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Original article

Accuracy of Prenatal Diagnosis of Common Aneuploidies Using QF-PCR Comparing with Standard Karyotyping in Thai Pregnant Women

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Abstract This study compared the efficacy of prenatal QF-PCR testing at a STR loci set and standard karyotyping for the detection of common fetal aneuploidy in Thai pregnant women. Genetic variations of the 21 STR markers were evaluated in a Thai population. Amniotic fluid or umbilical cord blood, together with buccal swabs, were collected from 648 volunteer pregnant women, all of whom were diagnosed as being at high risk of pregnancy with chromosomal abnormality. Twenty-two abnormal karyotyping-result samples were analyzed and compared with random 22 normal samples. QF-PCR was performed with the Devyser Compact v3 kit, while fragment analysis was conducted by capillary electrophoresis using the Applied Biosystems 3500 Genetic Analyzer, and genetic variation analysis was carried out with the forensic statistics analysis toolbox (FORSTAT) and GenAlEx 6.5. The QF-PCR and karyotyping results were identical, and no false positive or negative results were observed in either test. Heterozygosity, polymorphism information content (PIC), and power of discrimination (PD) were 76.1-95.2%, 0.54-0.92, and 0.744-0.970, respectively. All 21 STR markers of Thai pregnant women in this study displayed acceptable polymorphism and heterozygosity to be used as prenatal diagnosis. Further study in larger population about the polymorphism, cost-effectiveness and specimen transferring should be performed.

***Keywords*: QF-PCR; prenatal diagnosis; aneuploidy; karyotyping**

Introduction

Presently, there are 2 types of prenatal diagnostic testing methods commonly used for the determination of fetal chromosomal aberration. First, invasive method, which need amniocentesis or cordocentesis procedure. Second, non-invasive prenatal testing using next

generation genetic analyzer, which use maternal peripheral blood as examined specimen. Each method has their own advantages and disadvantages[1]. Important factors which indicate the method selection in each situation are the cost, economy of scale, time consuming and sample transferring capability. In this study, an invasive method was tested. A set of short tandem repeat loci using in a commercial QF-PCR test is examined for its suitability to be used in Thai population. Moreover, the method is also suitable for the situation when the rapid result is needed for the decision of pregnancy termination. Therefore, accuracy of the result is evaluated in the study. The data in this study maybe useful for the genetic counselor and further prenatal genetic testing study.

The estimated induced abortion ratio in Thailand is about 19.5 per 1,000 live births, with 15.4% of cases indicated by fetal abnormality, including congenital anomalies and hereditary diseases, identified with prenatal diagnosis techniques[2]. Aneuploidy is the most frequently found chromosomal abnormality in humans, occurring in at least 5% of all pregnancies, and it is the leading cause of pregnancy loss[3,4]. The most common chromosomal aneuploidy in newborns include Trisomy 21 (Down syndrome), Trisomy 18 (Edward syndrome), Trisomy 13 (Patau syndrome), monosomy X (Turner's syndrome) and other sex-chromosome aneuploidies. These aneuploidies can account for up to 95% of liveborn chromosome abnormalities world-wide[5]. In Southern Thailand, Down syndrome is the most common chromosomal abnormality, with an incidence rate of 1.21 per 1,000 newborns. Only 34.1% of cases are prenatally diagnosed, resulting in the need for induced abortions[6]. This reflects the lack of an effective national prenatal screening program in the country.

Legitimate termination of pregnancies of more than 12 weeks' gestation is allowed when there is evidence of serious fetal impairment; therefore, accurate and rapid prenatal diagnosis of fetal genetic abnormality is needed in order for pregnant women to make a decision about terminating their pregnancy. As this is related to criminal law and not human rights issues, the method of diagnosis should have the following essential features:

1. It should be accurate;
2. Its results should be repeatable and retraceable;
3. The access to testing should be fair and available to every Thai citizen;
4. Its turnaround time should be short.

