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ORIGINAL PAPER

Use of Quantitative Fluorescent Polymerase Chain Reaction (QF PCR) in Prenatal Diagnostic of Fetal Aneuploidies in a 17 Month Period in Parallel with Karyotyping

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ABSTRACT

Introduction: QF PCR has recently entered diagnostic practice as a possible way to bypass culturing of the fetal cells, as well as to provide a rapid response following amniocentesis. **Material and methods:** The effective value of the QF PCR remains a much debated issue, positions ranging from that it makes classic karyotyping obsolete except in special occasions, to that it is no more than a guideline for a mandatory karyotype. Current practices of the gynecology specialists generates samples in such fashion that karyotyping of samples quickly falls behind to the point of obsolescence, because, by the time a karyotype has been finished, a window of opportunity for termination of pregnancy has closed. **Results:** QF PCR provides a rapid response alternative, but it is necessary to establish its reproducibility, as well as an algorithm of its use along classic karyotyping. This study contains samples processed in a period from August 1, 2012 to December 31 2013 in both QF PCR and classic karyotype. Object of this study was compare results obtained by two methods, and establish confidence interval of the QF PCR testing. Overall, 661 amniotic fluid samples were processed and typed with QF PCR, out of which 221 were done in parallel with karyotyping, as an confirmation of results.

Key words: QF PCR, amniocentesis, karyotyping.

1. INTRODUCTION

In the practice of Clinical Center Sarajevo, karyotyping is the only definitive diagnostic method for the detection of fetal aneuploidies. Major drawbacks of classic karyotyping are long turnaround time, as well as labor intensive procedure which results in small number of samples that are parallel processed. (1) Average turnaround time for a karyotype is 45 days, but due to the staph and logistic issues can be as high as 90 days. On the other hand, QF PCR's to a good degree automated procedure, as well as the reliance on a chromosome specific markers enables turnaround time to be a fraction of what it is in karyotyping.(2)

QF PCR uses markers on a selective chromosomes, and by their profiling and data analysis we can establish presence of the aneuploidy in a tested sample. (3) Limitations of the QF PCR are primarily in its inability to detect structural abnormalities. Also, QF PCR testing is capable of detecting aneuploidies of

selected chromosomes, X, 13, 18 and 21, to be specific. If, for any reason, other chromosomes are of interest, different multiplexes need to be designed accordingly.(4)

2. MATERIALS AND METHODS

Amniotic fluid samples were received from the Clinic of Gynecology and Obstetrics, with proper paperwork requesting karyotyping. From those samples, 200 ul were taken for the QF PCR analysis. From thus generated samples, fetal DNA was extracted using QIAGEN QIAMP DNA Mini Kit, using the procedure recommended by the producer. Amplification on 21 loci on chromosomes X, Y, 13, 18 and 21 was conducted using Molgentix ANEUFASST QF PCR kit, PCR mix created as directed by the producer (table 1). The kit contains primer pairs for 6 markers on sex chromosomes, 5 markers for chromosome 13, 5 markers for chromosome 18, and 5 markers for chromosome 21, all in a single multiplex reaction. Kit also contains

single chromosome specific primer mixes, with additional loci for the respective chromosome (X, 13, 18, or 21), in case of a need for additional testing. Fragmental Analysis was conducted on a ABI 3130 Genetic Analyzer, using Run 3130 Data Collection software, using 36cm capillary array length, and Performance Optimizing Polymer (POP) 7. Run time was set to 1800 seconds. Sizing standard used was ABI LIZ 500. Data analysis and Electropherogram creation was done using GeneMapper ID v3.2 software (figure 1).

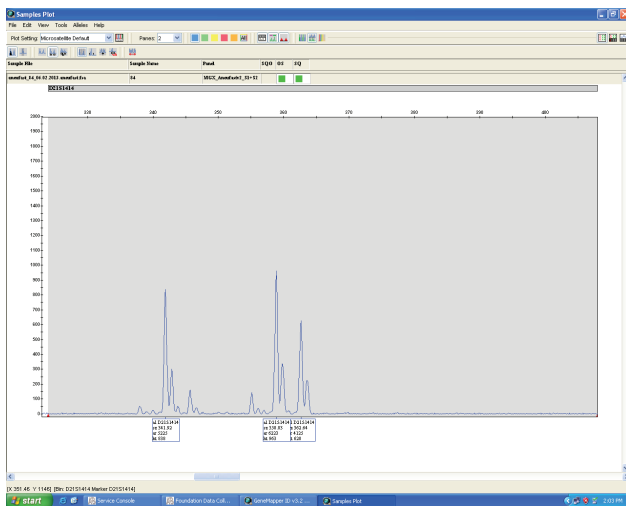


Figure 1: Electropherogram of a multiplex PCR reaction with a size standard table.

3. RESULTS

Overall, 661 amniotic fluids and 1 blood sample were tested for aneuploidies. Out of those, 569 were issued as normal after reviewing of the all-chromosome loci electropherograms. In 59 samples additional loci for chromosome 21 were tested (either due to the presence of the third peak on ch21 markers, peak area disbalance, homozygosity of all ch21 STR tested, or to the amplification failures on appropriate markers). Also, six samples were additionally tested for loci on chromosome 13, 16 for loci on chromosome 18, and 11 for loci on XY chromosomes, due to the same reasons as is the case with chromosome 21. Furthermore, for 6 samples mothers blood sample had to be obtained and profiled, due to the maternal contamination of the amniotic fluid. That puts the number of total profiles obtained to 758. Out of these, 221 samples were confirmed with karyotyping, including all positive ones for trisomy 21.

