RESEARCH ARTICLE

Non-Invasive Prenatal Testing with Next Generation Sequencing Methods in Birth Defect Pregnancy: A Pilot Study

Anom Suardika[1], Anak Agung Ngurah Jaya Kusuma[2], Ni Gusti Ayu Manik Ermayanti[3], Endang Sri Widiyanti[4,*], I Gusti Ngurah Agung Satria Wibawa[5], Divika Silvana[5], Anak Agung Gede Raka Budayasa[6], Ni Nyoman Ayu Dewi[7], I Made Jawi[8], H. Sunny Sun[9], Yen-An Tang[9]

[1]Fertility and Reproductive Endocrinologi Division, Department of Obstetrics and Gynecology, Prof. Dr. I.G.N.G. Ngoerah General Hospital/ Faculty of Medicine, Universitas Udayana, Jl. Diponegoro, Denpasar 80113, Indonesia
[2]Maternal Fetal Medicine Division, Department of Obstetrics and Gynecology, Prof. Dr. I.G.N.G. Ngoerah General Hospital/Faculty of Medicine, Universitas Udayana, Jl. Diponegoro, Denpasar 80113, Indonesia
[3]Faculty of Mathematics and Natural Sciences, Universitas Udayana, Jl. Kampus Bukit Jimbaran, Denpasar 80361, Indonesia
[4]Maternal Fetal Medicine Division, Department of Obstetrics and Gynecology, Universitas Udayana Hospital/Faculty of Medicine, Universitas Udayana, Jl. Rumah Sakit Unud, Denpasar 80361, Indonesia
[5]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Udayana, Jl. P.B. Sudirman, Denpasar 80232, Indonesia
[6]Maternal Fetal Medicine Division, Department of Obstetrics and Gynecology, Sanjiwani Hospital, Jl. Ciung Wanara No.2, Gianyar 80511, Indonesia
[7]Department of Biochemistry, Faculty of Medicine, Universitas Udayana, Jl. P.B. Sudirman, Denpasar 80232, Indonesia
[8]Department of Pharmacology, Faculty of Medicine, Universitas Udayana, Jl. P.B. Sudirman, Denpasar 80232, Indonesia
[9]Institute of Molecular Medicine, College of Medicine, National Cheng Kung University/Center for Genomic Medicine, National Cheng Kung University, Shengli Rd. 3F. No. 367, Tainan 704, Taiwan

*Corresponding author. Email: endang@unud.ac.id

Received date: Jul 15, 2025; Revised date: Aug 19, 2025; Accepted date: Aug 21, 2025

Abstract

BACKGROUND: Identification of cell-free foetal DNA (cffDNA) in maternal blood, combined with next-generation sequencing (NGS) advancement, has paved the way for non-invasive prenatal screening to detect foetal aneuploidies. However, there is limited evidence on its diagnostic accuracy when compared with gold-standard invasive tests specifically in pregnancies complicated by birth defects in Indonesia. This study was conducted to evaluate the precision of non-invasive prenatal testing (NIPT) using NGS and ultrasound findings compared with the established benchmarks of amniocentesis and neonatal karyotyping through G-banding analysis, which is an invasive procedures, in a private laboratory setting for pregnancies with birth defect.

METHODS: An observational cohort study involving pregnant women with foetal birth defects in central nervous system, facial, heart, gastrointestinal tract, urinary tract abnormalities and suspected Down Syndrome was conducted. The foetal birth defects were identified in the first trimester with ultrasound screening. Venous blood was drawn from the mother for NGS-based NIPT examination. As a gold standard, amniocentesis or neonatal G-banding karyotyping was conducted.

RESULTS: Using G-banding karyotyping as gold standard, the results indicated that NIPT using the NGS method and ultrasound findings achieved 100% sensitivity, 100% specificity, and 100% accuracy in detecting trisomy 13, 18, and 21, as well as foetal sex chromosome abnormalities. Additionally, a case of tetrasomy 9p was identified through G-banding karyotyping, which was associated with multiple clinical abnormalities.

CONCLUSION: NIPT with NGS methods and ultrasound findings demonstrated 100% accuracy for the screening of trisomy 13, 18, and 21 in birth defect pregnancy, which is comparable with G-banding analysis as a gold standard. Therefore, this suggest that these approaches offer a safe early detection, highly accurate alternative in high risk setting, compared to invasive procedure in Indonesia where access to such testing may be limited.

KEYWORDS: G-banding karyotyping, next generation sequencing, non-invasive prenatal testing

Indones Biomed J. 2025; 17(4): 407-15

Introduction

Chromosomal abnormalities can manifest in either the number or structure of chromosomes. Aneuploidy refers to an abnormal number of chromosomes, such as trisomy or monosomy. These abnormalities can lead to various pregnancy outcomes, including failure of the embryo to implant, fetal demise, miscarriage, or birth defects like Down Syndrome, which should be confirmed with the invasive tests of the internationally accepted gold standard such as chorionic villus sampling and amniocentesis chromosomal testing.

