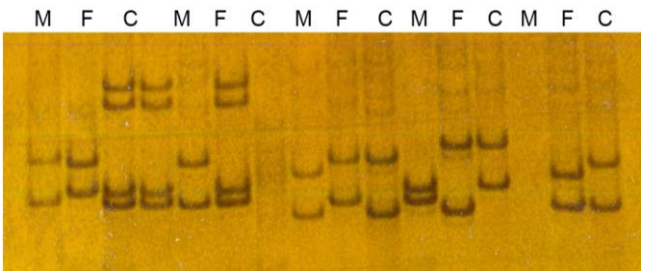

Figure-1: PCR amplification of STR marker D5S818 in five families.
M=Mother F=Father C=CVS
*Note that the marker is non-informative in Family 1 and 2.

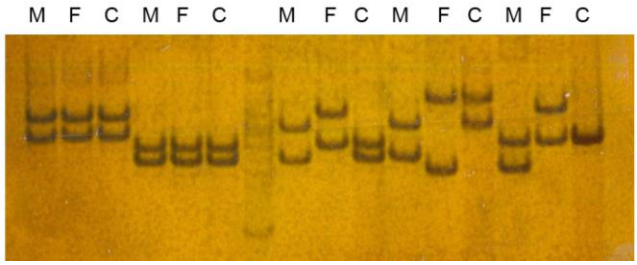

Figure-2: PCR amplification of STR marker D3S1358 in five families.

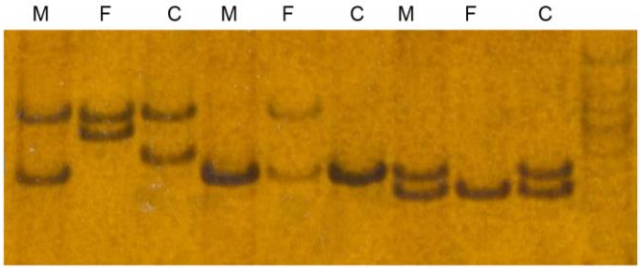

Figure-3: PCR amplification of STR marker D8S1179 in three families.

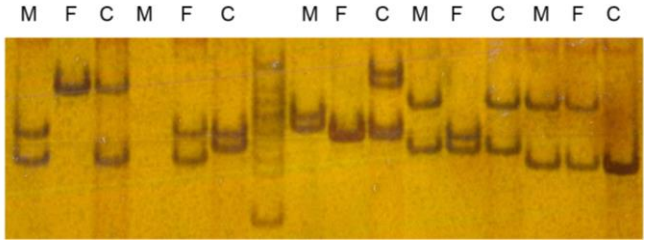

Figure-4: PCR amplification of STR markers D21S11 in five families.

DISCUSSION

After many legal, ethical and political disputes, the continuous clinical implementation of CVS started in 1983, developed by Denis Fairweather and Humphrey Ward, of the University College of London, for the diagnosis of the hemoglobinopathies. The CVS is a reliable method to obtain tissue reflecting the genetic constitution of the embryo and careful microdissection of aspirated villi can provide fetal tissue free of interfering amounts of

maternal decidual tissue [4]. Despite careful microdissection, a major potential cause of error in prenatal testing may be the presence of maternal cells in the samples; therefore, it is important to ensure that it should be excluded by testing for MCC whenever carrying out prenatal testing [5]. Prenatal diagnostic strategies applied today are mostly based on PCR analytical protocols. Even when sophisticated technique like microarray analysis is employed for prenatal diagnosis exclusion of MCC is essential [6].

A comprehensive survey of current diagnostic practices in 35 molecular diagnostic laboratories carried our by Iris *et al*. in 2007 showed that only 60 % of the labs performed MCC testing without exception and there was considerable variability in the utilization and interpretive criteria for MCC analysis. The same survey showed that the lower limit of MCC detection ranged from 1 % to 20 % [7]. Following a meeting of the clinical Molecular Genetics society in 2007, it is recommended that maternal and prenatal specimens should be tested and analyzed for MCC concurrently within the same analysis to allow for a direct comparison of results ideally by checking for polymorphic tetra/ penta repeat markers such as STR with sufficient number of markers to accurately rule out MCC at the level of sensitivity previously determined by the laboratory during its initial MCC assay validation process. It also states that at least 5 % MCC must be routinely detectable by the clinical laboratory. This is particularly important when the fetal genotype i.e. β globin gene mutation is the same as that of the mother. The same document gives guidelines for preanalytical checks as well as interpreting and reporting MCC in prenatal analyses [8].

