ว กรมวิทย พ 2565; 64 (1): 1-13

Prenatal Diagnosis of Down Syndrome and Common Chromosomal Disorders Using Molecular Karyotyping

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ABSTRACT For the prevention and control of Down syndrome and other chromosomal disorders, laboratory procedures for prenatal diagnosis should be established for high-risk pregnancies. A rapid molecular karyotyping assay, BACs-on-Beads (BoBs™), has been developed for diagnosis of Down syndrome and common aneuploidies of chromosomes 13, 18, X and Y as well as nine microdeletion/microduplication syndromes. The study evaluated the performance of BoBs™ assay for prenatal diagnosis of Down syndrome and other chromosomal disorders in amniotic fluid samples (n = 1,004) obtained *via* amniocentesis between the 15th and 22th weeks of gestation in comparison with the gold standard conventional karyotyping. Interpretable results were obtained with BoBs™ assay in detection of 26 chromosome abnormalities comprising 23 aneuploidies (Down syndrome, n = 10; Edwards syndrome, n = 7; Klinefelter syndrome, n = 1; Patau syndrome, n = 1; Triple X syndrome, n = 1; and Turner syndrome n = 3) with 100% agreement with conventional karyotyping and three cases of microdeletion/microduplication syndromes [22q11.2 microdeletion (DiGeorge syndrome), n = 1 and 22q11.2 microduplication, n = 2] missed by conventional karyotyping. The assay has been implemented for prenatal diagnosis service at the National Institute of Health according to ISO 15189:2012. In conclusion, molecular BoBs™ assay provides a rapid and reliable method for detection of common aneuploidies and microdeletion/microduplication syndromes in uncultured prenatal samples and should be helpful in the prevention and control of Down syndrome and other chromosomal disorders in the country.

Keywords: Chromosomal disorders, Down syndrome, Microdeletion/microduplication syndromes, Molecular karyotyping, Prenatal diagnosis

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Received: 25 November 2021 *Revised: 1 Feburaury 2022* *Accepted: 28 Feburaury 2022*

Introduction

Down syndrome is one of the most common genetic birth defects caused by presence of all or part of a third copy of chromosome 21. The World Health Organization (WHO) predicts Down syndrome prevalence to range from 1 in 1,000 to 1 in 1,100 live births worldwide.[1] Children with Down syndrome usually have a wide range of intellectual impairment accompanied by a variety of congenital anomalies and delayed growth, which require special care in monitoring and treatment of several physiological systems.[2-6]

The rate of live births with Down syndrome increases with maternal age: 0.61–1.46, 4.58, 15.7, and 33.50 per 1,000 births for mothers aged < 35, 35–39, 40–44, and 45–49 years, respectively;[7] however, the majority of children with Down syndrome are born to mothers aged < 35.[8-10] National public policies including additional financial and welfare support are recommended to provide adequate antenatal care for mothers and reduce economic burden of lifelong medical care for offspring with Down syndrome.[10]

Appropriate laboratory procedures for prenatal screening and diagnosis are required in programs for prevention and control of Down and other syndromes of chromosomal abnormalities. Screening tests should be carried out based on maternal age, ultrasound for measurement of nuchal translucency, serum assays and non-invasive prenatal tests.[11-14] Pregnant women with high risk of having fetus with Down and other chromosomal abnormality syndromes are usually offered prenatal diagnosis, which consists of fetal karyotyping to analyze numerical and structural changes of all observed chromosomes and is considered the gold standard.[15] The advantage of this type of karyotyping is that both imbalanced as well as balanced chromosome aberrations are detectable, but suffers from several disadvantages, such as requirement of specialized technique, lengthy duration (10–14 days) of cell culture, possibility of cell culture failure and limitation in detection of chromosome abnormalities ~5 Mb in length, e.g., microdeletion and microduplication.[16-20] The lengthy wait for laboratory results imposes high-risk mothers to stress and anxiety prior to genetic counseling and appropriate intervention measures.