The discovery of cell-free fetal DNA (cffDNA) in maternal blood, along with advancements in next-generation sequencing (NGS), has laid the groundwork for non-invasive prenatal testing (NIPT). This approach is particularly effective in detecting foetal aneuploidies, such as Patau syndrome (trisomy 13), Edward syndrome (trisomy 18), and Down syndrome (trisomy 21). NIPT analyzes the genetic material from the placenta, known as cffDNA, which is found in the mother's blood. This test can be conducted from the 10th week of pregnancy and is designed to detect chromosomal abnormalities in the developing baby.(1)

Since cffDNA was identified in the plasma of pregnant women in 1997 (2), the use of NGS-based NIPT for screening foetal chromosomal aneuploidies has become feasible. Nowadays, NIPT is extensively utilized to detect foetal chromosomal trisomies 13, 18, and 21, as well as sex chromosomal abnormalities, with exceptional sensitivity and specificity.(3) Most cffDNA in the maternal circulation is originated from maternal, with approximately 10% being of foetal origin, released from placental cells into the mother's bloodstream.(4)

Several studies have shown that NIPT technology has good accuracy and is reliable for use with DNA samples from pregnant women. Previous study reported NIPT's remarkable accuracy of 100% sensitivity and 99.9% specificity for detecting trisomy 13, 92.9% sensitivity and 100% specificity for trisomy 18, and 100% sensitivity and 99.9% specificity for trisomy 21. The positive predictive values (PPV) for trisomy 13, trisomy 18, and trisomy 21 were 90.0%, 100%, and 98.3%, respectively. Analysis by pregnancy trimester showed higher specificity in the first trimester compared to the second, while sensitivity remained 100% for trisomy 13 and 21 in both trimesters. Specificity remained consistently 100% for trisomy 18, although sensitivity improved in the second trimester.(5) In a comprehensive multicenter observational study, NIPT showcased impressive sensitivity rates of 99.60% for trisomy 21, 99.14% for trisomy 18, and 100% for trisomy 13. Specificities were similarly high, with 99.90% for trisomy 21, 99.94% for trisomy 18, and 99.95% for trisomy 13. However, PPV varied, namely 69.77% for trisomy 21, 47.24% for trisomy 18, and 22.36% for trisomy 13. Despite high sensitivity and specificity across clinical indications, PPV ranged from 9.09% to 66.46% depending on the indication. The highest PPVs were noted for trisomy 21 at 73.09% in cases of "advanced maternal age," trisomy 18 at 58.33% with " nuchal translucency (NT) thickening," and trisomy 13 at 47.37% also with "NT thickening".(6)

NGS enables precise measurement of specific DNA sequences by comparing sample DNA into a reference genome, facilitating the detection of foetal aneuploidy. It's important to note that the majority of the DNA sequence is maternal, and the presence of an extra chromosome in a foetus results in only a minor increase compared with a standard reference chromosome, as the abnormality constitutes an estimated 10% of the DNA sequence. For a reliable analysis, at least 2% of foetal DNA is required.

While the combination of cffDNA analysis via NGS and ultrasound offers a promising non-invasive approach for detecting foetal aneuploidies, there is limited evidence on its diagnostic accuracy when compared with gold-standard invasive tests (amniocentesis and neonatal karyotyping) specifically in pregnancies complicated by birth defects, particularly in the private laboratory setting in Indonesia. Based on patient visit records from the maternal fetal medicine outpatient clinic at Ngoerah General Hospital, Bali, between 2019 and 2022, the most prevalent congenital abnormalities were observed in the head-neck region and the digestive tract. From 2017 to 2022, amniocentesis karyotyping in pregnancies with birth defects at the same clinic revealed trisomy 18 in 9.4% (5 out of 53) of cases.(7) Ultrasonopgraphy have been used worldwide for screening birth defects, monitoring fetal growth, knowing position implantation of placenta and even intra labor monitoring. The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) has developed practices guidelines for health professional which is adopted by Indonesian Maternal Fetal Medicine Society. In the first trimester screening, soft marker of trisomy 13,18 and 21 are thick nuchal translucency, hypoplastic or absent of nasal bone, abnormal flow in the ductus venosus and tricuspid regurgitation.(8) In the second trimester morphology scan, complete scan was performed, include head, face, neck, chest/heart, abdomen, skeletal placenta, umbilical cord and genitalia.(9)

This study was conducted to evaluate the precision of NIPT using NGS and ultrasound findings compared with the established benchmarks of amniocentesis and neonatal karyotyping through G-banding analysis for birth defect pregnancy in a private laboratory setting in Indonesia. These approaches may offer a safe early detection, highly accurate alternative in high risk setting, other than the well-known invasive procedure, especially in Indonesia where access to such testing may be limited.