The gold standard technique for prenatal detection of fetal chromosomal abnormality is karyotyping analysis of cultured amniotic fluid cells (amniocentesis) or chorionic villi cells. This technique is currently the most accurate method with up to 99.5% accuracy[7-9]; however, the sensitivity of the karyotyping technique is limited by the number of cells in the specimen, and it can take about 2 weeks for results to be delivered[10-11]. Another restrictive factor is the need for skillful and expert genetic analysts in the analytical process, especially in cell culturing and interpretation of results[12]; furthermore, the karyotyping method is unrepeatable and requires for cell culturing. Large amounts of amniotic fluid or chorionic villi are normally needed for karyotyping analysis, so that all the amniotic fluid in the collected syringe is usually used in one go, making the method unrepeatable. Moreover, as the use of living cells is the most important factor for the success of cell culturing, amniocentesis samples have to reach the laboratory quite

quickly, and rapid sample transfer is still not possible in some areas of Thailand.

In recent years, quantitative fluorescence polymerase chain reaction (QF-PCR) has assisted greatly in the diagnosis of chromosomal aneuploidies directly from umbilical cord blood, amniotic fluid or CVS without the need for cell harvesting, and with a rapid turnaround time[7]. The sample transfer process is convenient and does not require cell culturing. The QF-PCR technique relies on quantitative multiplex PCR principles, using fluorescent labeled primers to amplify the small repetitive DNA sequences (STR, short tandem repeats). Currently, many countries include QF-PCR in their national prenatal screening programs[13]. There is no limitation in terms of the quantity and quality of fetal cells (amniocyte) in the sample of amniotic fluid because it does not require cell culture[14]; in addition, the remarkable superiority of QF-PCR methods over conventional cytogenetic and FISH methods derives from its ability to identify parental origin of the excess chromosomes[15], which can be useful in establishing guidelines for family planning genetics counselling.

DNA analysis of amniotic samples using the QF-PCR technique amplifies short tandem repeats base sequences (STR) located on chromosomes of interest to determine the copy numbers of those chromosomes in the cell[16]. Appropriate selection of STR markers for analysis needs to take into consideration the ethnicity of the population based on genetic variation parameters[17], so evaluation of the genetic variation of STRs in the population is required before applying QF-PCR in the national program[18]. This study examines the efficacy of assays for fetal chromosomal aneuploidies of chromosomes 13, 18, 21 and sex chromosomes, which together account for more than 90 percent of fetal chromosomal abnormalities. Analysis of amniotic fluid with QF-PCR technology at the evaluated STR markers should be the best option for the national prenatal screening program and in presenting confident legal evidence for abortive procedures. The aims of this study were to compare the efficacy of QF-PCR and standard karyotyping for the detection of common aneuploidy of the fetus in Thai pregnant women and evaluate genetic variation of a set of21 STR markers in Thai population.

Materials and Methods

The study involved pregnant women who were referred to the High-Risk Pregnancy Clinic of Rajavithi Hospital between January 2019 and January 2021.

Inclusion criteria:

1. Volunteer and her husband were Thais.
2. Volunteer was a pregnant woman over 18 years old.
3. Volunteer had a high risk of pregnancy with chromosomal abnormality which are:

1) advanced maternal age of at least 35 years old at the expected date of delivery
2) previous child or pregnancy with chromosomal abnormality
3) abnormal ultrasound findings
4) abnormal maternal serum biochemical markers

4. Volunteer provided written informed consent

Exclusion criteria:

1. Twin (or more) pregnancy
2. Heavily maternal blood contamination in the amniotic fluid
3. Volunteer had any difficulty or contraindication for buccal swab procedure

4. Volunteer had a prior diagnosis of pregnancy of chromosomal recombinant fetus

Withdrawal criteria:

1. The karyotyping result was not available

Samples collection and selection

Institutional Review Board of Rajavithi Hospital had reviewed and approved the study (EC. No.099/2561). Thai Pregnant women over 18 years old were asked to entry to the study. The subjects were evaluated as having a high risk of pregnancy with chromosomal abnormalities. All participants in the study provided written informed consent (n=648). Either 2 ml of amniotic fluid or 0.1 ml of umbilical cord blood was taken from each pregnant woman, and buccal swab samples were collected using a Whatman® sterile foam-tipped applicator. The fresh amniotic fluid or umbilical cord blood sample was divided into two parts; the first part was for regular cytogenetic diagnosis and the second part was for genomic extraction followed by QF-PCR for compare of the results.Second part samples were well keptat 20°C until analyzed. All aneuploidy samples by karyotyping method were analyzed with the QF-PCR (n=22). Twenty-two out of 624 normal karyotypic samples were randomly selected to analyze as the negative samples.