For ten samples, profiling of the additional markers on chromosome 21 confirmed the presence of the third chromosome, establishing the conditions for diagnosis of Down Syndrome. (Figures 2 and 3). Nine of these were confirmed by karyotyping, the remaining one did not produce sufficient number of mitosis, so, karyotype was inconclusive. One more sample was labeled as suspect by QF PCR, but the karyotype was normal. It needs to be mentioned that one of these samples was a blood from a newborn. No irregularities in number were observed for chromosomes 13,

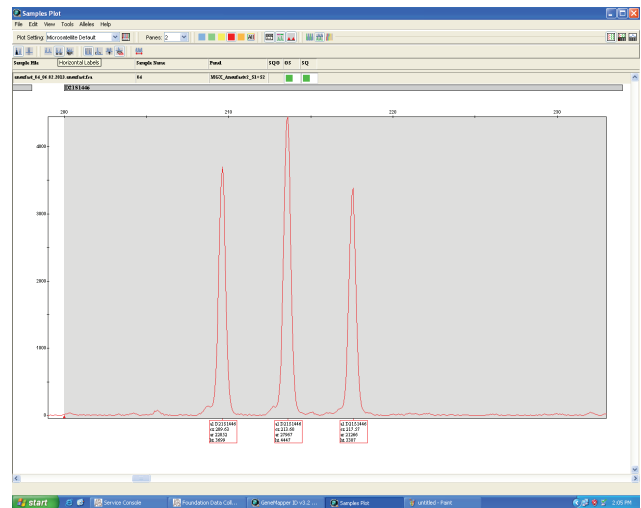


Figure 2: Individual loci on chromosome 21 in samples diagnosed as Down syndrome

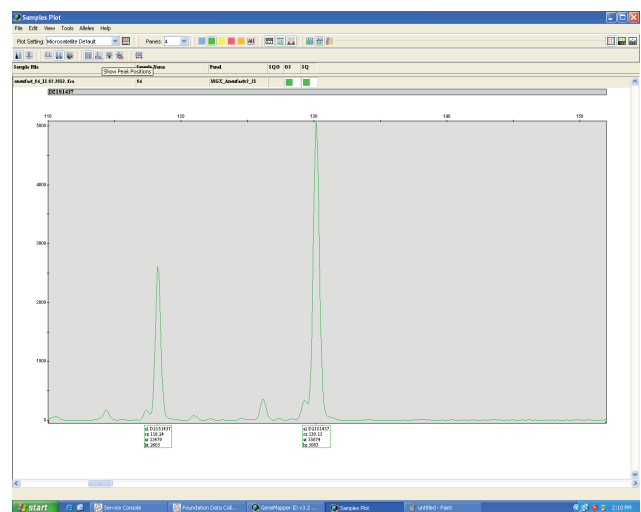


Figure 3: When trisomy is present, but two out of three chromosomes share the same loci, peak area is used to determine disbalance (one loci peak has peak area less than 35% of the two-loci peak), which is consistent with examples from literature (5)

18, or X in all of the processed samples. Overall, 1.51% of tested samples have shown an anomaly, exclusively on chromosome 21.

4. DISCUSSION

Results obtained from this study indicate that QF PCR is a dependable and accurate tool in the prenatal diagnostics of trisomies. In span of 17 months, parallel karyotype and QF PCR analysis have not shown any discrepancies. Furthermore, in the period covered, karyotyping has not shown any translocations in samples processed.

Sample generation per month has been 38.88 in the 17 months of observation, which would rate slightly higher than observed frequencies per capita from literature if we are to consider only population of Sarajevo, but one needs to bear in mind that Clinical Center processes patients from all over the country. (6,7,8). During the length of the study, 221 samples involved were karyotyped, which means that the capacity of QF PCR, with sample generation as de-

scribed, is close to three times bigger. This ratio only improves with higher number of samples.⁽⁹⁾ Out of 221 samples processed both with QF PCR and karyotyping, no discrepancies of results were observed.

Turnaround time for samples tested by QF PCR has stayed constant, between 3 and 5 days, while, due to the personnel and equipment requirements, labor intensiveness of the testing, as well as sample accumulation, turn around time for karyotyping has started at 15 days, and has grown to 45 days or more six months after. Also, the samples positive for trisomy 21 were karyotyped approximately 30–40 days earlier than if they waited their turn regularly, due to the QF PCR result. This time is of great value when termination of pregnancy options are considered. Experience in this study suggests that substituting karyotyping with QFPCR will not damage the accuracy of testing, but will significantly improve the rapidity of results achievement. Karyotyping would be performed to verify the aneuploidies, or if specifically asked by the gynecologist, due to the clinical observations.

Of course, fact remains that QF PCR will only answer questions regarding the number of chromosomes, it will not pick up on translocations, or structural abnormalities. (10,11) Karyotyping still remains the method of choice for complete diagnosis, but it does not need to be performed on all of the samples. Using QF PCR as a stand alone procedure actually makes karyotyping of the samples indicated for it quicker, due to the fact that it solves majority of samples indicated for testing for reasons such as older age of parents, which, in our experience, makes up more than 90% of samples. Observed rate of positivity for abnormalities of 1.51 % in this study further supports that claim.

CONFLICT OF INTEREST: NONE DECLARED

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