Methods

Study Design and Subjects Recruitment

A cohort analytical observational study was conducted from May to July 2024 at Ngoerah General Hospital, Bali, an Integrated Biomedical Laboratory, Faculty of Medicine, Universitas Udayana, Bali. The required sample size for this study was calculated using a single proportion sample formula (10), and 10 pregnant women over 10 weeks of gestation with foetus diagnosed with congenital birth defects in central nervous system, face, heart, gastrointestinal tract, urinary tract as well as suspected down syndrome was included in this study. The congenital birth defects in subjects were identified in the first trimester through ultrasound screening, The included mother subjects must also have a singleton alive baby and agreed to participate in this study. The subjects would be excluded if the baby passed away before the blood sample for karyotyping was obtained. The protocol of this study was ethically approved by the Research Ethics Committee, Universitas Udayana on April 30, 2024 (Approval No.: 1251/UN14.2.2.VII.14/LT/2024).

Detection of Congenital Birth Defects with Ultrasound

Ultrasound screening was performed by Maternal Foetal Medicine consultant using a General Electric Voluson E10 ultrasound machine (GE HealthCare, Chicago, IL, USA). The congenital birth defects included in this study were defects in central nervous system, facial, heart, gastrointestinal tract, urinary tract abnormalities and suspected down syndrome. The defects were assessed based on the structural findings of the foetus or by the findings of soft markers in the related organs.

cffDNA Extraction and NGS Library Preparation

Approximately 10 mL of peripheral venous blood was drawn from each participant and placed into EDTA tubes. Samples were centrifuged twice: first at 2000g for 600 seconds at room temperature, with supernatant and transferred to a 1.5 mL centrifuge tube. A second spin at 14,000g for 10 minutes was also performed at room temperature, after which plasma was stored at -80°C for future analysis. From each 2 mL plasma sample, DNA was extracted using the the QIAseq cfDNA Extraction Kit from QIAseqTM cffDNA All-in-one Kit (Qiagen, Hilden, Germany), following the specified protocols. Plasma was centrifuged again at 16,000g for 300 seconds, and extraction performed according to the manufacturer's procedure. The cffDNA quantity was measured using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The extracted cffDNA was stored at -20°C for NGS examination.

The QIAseq cffDNA Library Kit from QIAseqTM cffDNA All-in-one Kit (Qiagen) was used for the library preparation following the manufacturer protocol. After library purification and quality control was performed, the extracted samples were loaded onto the Illumina NextSeq550 (Illumina, San Diego, CA) for sequencing.

Bioinformatics Analysis

The complete cffDNA was sequenced in small segments and matched against a reference human genome database. Statistically, a Z score greater than 3 indicated significant deviation from the baseline, suggesting data likely from a triploid sample. Bioinformatics analysis focusing on chromosome 13, 18, and 21, as well as sex chromosome, was performed adhered to the protocol from the Centre for Genomic Medicine at National Cheng Kung University in Taiwan.(11)

G-banding Karyotyping Examination

The examination of G-banding karyotyping was conducted in Prodia Clinical Laboratory, Jakarta. Chromosomes were identified through an Olympus BX63 microscope (Olympus, Tokyo, Japan).(12) Amniocentesis with G-banding method was conducted for pregnancy less than 32 weeks; for those over 32 weeks, the G-banding analysis was performed using 6 mL of blood samples from the newborn. The results were available 4 to 6 weeks post-collection.

Data Analysis

These clinical data were accessible only to the first author, ensuring no direct contact between the two laboratories performing NIPT and G-banding karyotyping analyses. The data were then statistically analyzed using IBM SPSS Statistics version 30.0 (IBM Corporation, Armonk, NY, USA) for the calculation of sensitivity, specificity, and accuracy of the two methods.

Results

Characteristics of Subjects

In this study, 10 samples were analyzed, and the average age of subjects was 30.3±5.74 years old, with average gestational age of 34±5.79 (Table 1). Clinical findings and abnormalities found in each subject were also assessed. There were 2 identified cases of trisomy 13, 2 cases of trisomy 21, and 6 cases of euploidy from NGS results. Meanwhile, the G-banding karyotyping revealed 2 cases of trisomy 13, 2 cases of trisomy 21, 5 cases of euploidy, and 1 case of tetrasomy of chromosome 9p, which was associated with clinical signs such as foetal growth restriction (FGR), bilateral cleft lips, bilateral ventriculomegaly, and an atrial septal defect. The NIPT results showed risk of miscarriages and many difficulties during sampling a premature baby in birth defect population (Table 2).

Table 1. Characteristics of research subjects.

Characteristics	Result
Age (year)	
Mean±SD	30.3±5.736
Median (Min-Max)	32 (21-39)
Gravida	
Mean±SD	2.3±1.16
Median (Min-Max)	2 (1-4)
Gestational Age (weeks)	
Mean±SD	34±5.793
Median (Min-Max)	37 (23-28)

NGS-based NIPT *vs.* G-Banding Karyotyping

NGS-based NIPT performed was a non-invasive testing from mother's blood, while G-banding karyotyping was an invasive testing using amniotic fluid sample during pregnancy or neonatal blood. The NIPT examination using the NGS method had a sensitivity of 100%, specificity of 100%, and accuracy of 100% in detecting trisomy 13, 18, and 21 (Figure 1), compared with G-banding karyotyping (Table 3 and Figure 2). In detecting sex chromosomes, the NGS-based NIPT achieved a sensitivity, specificity, and accuracy of 100% (Table 4).