DNA extraction

DNA was extracted from a prenatal sample (amniotic fluid or umbilical cord blood) using a QIAamp DNA Blood Mini kit (Qiagen, Germany), and PrepFiler Express™ Forensic DNA Extraction Kit on Automate Express (Thermo Fisher Scientific, Waltham, MA, USA) was used to extract DNA from the buccal swabs in accordance with the instructions on the kit. After extraction, the DNA quantity of the samples was determined using the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, USA) of the Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, USA).

Markers used

This experiment was performed with Multiplex PCR using the Devyser Compact v3 kit (Hagersten, Sweden), which was tested on 26 markers as follows: five STRs from chromosome 13 (D13S742, D13S634, D13S628, D13S305, D13S1492); five from chromosome 18 (D18S978, D18S535, D18S386, D18S976, GATA178F11); six from chromosome 21 (D21S1435, D21S11, D21S1411, D21S1444, D21S1442, D21S1437); and 10 STRs from chromosome X and Y (DXS1187, XHPRT, DXS2390, SRY, DXYS267, DXYS218, AMELXY, ZFYX, T1, T3). As an extra marker for confirmation of inconclusive results,we utilised Devyser Resolution v2 consisting of Devyser Resolution 21 v2, Devyser Resolution 18 v2, Devyser Resolution 13 v2 and Devyser Resolution XY v2.The list of markers and labeling information are available in the Devyser kit.

Multiplex QF-PCR

QF-PCR was performed with the Devyser Compact v3 kit (Hagersten, Sweden) in accordance with the manufacturer's instructions. The PCR program was used as follows: pre-denaturation at 95°C for 15 min, and 94°C for 30 s; annealing at 58°C for 1 min 30 s and extension at 72°C for 1 min 30 s for 27 cycles, and final extension at 72°C for 30 min. The reaction was performed in a thermal cycler (Thermofisher, USA). The protocol was applied for the amplification of both prenatal and buccal swab samples.

Fragment and data analysis

Fragment analysis was performed by capillary electrophoresis using the Applied Biosystems 3500 Genetic Analyzer (Thermofisher, USA), with 3500 Data Collection software, 36 cm capillary array length, and Performance Optimized Polymer 4 (Applied Biosystems) for electrophoresis. Data were analyzed and electropherograms were constructed using Gene Mapper IDX software v 1.5.A normal control sample was consistently analyzed with each run, and the results were compared with routine karyotyping obtained from cultured cells.

The interpretation results were defined in terms of peak-areas ratios. For the presence of normal heterozygous markers, the allele ratio was from 0.8 - 1.4 and up to 1.5 if the alleles were separated by more than 24 bp. The presence of either three alleles in a 1:1:1 ratio or two alleles with a ratio of <0.65 or >1.8 was considered to represent a trisomic pattern, while the presence of only one peak was classified as non-informative. Markers demonstrating allele ratios falling in the intermediate ranges (1.4 - 1.8 and 0.65 - 0.8) were classified as inconclusive results. At least two informative markers were chosen to give normal results for each chromosome, and at least 3 markers were needed to report an abnormality. When a trisomic pattern (2:1 or 1:1:1 ratios) was detected in only one marker, extra markers were used for confirmation of inconclusive results. In the case of amplification failure, the study was repeated at DNA isolation level at least twice.

Genetic variation analysis

Heterozygosity, typical paternity index, polymorphism information content (PIC), power of exclusion (PE) and power of discrimination (PD) were calculated using the forensic statistics analysis toolbox (FORSTAT)[19], and probability of identity (matching probability) was calculated using the GenAlEx 6.5.

Results

Sensitivity and specificity

A total of 44 prenatal samples (22 abnormal and 22 normal) were investigated, and the results of QF-PCR samples were compared to those of cytogenetic tests. All the result in this study was interpretable. Inconclusive result and amplification failure were not found. QF-PCR results of buccal swab from the pregnant women were used as maternal cell contamination control. All results from amniotic fluid or CB, were compared with results from the buccal swab. Maternal cell contamination was not found in the study. The results of QF-PCR were as follows: trisomy 21 (eight cases), trisomy 18 (seven cases), trisomy 13 (three cases), triploidy (one case), and sex chromosome trisomy (three cases). Trisomic pattern in only one marker was not found in the study. Therefore, extra markers examination was not performed. The results of the QF-PCR were consistent with those of cytogenetic analyses in 22 out of 22 total abnormalities tested. All 22 normal samples were successfully assigned as normal copy numbers of chromosomes 13, 18, 21 and sex chromosome by QF-PCR. Therefore, the sensitivity and specificity were both 100% (Table 1). An example of the abnormal electropherogram is shown in figure 1.