Rapid aneuploidy diagnoses (RAD), such as bacterial artificial chromosomes (BACs)-on-Beads (BoBs™), fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification and quantitative fluorescence polymerase chain reaction (QF-PCR), have been developed to detect common aneuploidies.[16,17,21] In several countries, implementation of RAD services for all prenatal samples are considered a necessary requirement in optimization of prenatal services.[22] For instance, in the UK, RAD is recommended as a stand-alone approach in a Down syndrome screening program.[17,23]

A rapid molecular karyotyping BACs-on-Beads (BoBs™) assay is a multiplex assay employing beads impregnated with different concentrations of two different fluorochromes to create an array of up to 100 different unique probes to measure DNA copy numbers at chromosome arm resolution, such as DNA gain or loss and genomic rearrangement.[21] This molecular karyotyping assay has been developed to detect common aneuploidies of chromosomes 13, 18, 21, X and Y, as well as nine common

chromosomal microdeletions/microduplications responsible for Angelman, Cri du Chat, DiGeorge, Langer-Giedion, Miller-Dieker, Prader-Willi, Smith-Magenis, Williams-Beuren, and Wolf-Hirschhorn syndromes, selected based on their relatively high prevalence (1/2,000–1/300,000 population), significant morbidity/mortality, mild or unspecific ultrasound findings, strong genotypic and phenotypic correlation and changes typically too small to be detected by conventional karyotyping.[24,25] The assay is accepted by European countries, Australia and New Zealand.[26,27]

The Department of Medical Sciences, Ministry of Public Health, Thailand, in collaboration with the Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University, has the goal of developing a molecular karyotyping service for rapid detection of Down syndrome in prenatal amniotic fluid samples. In this study, molecular karyotyping BACs-on-Beads (BoBs™) assay was evaluated as a potential rapid and reliable prenatal diagnosis for common aneuploidies of chromosomes 13, 18, 21, X and Y, as well as the nine common chromosomal microdeletions/microduplications. The findings should provide baseline data for development of a prevention and control program for syndromes associated with common chromosomal abnormalities present in the country.

Materials and Methods

Participants and sample collection

Amniotic fluid samples (n = 1,008; 3–5 mL) were obtained from pregnant mothers at 15–22 weeks of pregnancy attending an antenatal clinic, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Chiang Mai, from August 2016 through October 2017. All subjects had undergone prenatal diagnosis by amniocentesis and chromosome study with an indication of high risk of having fetus with Down syndrome. All cases have been counseled and informed that amniotic fluid samples would be tested by using two techniques: the conventional one and the molecular karyotyping assay. The samples for the conventional method were sent to the cytogenetic unit of the Department of Anatomy, Faculty of Medicine, Chiang Mai University, as usual. The others (3–5 mL of amniotic fluid samples) were stored and transported at 2–8°C within 7 days after collection to the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand.

The study protocol was approved by the Ethics committee, Faculty of Medicine, Chiang Mai University (approval no. 188/2559, research ID 3862, study code OBG-2559-03862). Prior written consent was obtained from all participants.

Molecular karyotyping protocol

DNA was extracted from amniotic fluid using a QIAamp DNA Blood Mini Kit (QIAGEN, Germany), concentrated and purity determined by measurement of A260 nm:A280 nm (NanoDrop 1000 spectrophotometer; Thermo Scientific, USA). Molecular karyotyping was carried out using a Prenatal-BoBs™ BACs-on-Beads assay (Perkin Elmer, Finland) to detect aneuploidy of chromosomes 13, 18, 21, X and Y and nine microdeletion/microduplication regions. In brief, genomic DNA samples and reference DNA (male and female) samples (Promega, USA)

were labeled with biotin, purified based on ultrafiltration (NucleoFast® 96 PCR; Macherey-Nagel GmbH & Co., Germany) and hybridized to BoBs™ beads overnight at 52°C in a 96-well plate (Perkin Elmer, Finland), washed with wash buffer (Perkin Elmer, Finland), and then transferred to a 96-well filter plate (MultiScreen® HTS HV Sterile plate; Merck KGaA, Germany). The reactions were washed as described above, incubated at 37°C for 30 minutes with phycoerythrin-labeled streptavidin (Perkin Elmer, Finland), washed as described above, and then fluorescent signals were measured using a Luminex™ 200 spectrofluorometer (Luminex Corp., USA) (λ532 nm excitation, λ575 nm emission). Molecular karyotyping results were analysed by BoBsoft 2.0 analysis software (Perkin Elmer, Finland) and reported as fluorescence ratio relative to both normal female and male reference DNA at each chromosome locus of interest, acceptance requiring a coefficient of variation (CV) less than 6%. A sample is defined as normal disomic when fluorescence ratio for a chromosome region has a value within the lower and upper threshold limits (mean fluorescence ratio±2 SD) and as having a deletion or duplication at a specific chromosomal locus when fluorescence ratio is below lower or above upper threshold, respectively.