Ultrasound Findings *vs.* G-Banding Karyotyping

Through statistical analysis, we found that using ultrasonography to assess foetal clinical signs was also

Table 2. Clinical findings of each research subjects.

No of Subjects	Age (Years)	Gestational Age at NIPT	Clinical Diagnosis
1	21	37W6D	G2P0100 37W6D singleton live, previous CS, obesity grade 1, congenital anomaly (bilateral ventriculomegaly, cisterna magna enlargement), estimated foetal weight (EFW): 3062g
2	33	32W1D	G2P1001 33W3D singleton live, previous CS, suspect trisomy 13, congenital anomaly (foetal microcephaly, holoprosencephaly, proboscis), EFW: 825g
3	32	37W5D	G3P1102 37W5D singleton live, transverse lie, previous CS, congenital anomaly (cardiomegaly, left ventricle dilatation, dextrocardia, hydrocele, polyhydramnios), EFW: 4512g
4	34	37W2D	G4P2012 37W2D singleton live, previous CS, multiple congenital anomaly (congenital talipes equinovarus, atrial septal defect, susp. atresia oesophagus), foetal growth restriction (FGR), polyhydramnios, EFW: 2373g
5	36	37W2D	G4P3013 37W2D, previous CS 2x, multiple congenital anomaly (polyhydramnios, double bubble, absent nasal bone, dysmorphic face, macroglossia), suspect down syndrome (trisomy 21), transverse lie
6	32	38W6D	G1P0000 38W6D singleton live, obesity grade I, congenital anomaly (multicyclic dysplastic kidney bilateral)
7	26	23W6D	G1P0000 34W3D, singleton live, breech presentation, congenital anomaly (trisomy 13, bilateral cleft lips, hydronephrosis right kidney), EFW: 2048g
8	25	38W6D	G2P1001 38W 6D singleton live, congenital anomaly (hydrocephaly), EFW : 2783g
9	25	37W3D	G1P0000 37W3D singleton live, intrauterine growth restriction stage 0, multiple congenital anomaly (bilateral cleft, bilateral ventriculomegaly, atrial septal defect), EFW: 1817g
10	39	24W6D	G3P0020 24W6D singleton live foetal trisomy 21 premature rupture of membrane (Thick NT at 1st trimester), EFW 512g

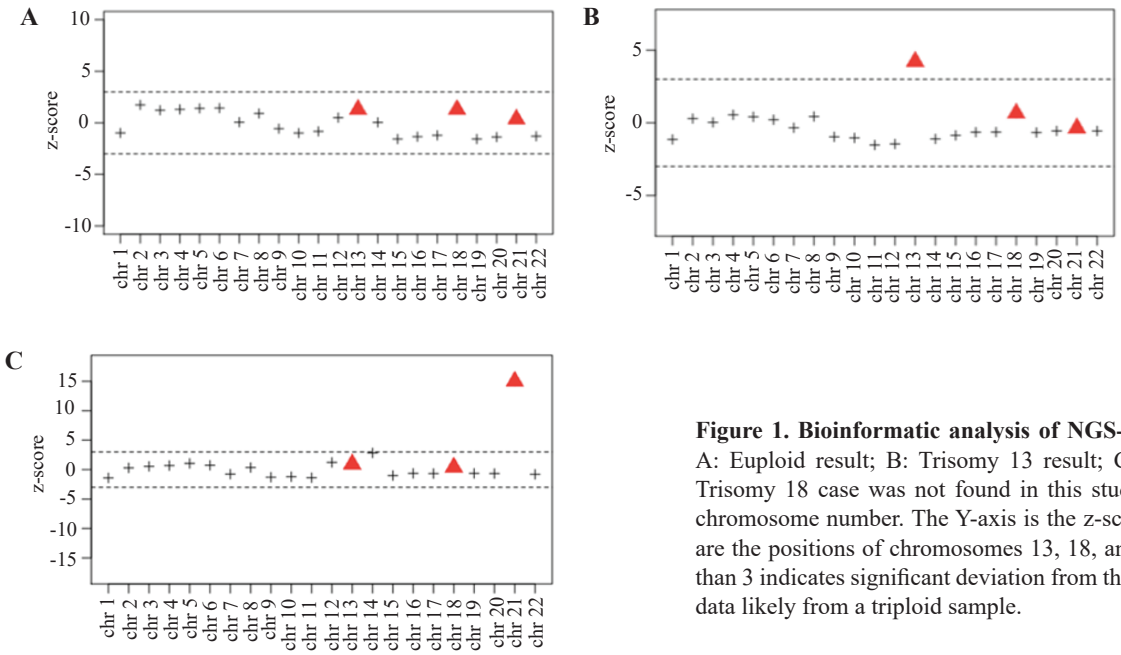

Figure 1. Bioinformatic analysis of NGS-based NIPT results. A: Euploid result; B: Trisomy 13 result; C: Trisomy 21 result. Trisomy 18 case was not found in this study. The X-axis is the chromosome number. The Y-axis is the z-score. The red triangles are the positions of chromosomes 13, 18, and 21. Z-score greater than 3 indicates significant deviation from the baseline, suggesting data likely from a triploid sample.

remarkably effective in identifying trisomy 13, 18, and 21. It demonstrated perfect sensitivity, specificity, and accuracy, each at 100%, when compared to the traditional G-banding karyotyping method (Table 5). The ultrasound screening in these cases were performed mostly in second and third trimester screening, only one case was conducted in the first trimester visit.