Table 1 shows the sensitivity, specificity, and positive and negative predictive values for all cases. No false positive or negative results were observed in either test. All the parents' samples were found to be negative for aneuploidy and produced either two (het-

Figure 1 An example of the abnormal electropherogram (Down's syndrome), showing a triallelic pattern at 21B locus and peak ratios of 2:1 at 21A and 21D loci

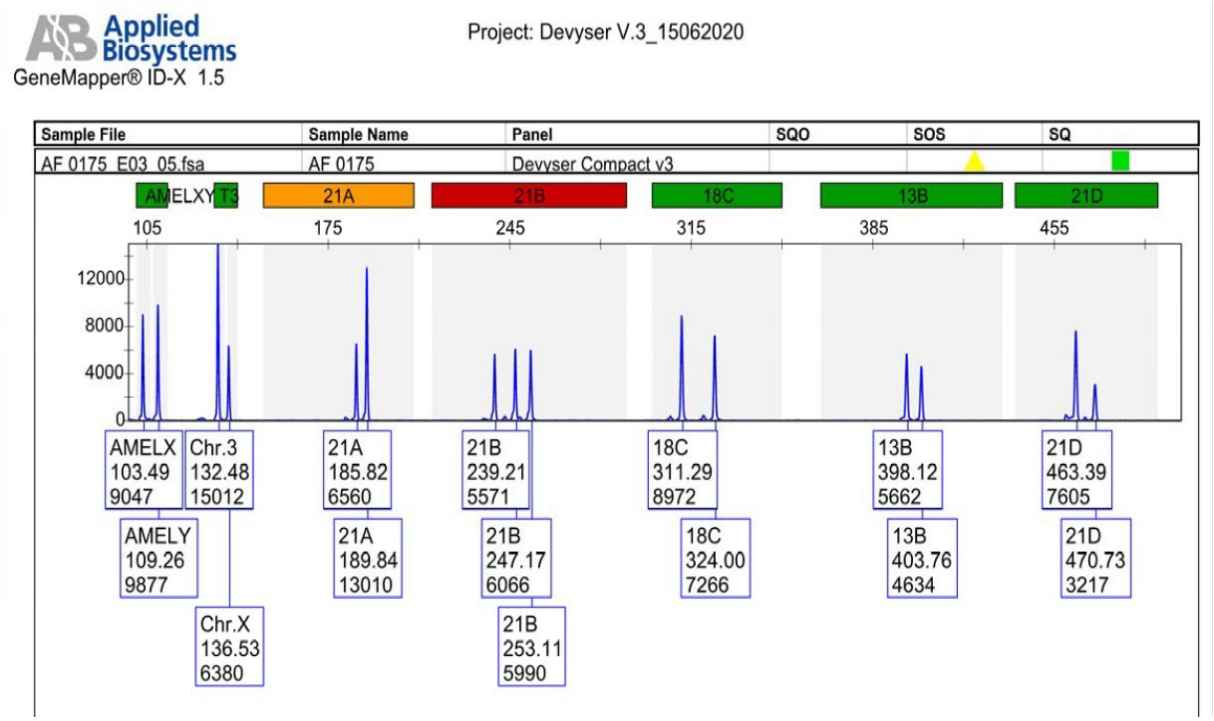

Table 1 Statistical analysis of AF and CB samples tested using QF-PCR in high-risk pregnancies with an aneuploid fetus.

Statistical analysis	AF and CB samples without rearrangements cases (n=44) (%)	AF and CB samples without mosaicism cases (n=44) (%)
Sensitivity	100	100
Specificity	100	100
Positive predictive value	100	100
Negative predictive value	100	100
Accuracy	100	100

erozygous) or a single allele (homozygous) peak for the markers used.

Allelic size range

The observed allele size ranges are shown in Table 2. Observed allelic size ranges were within the expected size range recommended by the manufacturer at all examined loci.