Pakistan is a developing country where 40 % of the population is living below poverty line. The hemoglobinopathies are one of major genetic problem in Pakistan with an overall carrier rate of β thalassemia is about 5.5 %. Access to prenatal diagnostic services is available only to few because of its limited availability and lack of both specialized centers and technical expertise in this regard. Moreover, most of the individuals are reluctant for this analysis because of the high test cost i.e., Rs. 4000/ ($50) for direct mutation analysis and Rs. 8000/ ($100) if subsequent STR analysis is done [9]. Nadeem Ikram *et al*. have reported that majority (98.7 %) of couples opt for termination of pregnancy (TOP) with thalassemia major diagnosis and that prenatal diagnosis for ß thalassaemia by CVS analysis is a safe and cost-effective procedure. They reported two false negative cases among the 620 procedures carried out, however it is not reported if these cases were due to MCC [10]. In a latest report by Italy Giovanni *et al*. a similar rate of TOP was reported. According to them 98.2 % cases of ß-thalassaemia major diagnosis opted for termination, and they also state that it has led to a significant decrease in the incidence of ß-thalassaemia major over the last 40 years [11]. The effectiveness of CVS in thalassemia prevention has also been documented by Dasgupta *et al* [12].

In our study, none of the 70 samples showed any molecular evidence of maternal contamination. Four loci including D3S1358, D5S818, D8S1179 and D21S11 were amplified in the present study and we were able to completely exclude maternal contamination by obtaining unique fingerprint of CVS using these markers using tetra-primer amplification refractory mutation system-polymerase chain (ARMS PCR) technique. D21 proved to be the most informative marker. It was informative in 90 % of the cases. Careful microdissection was sufficient to exclude MCC in 100 % of the cases in this study. Here we suggest that the need of further STR analysis can be alleviated if cost of the complete package of prenatal diagnosis including fetal testing as well as MCC testing is an issue. A study by Ahmed S in 2007 which was based on 12 years experience of prenatal diagnosis of ß thalassaemia revealed an overall rate of misdiagnosis of 0.37 % out of 2174 prenatal diagnosis. Investigations for errors included maternal contamination, non-paternity, clerical mistakes and technical problems with PCR. It was found out that the causes of misdiagnosis included two maternal contaminations in CVS, one clerical mistake and three false positive PCR results. It was concluded that low error rate can be achieved by strict quality assurance [13,14].

Contrary to our suggestion a study by Garewal *et al*. in 2005 showed that in 5 out of 99 cases (5 %) a definitive report could not be given due to maternal contamination even after microdissection [15]. Similarly, in a study by T. Antoniadi *et al*. MCC was detected in four cases out of 135 cases where 44 samples needed to be tested for MCC. He concluded that it is difficult to thoroughly remove the maternal decidual tissue from the fetal cells and that the risk of MCC in CVS may be minimized by optimal sampling procedures and careful microdissection. According to him in almost 90 % of cases a simple test of one polymorphic locus provided sufficient information about MCC [16]. According to a study of prenatal testing practices in UK by Fiona Macdonald

in 2008, it was shown that it is important to rule out maternal contamination by rigorous microdissection by an experienced cytogeneticist whenever carrying out prenatal testing. She stressed upon testing for the evidence of MCC despite the fact that it is rare to find significant levels of MCC in the cleaned material [17].

Our suggestion is further supported by Keser *et al*. who carried out study on the prenatal diagnosis of ß thalassaemia in the Antalya province in Italy. Out of 103 fetuses 25 were found affected. After careful microdissection to eliminate the risk of MCC, VNTR analysis using Apo B, MCT, IgJH and D4S95 alleles was performed when the genotype of fetus was found identical to that of the mother. No misdiagnosis was recorded [18]. Similar results were reported by Rahim *et al*. from Iran with 127 samples also by using VNTR analysis [19].

In summary CVS provides a good source of DNA and the results are available early in pregnancy however the potential risk of MCC is the main issue which can be resolved by careful microdissection as shown by our study.

CONCLUSION

Our results of prenatal diagnosis of thalassaemia using TA-CVS followed by microdissection are reliable and we can safely conclude that careful microdissection of chorionic villi to remove maternal decidual tissue is sufficient to nullify the chances of misdiagnosis because of MCC.

AUTHORS CONTRIBUTION

Shawana Kamran: Entire research work, sample collection and processing, write up.
Suhaib Ahmed: Concept, supervision.
Kamran Nazir Ahmad: Literature review, write up.
Asad Abbasi: Data analysis.
Aniqa Bano and Farah Bano: Literature review.

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