Discussion

The field of detecting cytogenetic abnormalities has seen significant advancements in line with research into preeclampsia, various biomarkers have been studied to reveal the pathogenesis (13-15), even research in gene therapy (16), but to date, still remains a disease of theory. Initially, techniques like solid staining and Giemsa banding (G-banding), nowadays, next-generation sequencing methods allow for a comprehensive analysis of genomic gains, losses, and rearrangements.(17)

Initially, placental cytotrophoblasts combine with syncytiotrophoblasts, and as they mature, they enter the maternal circulation through structures called syncytial knots.(18) When these knots break down, they release foetal DNA, which is typically shorter than the maternal DNA typically under 313 base pairs compared to 400-500 base pairs of the maternal DNA.(19) This size difference facilitates the detection of cffDNA in maternal blood using simple methods like blood draws. Detectable as early as 4 weeks into pregnancy (20), cffDNA increases to about 10-15% of the mother's plasma between 10 and 20 weeks of gestation (21) and is quickly cleared after childbirth. This makes cffDNA an excellent biomarker for identifying chromosomal abnormalities, even in the first trimester.(22)

The cffDNA holds great potential in prenatal diagnostics by not only predicting obstetric conditions like pre-eclampsia (23), monogenic disorders (24), and placenta accreta (25) but also identifying foetal chromosomal anomalies early in pregnancy (26). NIPT uses NGS to analyse these small cffDNA fragments to detect

Table 3. Comparison of NGS-based NIPT with gold standard of G-banding karyotyping in detecting trisomy 13, 18 and 21.

NGS-based NIPT Trisomy 13,18,21	G-Banding Karyotyping Trisomy 13,18,21			
	Yes	No	Total	
Yes	4	0	4	Sensitivity : 100%
No	0	6	6	Specificity : 100%
Total	4	6	10	PPV : 100%; NPV:100%, Accuracy 100%

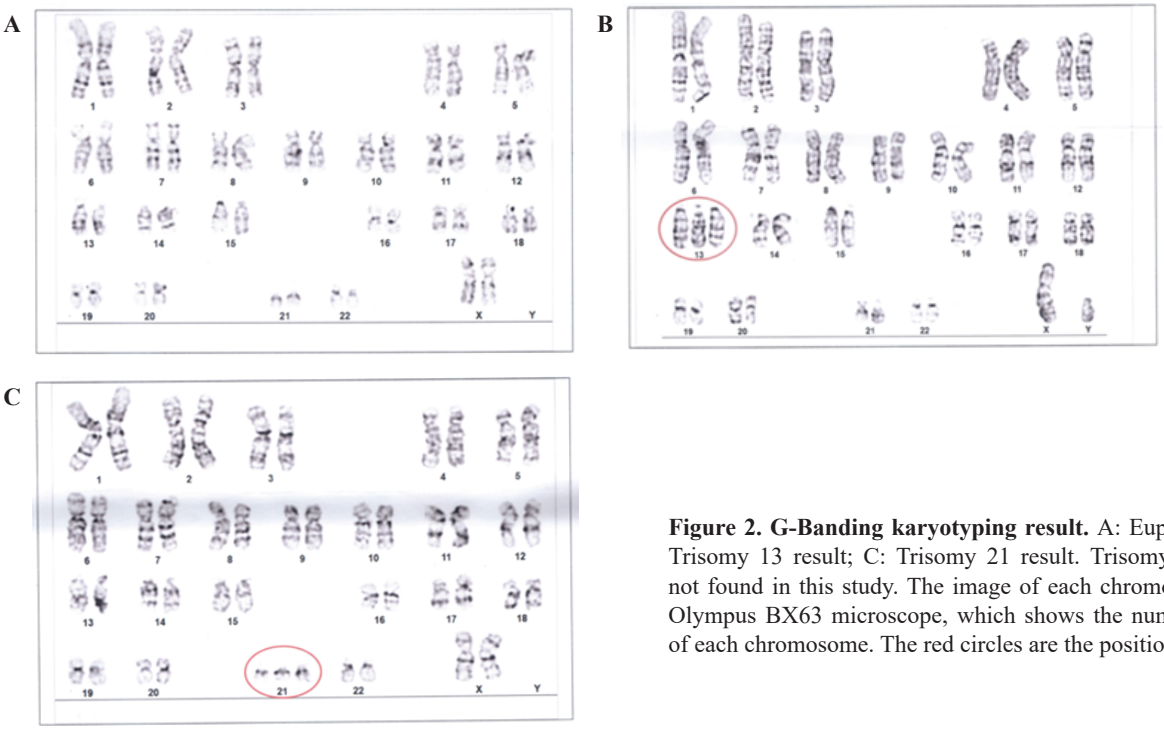

Figure 2. G-Banding karyotyping result. A: Euploid result; B: Trisomy 13 result; C: Trisomy 21 result. Trisomy 18 case was not found in this study. The image of each chromosome as seen Olympus BX63 microscope, which shows the number of copies of each chromosome. The red circles are the positions of trisomy.