Performance characteristics evaluation

Performance characteristics including accuracy and precision were evaluated. Accuracy is defined as degree of compliance with standard karyotyping method (n = 1,004) carried out at the cytogenetic unit of the Department of Anatomy, Faculty of Medicine, Chiang Mai University. Precision of the assay is defined as between-run variation (n = 44) in fluorescence ratio of six chromosome loci (13C, 18C, 21C, 22q11.2 (DiGeorge syndrome, DGS), X and Y) compared to male and female DNA reference samples performed by three different operators in the same laboratory setting.

Statistical analysis

Primary output measure is concordance of any numerical, structural or submicroscopic chromosomal abnormalities between molecular karyotyping assay and conventional karyotyping. Data are presented as numbers and percentages. Maternal age and gestational age were expressed as median, range and interquartile range (IQR). Mean, standard deviation (SD) and percent coefficient of variation (CV) were used to estimate precision.

Results

Participant ages, gestation status and DNA samples

Participants (n = 1,008) had a median age of 36 years (range 15–48 years) with a median gestational age of 17 weeks (range 15–22 weeks). DNA concentration and A260 nm:A280 nm values of amniotic fluid samples were 9.7±5.78 ng/μL and 2.0±0.4, respectively.

Molecular karyotyping assay

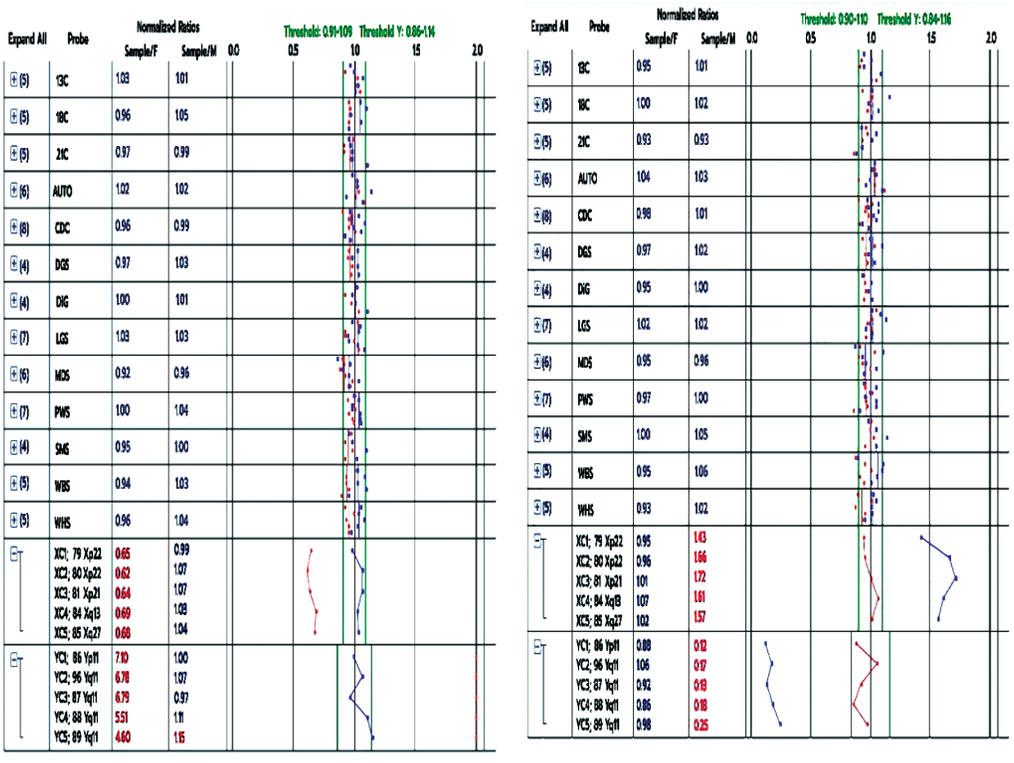
Molecular karyotyping assay gave conclusive results for 1,004 samples; failure in four samples was due to maternal cells contamination. Among the samples karyotyped by molecular assay, 978 (97.4%) were normal disomic, 23 (2.3%) aneuploidy and 3 (0.3%) microdeletion/microduplication (Table 1). As expected, Down