Genetic variation parameters

In this study, heterozygosity and other genetic variation parameters were calculated for evaluation of the polymorphism quality of 21 STR markers (Table 3). Non-polymorphic marker of the amelogenin gene (AMELXY, ZFYX, T1, T3) and SRY were included for fetal sex determination purpose and not taken to

Table 2 Allele size range of the STR markers included in this study compared with those reported by the commercial kit manufacturer.

Marker	Location	Expected size (bp)	Observed size (bp)	Observed alleles (bp)
D13S742	13q12.12	222 - 334	248-285	248, 252, 255, 256, 259, 260, 263, 264, 265, 267, 268, 269, 271, 272, 273, 275, 276, 277, 281, 285
D13S634	13q21.32-q21.33	365 - 435	388-423	388, 394, 398, 400, 402, 404, 406, 408, 410, 411, 412, 415, 416, 417, 418, 419, 423
D13S628	13q31.	420 - 475	433-468	433, 434, 445, 453, 454, 457, 458, 460, 461, 464, 468
D13S305	13q13.3	435 - 505	446-469	446, 447, 450, 451, 454, 455, 458, 459, 461, 462, 465, 466, 469
D13S1492	13q21.1	100 - 175	112-164	112, 113, 116, 120, 124, 131, 132, 135, 136, 139, 140, 143, 144, 147, 148, 151, 155, 156, 159, 160, 164
D18S978	18q12.3	195 - 230	208-228	208, 209, 212, 216, 217, 220, 224, 228
D18S535	18q12.3	300 - 350	307-336	307, 311, 316, 320, 324, 328, 332, 336
D18S386	18q22.1	338 - 430	343-407	343, 347, 348, 351, 352, 355, 356, 359, 360, 361, 363, 364, 367, 368, 369, 371, 372, 375, 377, 380, 381, 385, 387, 390, 391, 394, 395, 396, 399, 400, 407
D18S976	18p11.31	440 - 495	452-478	452, 456, 459, 460, 462, 463, 464, 466, 467, 474, 478
GATA178F11	18p11.32	350 - 410	363-394	363, 367, 370, 374, 378, 382, 383, 385, 386, 389, 390, 394
D21S1435	21q21.3	150 - 208	178-198	178, 179, 182, 186, 190, 194, 198
D21S11	21q21.1	215 - 290	235-261	235, 239, 243, 247, 249, 251, 253, 255, 257, 259, 261
D21S1411	21q22.3	245 - 345	285-336	285, 290, 294, 298, 299, 302, 303, 306, 307, 308, 310, 311, 312, 315, 316, 319, 320, 336
D21S1444	21q22.13	440 - 495	456-486	456, 460, 462, 463, 464, 465, 466, 467, 468, 471, 474, 475, 477, 478, 479, 483, 486
D21S1442	21q21.3	362 - 420	374-414	374, 378, 382, 383, 386, 387, 390, 394, 395, 398, 402, 410, 414
D21S1437	21q21.1	105 - 152	115-150	115, 116, 119, 120, 123, 127, 130, 131, 134, 135, 136, 138, 139, 142, 143, 150
DXS1187	Xq26.2	120 - 170	138-155	138, 142, 146, 147, 150, 151, 155
XHPRT	Xq26.2-q26.3	265 - 308	281-306	281, 282, 285, 286, 289, 290, 293, 294, 298, 306
DXS2390	Xq27.1-q27.2	312 - 357	321-342	321, 322, 326, 329, 330, 334, 338, 342
DXYS267	Xq21.31, Yp11.31	175 - 217	191-207	191, 194, 195, 199, 203, 207
DXYS218	Xp22.33, Yp11.32	215 - 260	234-255	234, 235, 238, 239, 242, 243, 246, 247, 255

Table 3 Genetic variation parameters of the 21 STRs on chromosomes 13, 18, 21, X, and Y in the Thai population.

Marker	Heterozygosity %	Typical paternity index	Polymorphism information content (PIC)	Power of exclusion (PE)	Power of discrimination (PD)
D13S742	95.2	5.50	0.91	0.814	0.966
D13S634	92.7	3.67	0.85	0.722	0.956
D13S628	81.1	1.57	0.75	0.401	0.901
D13S305	95.2	5.50	0.84	0.814	0.939

Table 3 Genetic variation parameters of the 21 STRs on chromosomes 13, 18, 21, X, and Y in the Thai population (cont.)