genetic variants signalling chromosomal abnormalities. First introduced commercially NIPT showed remarkable sensitivity and specificity for screening high risk trisomy 21 pregnancies.(27) Positive results lead to further invasive diagnostic procedures, minimizing unnecessary exposure to procedural risks.(22)

Sequencing of cffDNA base pairs would be exceedingly difficult without NGS. Conventional DNA analysis methods, like polymerase chain reaction (PCR) and gel electrophoresis, played crucial roles in the Sanger and Maxam–Gilbert sequencing methodologies. This sequencing employs the chain termination approach, producing DNA fragments of varying lengths when a dideoxy nucleotide (ddNTP) bind to the DNA, stopping replication. These fragments are then analysed via gel electrophoresis.(28) NGS advances with sophisticated techniques like pyrosequencing and bridge amplification (29), reducing the time and expense of nucleic acid analysis compared to traditional methods. This progress enhances research capabilities and diagnostic potential for NIPT using cffDNA.(30)

Compared with previous studies, the results of this study showed higher sensitivity, specificity, and accuracy. In this study, NIPT sensitivity, specificity and accuracy were found at 100%. Meanwhile in a study in general population reported that the sensitivity of NIPT in detecting trisomy 13, 18 and 21 were respectively 90.3-100%, 90-100%, and 96.3-100%. The specificity of NIPT in detecting trisomy 13,18 and 21 were respectively 99.4-100%, 99.9-100%, and 98.9-100%. The positive predictive value in trisomy 13, 18, 21 were respectively 41.94-100%, 58.7-100%, and 80.9-100%. The false positive rate for trisomy 13,18 and 21 were respectively 0-28.5%, 0-0.13%, and 0-4.65%.(22)

In this study, one case of tetrasomy 9p was detected by G-banding karyotyping, with clinical sign of foetal intrauterine growth restriction (IUGR), bilateral cleft

Table 4. Comparison of NGS-based NIPT with gold standard of G-banding karyotyping in detecting sex chromosome.

NGS NIPT	G-Banding Karyotyping			
	XX	XY	Total	
XX	4	0	4	Sensitivity : 100%
XY	0	6	6	Specificity : 100%
Total	4	6	10	PPV : 100%; NPV:100%, Accuracy 100%

Table 5. Comparison of ultrasound findings with gold standard of G-banding karyotyping in detecting trisomy 13,18 and 21.

Ultrasound Sonography Trisomy 13,18,21	G-Banding Karyotyping Trisomy 13,18,21			
	Yes	No	Total	
Yes	4	0	4	Sensitivity : 100%
No	0	6	6	Specificity : 100%
Total	4	6	10	PPV : 100%; NPV:100%, Accuracy 100%

lips, bilateral ventriculomegaly and an atrial septal defect. Tetrasomy 9p was first published in 1973 (31), with detection rate during amniocentesis around 0.002% (32). The most common clinical findings of tetrasomy 9p reported including central nervous system abnormalities (59%), IUGR (57%), cleft lip and or palate (45%), cardiac defect (29%), genitourinary and renal anomalies (29%) and skeletal anomalies (29%). During the first trimester screening for tetrasomy 9p, increased NT was detected in 67% of cases, though diagnosis was confirmed through amniocentesis and karyotyping. This abnormality was not optimally detected by NIPT, as shown in this current study. Due to the wide variation of clinical signs and many chromosomal abnormalities shows a broad phenotypes in common, the only gold standard for diagnosis tetrasomy 9p is by an invasive genetic test.(33)

Maternal mosaic tetrasomy 9p possibly become a cause of false positive NIPT result in normal carriers. In 2021, the first instance tetrasomy 9p was detected through NIPT testing at both 11 and 15 weeks of pregnancy. Despite these findings, the foetus had normal results from amniocentesis and chromosomal microarray analysis (CMA). Further testing using multiplex ligation-dependent probe amplification (MLPA) on a buccal swab from the newborn and uncultured maternal blood revealed normal results in the neonate's swab, while revealing mosaic tetrasomy 9p in the mother's blood. Remarkably, both the mother and child displayed no physical abnormalities. (34) Several researchers have also reported some cases related with carriers of mosaic tetrasomy.(35-38) In cases of mosaic tetrasomy 9p identified during amniocentesis, differences in genetic results could arise from tissue-specific mosaicism and cytogenetic discrepancies between cultured and uncultured amniocytes. highlights that mosaic levels identified through traditional cytogenetic analysis in cultured amniocytes may differ from those found through uncultured amniocytes, often with the latter showing higher mosaicism levels.(39,40) For prenatal diagnosis involving mosaicism such as i(9p), i(9q), small supernumerary marker chromosomes (sSMC)9, and trisomy 9, it is essential to consider a differential diagnosis for uniparental disomy (UPD).(40)