syndrome was the most common, followed by Edwards, Turner and 22q11.2 microduplication syndrome, while there was only one case each of DiGeorge, Klinefelter, Patau, and Triple X syndromes. For Patau, Edwards, Down and 22q11.2 microduplication syndromes, mean fluorescence ratios of chromosomes 13, 18, 21 and 22q11.2 marker, respectively, were above the upper threshold limit, whereas that of DGS marker in DiGeorge syndrome was below the lower threshold (Figures 1–3, Table 2).

Table 1 Molecular karyotyping assay was compared to conventional karyotyping of amniocentesis samples obtained from mothers attending the Antenatal Clinic, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (August 2016–October 2017).

Syndrome	Number of samples (%) (n = 1,004)	Maternal age (years)		Molecular karyotype	Conventional karyotype
		Median	Range/ IQR		
Normal, female	451 (44.9)	36	15–47/35–38	XX	46, XX
Normal, male	527 (52.5)	36	17–48/35–39	XY	46, XY
Down syndrome, female	2 (0.20)	34	30–38	XX,+21	47, XX,+21
Down syndrome, male	8 (0.8)	41	34–46/39–45	XY,+21	47, XY,+21
Edwards syndrome, female	1 (0.1)	38	38	XX,+18	47, XX,+18
Edwards syndrome, male	6 (0.6)	37	27–41/33–40	XY,+18	47, XY,+18
Patau syndrome, male	1 (0.1)	36	36	XY,+13	47, XY,+13
Turner syndrome	2 (0.2)	25	21–29	X	45, X
Mosaic Turner syndrome	1 (0.1)	36	36	X/XX	mos 45, X/46, XX (45%/55%)
Triple X syndrome	1 (0.1)	36	36	XXX	47, XXX
Klinefelter syndrome	1 (0.1)	45	45	XXY	47, XXY
DiGeorge syndrome, female	1 (0.1)	38	38	XX, del(22) (q11.2)	46, XX
22q11.2 microduplication syndrome, female	1 (0.1)	26	26	XX, dup(22) (q11.2q11.2)	46, XX
22q11.2 microduplication syndrome, male	1 (0.1)	27	27	XY, dup(22) (q11.2 q11.2)	46, XY

IQR, Interquartile range
mos, mosaic
del, deletion
dup, duplication



A) Normal male B) Normal female

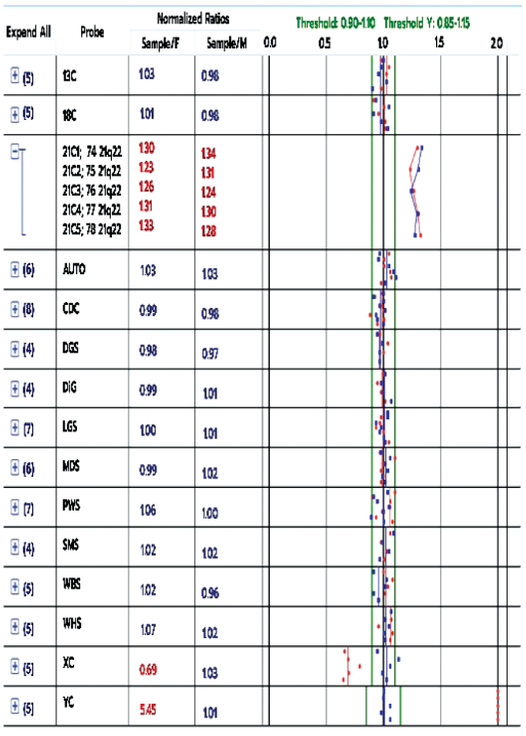
Figure 1 Molecular karyotyping profiles of normal male (A) and normal female (B). Red and blue spot represents sample:reference female and sample:reference male fluorescence ratio respectively.