Marker	Heterozygosity %	Typical paternity index	Polymorphism information content (PIC)	Power of exclusion (PE)	Power of discrimination (PD)
D13S1492	91.4	3.14	0.89	0.677	0.961
D18S978	84.2	1.83	0.70	0.472	0.890
D18S535	82.7	1.69	0.74	0.435	0.905
D18S386	95.2	5.50	0.92	0.814	0.970
D18S976	85.7	2.00	0.81	0.510	0.928
GATA178F11	85.7	2.00	0.81	0.510	0.941
D21S1435	85.7	2.00	0.74	0.510	0.908
D21S11	88.6	2.44	0.81	0.591	0.942
D21S1411	94.0	4.40	0.90	0.768	0.966
D21S1444	88.6	2.44	0.85	0.591	0.954
D21S1442	84.2	1.83	0.81	0.472	0.935
D21S1437	87.2	2.20	0.85	0.549	0.937
DXS1187	91.4	3.14	0.78	0.677	0.912
XHPRT	92.7	3.67	0.83	0.722	0.921
DXS2390	91.4	3.14	0.78	0.677	0.919
DXYS267	76.1	1.29	0.54	0.308	0.774
DXYS218	81.08	1.57	0.77	0.401	0.884

the heterozygosity evaluation. All 21 STR markers displayed acceptable polymorphism and heterozygosity in our population. Heterozygosity, PIC, and PD were 76.1-95.2%, 0.54-0.92, and 0.744-0.970 respectively. Among all the STR markers, D13S742, D13S305, D18S386, D21S1411, XHPRT, D13S634, D13S1492, DXS1187, DXS2390 showed high heterozygosity of more than 90%, with D13S742, D13S305, and D18S386 showing the highest heterozygosity of 95.2%, while D21S1411 had 94%, XHPRT and D13S634 showed 92.7%, and DS13S1492, DXS1187, and DXS2390 had 91.4%. DXYS267 STR showed the lowest heterozygosity at 76.1%. The results obtained for heterozygosity and PIC of each of the 21 STR markers showed that locus D18S386 was the most polymorphic marker, and that DXYS267 was the least.

Discussion

When using QF-PCR for prenatal diagnosis, careful consideration should be given to the selection of the appropriate STR markers and also to the best number of markers to use for amplification[20].

Validation of the QF-PCR method requires extensive information about STR markers, such as heterozygosity, number, distribution and the size of possible alleles. Data validation is a necessary step before the testing can be used in prenatal diagnosis[21]. All markers have to be examined for heterozygosity and polymorphism characteristics specific to each

population[22].This is a preliminary genetic variation study of 21 STR markers in Thai population, and the results show that these 21 loci have high heterozygosity in the population in this study.

In our study, the results obtained by QF-PCR and karyotyping techniques were compared in 44 cases and showed a 100% match up. This result is concordance with other study in other ethnicity such as Huo et al[23], which reported 100% of concordance rate of the 428 samples without any falsepositive or false-negative results in Chinese population. However, there are some limitations of the QF-PCR method in prenatal diagnosis, especially cases with chromosomal rearrangements. De Moraes et al[4] and Rostami et al[24] worked on 162 and 4,058 samples, and reported a concordance rate of 93 and 98.6% respectively. Concordance rate of the earlier studies is varied between 93 to 100% according to the number of chromosomal structural abnormalities and mosaicisms cases found in each study. As a result, accuracy of "not less than 90%" should be given by the genetic counsellor rather than 100%. Advantages of prenatal QF PCR are the rapid laboratory turn around time[7], the minimal amount of specimen requirement and no tissue culture is required [14].Significant method limitation of QF-PCR is the incapability to detect the abnormality in balanced rearrangement and mosaicism proportion of <30%[25].According to the Covid 19 pandemic during the study, the number of participants in this study is quite small. Further study in a larger population is needed to evaluate the suitability for nation-wide implementation. The cost effectiveness and specimen robustness should be also evaluated.

In Thailand, there is as yet no clear policy regarding recommended diagnostic methods for identifying factors which justify legitimate abortion. As a rapid method used in genetic counseling, QF-PCR's advantages and disadvantages in comparison with karyotyping should be clearly explained to the patient. Our study indicates that the QF-PCR method using21 markers of Devyser Compact v3 kit for prenatal testing trend to be useful in Thai pregnant women, according to the rapid turnaround time and sufficient polymorphism.