The high sensitivity, specificity, and accuracy of non-invasive NGS-based NIPT combined with ultrasonography suggest that it is comparable with the invasive G-bending analysis as the gold standard for the detection of congenital birth defects. To overcome the occurrence of false positive and false negatives in NIPT, it is suggested to combine NIPT with ultrasonography. This study focused on pregnancies with birth defects, which does not accurately represent the general use of NIPT as a screening tool in the wider population of pregnant women. While the results are promising, the small sample size necessitates larger, multicentre investigation to confirm these findings and assess cost-effectiveness, feasibility and integration into national prenatal screening guidelines. Nonetheless, this study provides a crucial foundation for promoting the implementation of NIPT in the management of pregnancies complicated by foetal birth defects both in Bali, Indonesia, and in other similar healthcare environments.

Conclusion

Using G-banding analysis as the gold standard for diagnosis, NIPT with the NGS method and the combination with ultrasonography findings has proven to be a highly promising screening method for congenital birth defects. It demonstrates excellent sensitivity and specificity in detecting trisomy 13, 18, and 21, as well as sex chromosome anomalies in pregnancies with birth defects. This approach offers a safe, accurate and rapid alternative to invasive testing in high-risk pregnancies, reducing procedural risks and patient anxiety while enabling earlier clinical decision making. Integrating NIPT into prenatal screening for high risk pregnancies in Indonesia could increase access to advanced diagnostics, guide perinatal management and ultimate improve maternal and neonatal outcomes.

Acknowledgments

This research was funded by Research Grant DIPA PNBP Universitas Udayana 2024 (No: B/255.55/UN14.4.A/PT.01.03/2024, dated 17 April 2024).

Authors Contribution

AS, AANJK, NGAME, and ESW were involved in concepting and planning of the research. AS, AANJK, ESW, AAGRB, IGNASW, and DS performed the blood sample collection. NNAD performed NIPT analysis and designed the figures under assistance of HSS and YAT. AS, ESW, and IMJ drafted the manuscript and interpreted the results. All authors took parts in giving critical revision of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest or competing interests related to the content of this manuscript.