Performance characteristics of molecular karyotyping

In the accuracy study, all samples showing aneuploidy were in agreement with conventional karyotyping, with no false-positive or false-negative results. Molecular karyotyping assay successfully detected three microdeletion/microduplication syndromes missed in the conventional method, including DiGeorge syndrome (22q11.2 microdeletion syndrome) (n = 1) and 22q11.2 microduplication syndrome (n = 2). Precision of the assay was determined as between-run variations of mean fluorescence ratio of six chromosome loci (13C, 18C, 21C, DGS, XC and YC) in male and female DNA internal control samples. Variability of between-run precision ranged 1.52-7.81% CV, considered satisfactory (Table 3).

Application of molecular karyotyping for prenatal diagnosis

Preliminary molecular karyotyping for prenatal diagnosis of Down syndrome and other chromosomal disorders was implemented at the Hematology Laboratory, Thailand National Institute of Health (Thai NIH) according to ISO 15189, 'Medical laboratories-particular requirements for quality and competence'.[28,29] Three medical scientists were trained to perform the analysis according to standard operating procedures. Of 19 samples investigated, 2 Down syndrome fetuses were identified. Performance specifications of the procedure were monitored for each analytical batch using male and female DNA internal quality controls and the findings of the participants in inter-laboratory comparison

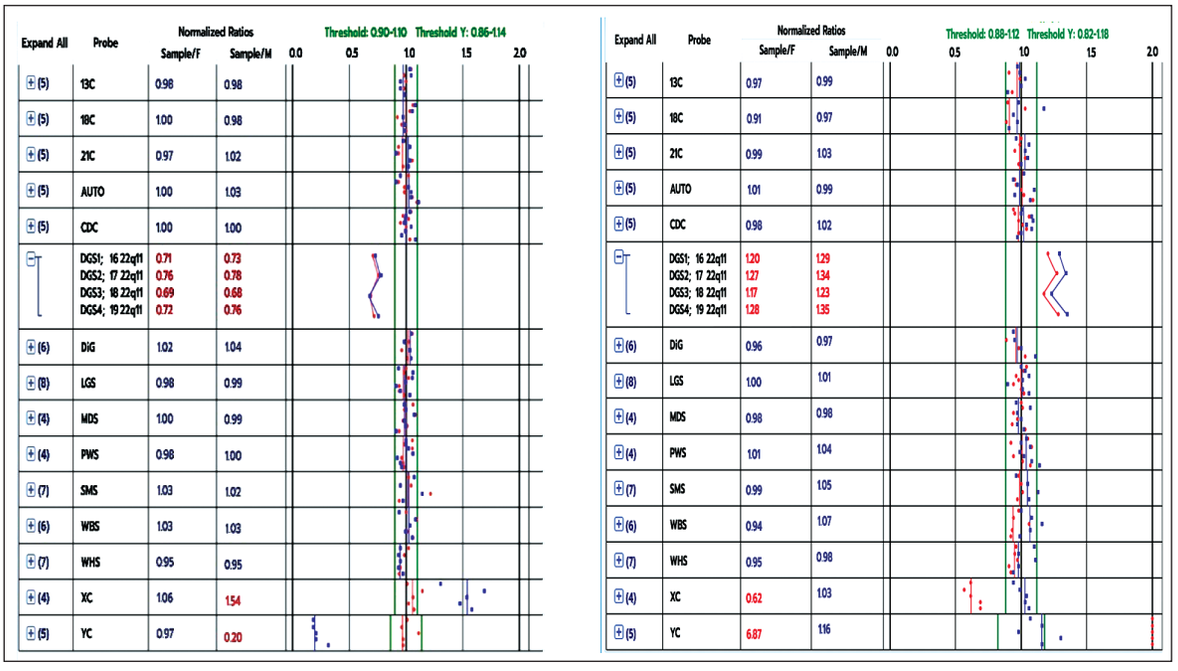


A) Trisomy 21 (Down syndrome)

B) Trisomy 18 (Edwards syndrome)

C) Trisomy 13 (Patau syndrome)

Figure 2 Molecular karyotyping profiles of Trisomy 21 (A), Trisomy 18 (B) and Trisomy 13 (C). Red and blue spot represents sample:reference female and sample:reference male fluorescence ratio respectively.



A) 22q11.2 microdeletion (DiGeorge syndrome) B) 22q11.2 microduplication

Figure 3 Molecular karyotyping profiles of 22q11.2 microdeletion (A) and 22q11.2 microduplication (B). Red and blue spot represents sample:reference female and sample:reference male fluorescence ratio respectively.