Conclusion

Devyser Compact v3 QF-PCR kit trend to be useful for prenatal aneuploidy diagnosis in Thai population. Further research should be considered for other sub-population ethnics and the using of other reagent kit. Cost effectiveness assessment and study in larger population are needed before nation-wide implementation.

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Conflict of interest: none

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บทคัดย่อ: การวินิจฉัยก่อนคลอดของทารกที่มีความผิดปกติของโครโมโซมชนิด aneuploidy ที่พบได้บ่อยโดยการใช้เทคนิค QF-PCR เทียบกับการตรวจแคริโอไทป์มาตรฐานในหญิงไทยตั้งครรภ์

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การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของการตรวจตัวอย่างน้ำคร่ำหรือเลือดจากสายสะดือด้วยเทคนิค QF-PCR ในการวินิจฉัยทารกในครรภ์ที่มีความผิดปกติของโครโมโซมชนิด aneuploidy สำหรับหญิงไทยตั้งครรภ์โดยทำการประเมินประสิทธิภาพของตำแหน่งจำเพาะบนโครโมโซมคู่ที่ 13, 18, 21 และโครโมโซมเพศในกลุ่มประชากรไทย โดยเก็บตัวอย่างน้ำคร่ำ/เลือดจากรก (cord blood) และเยื่อบุกระพุ้งแก้ม จำนวน 648 ตัวอย่าง จากอาสาสมัครที่ได้รับการวินิจฉัยว่าตั้งครรภ์เสี่ยงสูงจากนั้นทำการตรวจวิเคราะห์ตัวอย่างจำนวน 22 ตัวอย่างที่มีผลการตรวจด้วยวิธี karyotyping ว่ามีความผิดปกติ เปรียบเทียบกับ 22 ตัวอย่างที่ทำการสุ่มมาจากตัวอย่างที่ผล karyotyping รายงานว่าปกติ โดยการตรวจวิเคราะห์ทำโดยการเพิ่มปริมาณสารพันธุกรรมด้วยชุดน้ำยา Devyser Compact v3 kit แล้วนำไปแยกสารพันธุกรรมด้วยกระแสไฟฟ้า (capillary electrophoresis) ด้วยเครื่อง Applied Biosystems 3500 Genetic Analyzer และหาค่าความแปรปรวนทางพันธุกรรม (genetic variation) ด้วยโปรแกรม Forensic statistics analysis toolbox (FORSTAT) และ GenAlEx 6.5. จากการศึกษาพบว่า QF-PCR ในตำแหน่งที่ทำการศึกษานี้มีความสอดคล้องกับผลการตรวจ karyotype ทั้งหมด ไม่พบผลบวกปลอมและผลลบปลอม ค่า polymorphism และ heterozygosity ของ short tandem repeat ที่ทำการตรวจทั้ง 21 ตำแหน่งอยู่ในเกณฑ์ที่ยอมรับได้ โดยมีค่า Heterozygosity ตั้งแต่ 76.1-95.2% มีค่า polymorphism information content (PIC) อยู่ระหว่าง 0.54-0.92 และค่า power of discrimination (PD) อยู่ระหว่าง 0.744-0.970 ซึ่งเป็นค่าที่แสดงให้เห็นได้ว่าตำแหน่งที่ทำการตรวจในการศึกษาครั้งนี้มีแนวโน้มการกระจายตัวที่น่าจะเหมาะสมสำหรับใช้ในการตรวจในกลุ่มประชากรไทย ข้อเสนอแนะสำหรับการศึกษาเพิ่มเติมต่อไปคือการศึกษา polymorphism ในกลุ่มประชากรที่มีขนาดใหญ่ขึ้น รวมทั้งการศึกษาเกี่ยวกับความคุ้มค่าคุ้มทุนและความสะดวกในการส่งต่อสิ่งส่งตรวจของการตรวจวินิจฉัยความผิดปกติของโครโมโซมชนิด aneuploidy ของทารกในครรภ์ด้วยเทคนิค QF-PCR โดยชุด Devyser compact v3 หรือชุดตรวจอื่น ๆ ต่อไป

คำสำคัญ: เทคนิค QF-PCR; การวินิจฉัยก่อนคลอด; โครโมโซม aneuploidy; คาริโอไทป์