Table 2 Molecular karyotyping assay fluorescence ratios in chromosomal disorders of 13C, 18C, 21C and 22q11.2 DiGeorge syndrome (DGS) from amniocentesis samples of mothers attending the Antenatal Clinic, Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (August 2016–October 2017).

Syndrome	Number of samples	Fluorescence ratio (mean±SD)			
		13C	18C	21C	22q11 (DGS)
Down	10	0.98±0.03	1.01±0.02	1.29±0.03	0.99±0.02
Edwards	7	0.98±0.02	1.30±0.04	0.99±0.02	1.00±0.02
Patau	1	1.29	0.99	0.99	0.98
DiGeorge	1	1.03	0.99	0.96	0.69
22q11.2 microduplication	2	0.99±0.01	0.97±0.04	0.99±0.01	1.27±0.02
Normal	978	0.99±0.03	1.00±0.03	0.98±0.03	1.00±0.03

Table 3 Between-run precision of molecular karyotyping assay (n = 44).

Control	Probe	Min	Max	Mean	SD	%CV
Normal, male	13C	0.96	1.05	1.01	0.02	1.87
Normal, female	13C	0.98	1.05	1.01	0.02	1.52
Normal, male	18C	0.95	1.03	0.99	0.02	2.10
Normal, female	18C	0.96	1.04	0.99	0.02	2.03
Normal, male	21C	0.93	1.07	1.01	0.03	2.55
Normal, female	21C	0.95	1.05	1.00	0.03	2.62
Normal, male	DGS	0.96	1.04	1.01	0.02	1.97
Normal, female	DGS	0.95	1.04	0.99	0.02	1.73
Normal, male	X	0.79	0.89	0.84	0.02	2.64
Normal, female	X	0.96	1.15	1.01	0.03	3.08
Normal, male	Y	0.94	1.07	0.99	0.04	3.92
Normal, female	Y	0.46	0.71	0.59	0.05	7.81

were evaluated according ISO15189:2012 requirements. Turnaround time was reduced from three weeks using conventional method to three days with molecular karyotyping assay.

Discussion

In Thailand, the prevalence of Down syndrome is 1.21 per 1,000 births[9] and in 2016 there were 1,100 newborns born with Down syndrome.[3] A national Down syndrome prenatal screening program was implemented in the country by the National Health Security Office (NHSO) in 2019 whereby mothers > 35 years of age in the second trimester are offered free blood serum screening tests and prenatal diagnosis for at risk mothers to determine chromosome abnormality.[30] Mothers carrying Down syndrome fetuses are counseled on the choice of carrying to term or having an abortion.[9] Although advanced maternal age is the most important risk factor for Down syndrome, Adams MM et al.[8] reported 80% of infants with Down syndrome were born to mothers under 35 years of age and hence, from October 2020 the program was extended to all Thai pregnant women.[31]

In order to assist in the national Down syndrome prevention program, the Department of Medical Sciences, Ministry of Public Health, Thailand has collaborated with the Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University to assess the reliability of molecular karyotyping assay for detection of common aneuploidy and microdeletion/microduplication syndromes, in comparison with conventional gold standard karyotyping technique. Aneuploidies detected by molecular karyotyping assay was in concordance with those observed by the gold standard karyotyping technique and in addition three cases of microdeletion syndromes missed by conventional karyotyping were detected. Precision of the assay was quite high, with an overall %CV of between-run precision within the required range.

The major advantage of molecular karyotyping is its ability to provide more informative results than FISH and QF-PCR

methods that can only reveal common aneuploidies,[20] namely detection of microdeletion/microduplication syndromes. Total reagent and consumable costs per sample in molecular karyotyping assay are comparable with FISH and QF-PCR but less than array-comparative genomic hybridization (CGH).[18,21,32] In addition, molecular karyotyping assay is less labor-intensive and suitable for a high throughput platform, with a turnaround time within three days. However, molecular karyotyping assay cannot detect chromosome inversions, balanced translocations, point mutations, polyploidies, alterations in methylation and low-level mosaicism.[18,33,34] Both molecular karyotyping assay and conventional karyotyping alone could not detect all fetal chromosomal abnormalities. A combination of these techniques should improve the detection and accuracy of prenatal diagnosis.[19,25,33] In 2019, Miao Z et al[25] reported that the combined use of molecular karyotyping assay and conventional karyotyping could detect more fetal chromosomal abnormalities (4.51%) than either molecular karyotyping assay (2.97%) and conventional karyotyping (4.04%) alone.

Conclusion

Molecular karyotyping assay is as reliable as conventional gold standard karyotyping in detecting common aneuploidies and is able to identify common microdeletion/microduplication syndromes. Its high accuracy, low sample volume requirement, ease in implementation, adaptation to a high throughput platform and rapid turnaround time should assist in improving acceptance of the national Down syndrome prenatal screening program especially among late antenatal care pregnant women.

Acknowledgements

The research was supported by a grant from the Department of Medical Sciences, Ministry of Public Health, Thailand. The authors gratefully thank Professor Dr. Prapon Wilairat for valuable suggestions and assistance in editing the manuscript. The authors thank Ms. Panyakamol Chandrasakha and hematology laboratory staffs for their kind assistance.

Conflicts of Interest Declaration

The authors declare no conflicts of interest.

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การตรวจวินิจฉัยก่อนคลอดสำหรับกลุ่มอาการดาวน์และโครโมโซมผิดปกติที่พบบ่อย โดยวิธี Molecular Karyotyping

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บทคัดย่อ การตรวจวินิจฉัยก่อนคลอดมีความสำคัญในการควบคุมและป้องกันกลุ่มอาการดาวน์และโครโมโซมผิดปกติในหญิงตั้งครรภ์ที่มีความเสี่ยงสูง การตรวจวินิจฉัยก่อนคลอดโดยวิธีทางอณูพันธุศาสตร์ BACs-on-Beads (BoBs™) เป็นวิธีตรวจวินิจฉัยกลุ่มอาการดาวน์และโครโมโซมผิดปกติคู่ที่ 13, 18, X และ Y รวมถึง microdeletion/microduplication syndromes 9 ชนิด อย่างรวดเร็ว การศึกษานี้มีวัตถุประสงค์เพื่อประเมินประสิทธิภาพการตรวจวินิจฉัยโดยวิธีอณูพันธุศาสตร์ในตัวอย่างน้ำคร่ำของหญิงตั้งครรภ์ที่มีอายุครรภ์ 15-22 สัปดาห์ จำนวน 1,004 ราย ศึกษาเปรียบเทียบกับวิธี conventional karyotyping จากการเพาะเลี้ยงน้ำคร่ำซึ่งเป็นวิธีมาตรฐาน ผลการศึกษาพบความผิดปกติทางโครโมโซม 26 ตัวอย่าง เป็น aneuploidies 23 ตัวอย่าง (Down syndrome 10 ตัวอย่าง Edwards syndrome 7 ตัวอย่าง Klinefelter syndrome 1 ตัวอย่าง Patau syndrome 1 ตัวอย่าง Triple X syndrome 1 ตัวอย่าง และ Turner syndromes 3 ตัวอย่าง) ผลทั้งหมดสอดคล้องกับวิธีมาตรฐาน นอกจากนี้ยังสามารถวินิจฉัย microdeletion/microduplication syndromes ได้ 3 ตัวอย่าง (22q11.2 microdeletion 1 ตัวอย่าง และ 22q11.2 microduplication 2 ตัวอย่าง) ซึ่งวิธีดั้งเดิมตรวจไม่พบ วิธีการนี้ได้บูรณาการสู่การให้บริการตามมาตรฐานสากล ISO 15189:2012 ณ สถาบันวิจัยวิทยาศาสตร์สาธารณสุข การตรวจทางอณูพันธุศาสตร์นับเป็นวิธีที่น่าเชื่อถือและรวดเร็วในการตรวจวินิจฉัยก่อนคลอดสำหรับโครโมโซมผิดปกติที่พบบ่อย ทั้งชนิด aneuploidies และ microdeletion/microduplication syndromes โดยไม่ต้องเพาะเลี้ยงน้ำคร่ำ นับเป็นการสนับสนุนการควบคุมและป้องกันกลุ่มอาการดาวน์และโครโมโซมผิดปกติของประเทศ

คำสำคัญ: โครโมโซมผิดปกติ, กลุ่มอาการดาวน์, Microdeletion/microduplication syndrome, อณูพันธุศาสตร์, การตรวจวินิจฉัยก่อนคลอด