References

1. Yang J, Ding X, Zhu W. Improving the calling of non-invasive prenatal testing on 13-/18-/21-trisomy by support vector machine discrimination. PLoS ONE. 2018; 13(12): e0207840. doi: 10.1371/journal.pone.0207840.
2. Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, *et al.* Presence of fetal DNA in maternal plasma and serum. Lancet. 1997; 350(9076): 485-7.
3. Xue Y, Zhao G, Li H, Zhang Q, Lu J, Yu B, *et al.* (2019). . Non-invasive prenatal testing to detect chromosome aneuploidies in 57,204 pregnancies. Mol Cytogenet. 2019; 12: 29. doi: 10.1186/s13039-019-0441-5.
4. Manegold-Brauer G, Lapaire O. Clinical implementation of next-generation sequencing in the field of prenatal diagnostics in next generation sequencing. In: Kulski JK, editor. Next Generation Sequencing - Advances, Applications and Challenges. London: InTech; 2016. doi: 10.5772/61799.
5. Lee DE, Kim H, Park J, Yun T, Park DY, Kim M, *et al.* Clinical validation of non-invasive prenatal testing for fetal common aneuploidies in 1,055 Korean pregnant women: A single center experience. J Korean Med Sci. 2019; 34(24): e172. doi: 10.3346/jkms.2019.34.e172.
6. Xiang L, Zhu J, Deng K, Li Q, Tao J, Li M, *et al.* Non-invasive prenatal testing for the detection of trisomies 21, 18, and 13 in pregnant women with various clinical indications: A multicenter observational study of 1,854,148 women in China. Prenat Diagn. 2023; 43(8): 1036-43.
7. Prof. Dr. Ngoerah General Hospital. Birth Defect Register 2017-2022 of Ngoerah General Hospital. Bali: Prof. Dr. Ngoerah General Hospital; 2022.
8. The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG). ISUOG practice guidelines (updated): Performance of 11–14-week ultrasound scan. Ultrasound Obstet Gynecol. 2023; 61: 127-43.
9. The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG). ISUOG practice guidelines (updated): Performance of the routine mid-trimester fetal ultrasound scan. Ultrasound Obstet Gynecol. 2022; 59: 840-56.
10. Lemeshow S, Hosmer Jr DW, Klar J, Lwanga SK. Adequacy of Sample Size in Health Studies. Chicester: John Wiley&Sons; 1990.
11. Qi QG, Tuo Y, Liu LX, Yu CX, Wu AN. Amniocentesis and next generation sequencing (NGS)-based noninvasive prenatal DNA testing (NIPT) for prenatal diagnosis of fetal chromosomal disorders. Int J Gen Med. 2021; 14: 1811-7.
12. Bayani J, Squire JA. Traditional banding of chromosomes for cytogenetic analysis. Curr Protoc Cell Biol. 2004; Chapter 22: Unit 22.3. doi: 10.1002/0471143030.cb2203s23.
13. Nelson N, Yusrawati, Serudji J, Jamsari, Amir A, Afriwardi, et al. Elevation of miR-210 expression and mean arterial pressure as early-onset pre-eclampsia biomarkers, while elevation of matrix metalloproteinase-2 as late-onset pre-eclampsia biomarker. Indones Biomed J. 2025; 17(2): 180-7.
14. Karmia HR, Yusrawati, Jamsari, Hadisman. miR-200a as potential early-onset biomarker, while high nitric oxide as potential late-onset biomarker in preeclampsia. Indones Biomed J. 2025; 17(3): 207-316.
15. Puspasari A, Enis RN, Herlambang. Genetic variant of vascular endothelial growth factor (VEGF)-A rs699947 is associated with preeclampsia. Mol Cell Biomed Sci. 2022; 6(2): 70-6.
16. Riu DV, Sunarno I, Lukas E. MCM6 rs4988235 allele G, AGT rs699 allele C, ACE rs4343 allele A, FADS1 rs174547 allele C, DCHR7 rs12785878 allele G, and GC rs7041 allele T: Candidate genes for preeclampsia prevention. Indones Biomed J. 2025; 17(2): 109-206.
17. Martin CL, Warburton D. Detection of chromosomal aberrations in clinical practice: From karyotype to genome sequence. Annu Rev genomics hum genet. 2015; 16: 309-26. doi: 10.1146/annurev-genom-090413-025346.
18. Sifakis S, Koukou Z, Spandidos DA. Cell-free fetal DNA and pregnancy-related complications (review). Mol Med Rep. 2015; 11(4): 2367-72.
19. Chan KC, Zhang J, Hui AB, Wong N, Lau TK, Leung TN, *et al.* Size distributions of maternal and fetal DNA in maternal plasma. Clin Chem. 2004; 50(1): 88-92.
20. Karakas B, Qubbaj W, Al-Hassan S, Coskun S. Noninvasive digital detection of fetal DNA in plasma of 4-week-pregnant women following in vitro fertilization and embryo transfer. PLoS One. 2015; 10(5): e0126501. doi: 10.1371/journal.pone.0126501.
21. Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, *et al.* DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study. Genet Med. 2011; 13(11): 913-20.
22. Jayashankar SS, Nasaruddin ML, Hassan MF, Dasrilsyah RA, Shafiee MN, Ismail NAS, *et al.* Non-invasive prenatal testing (NIPT): Reliability, challenges, and future directions. Diagnostics. 2023; 13(15): 2570. doi: 10.3390/diagnostics13152570.
23. Sahai K, Saraswathy S, Yadav TP, Arora D, Krishnan M. Pre-eclampsia: Molecular events to biomarkers. Med J Armed Forces India. 2017; 73(2): 167-74.
24. Scotchman E, Shaw J, Paternoster B, Chandler N, Chitty LS. Non-

invasive prenatal diagnosis and screening for monogenic disorders. Eur J Obstet Gynecol Reprod Biol. 2020; 253: 320-7.
25. Samuel A, Bonanno C, Oliphant A, Batey A, Wright JD. Fraction of cell-free fetal DNA in the maternal serum as a predictor of abnormal placental invasion-a pilot study. Prenat Diagn. 2013; 33(11): 1050-3.
26. Carbone L, Cariati F, Sarno L, Conforti A, Bagnulo F, Strina I, *et al.* Non-invasive prenatal testing: Current perspectives and future challenges. Genes. 2020; 12(1): 15. doi: 10.3390/genes12010015.
27. Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, *et al.* Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ. 2011; 342: c7401. doi: 10.1136/bmj.c7401.
28. Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. J Mol Biol. 1975; 94(3): 441-8.
29. Behjati S, Tarpey PS. What is next generation sequencing? Arch Dis Child Educ Pract Ed. 2013; 98(6): 236-8.
30. Schuster SC. Next-generation sequencing transforms today's biology. Nat Methods. 2008; 5(1): 16-8.
31. Ghymers D, Hermann B, Distèche C, Frederic J. Partial tetrasomy of number 9 chromosome, and mosaicism in a child with multiple malformations. Humangenetik. 1973; 20(3): 273-82.
32. Chen CP. Genetic counseling of mosaic and non-mosaic tetrasomy 9p at prenatal diagnosis. Taiwan J Obstet Gynecol. 2024; 63: 633e636. doi: 10.1016/j.tjog.2024.07.004.
33. Vinkšel M, Volk M, Peterlin B, Lovrecic L. A systematic clinical review of prenatally diagnosed tetrasomy 9p. Balkan J Med Genet. 2019; 22(1): 11-20. doi: 10.2478/bjmg-2019-0012.
34. Shu W, Cheng SSW, Xue S, Chan LW, Soong SI, Kan ASY, *et al.* First case report of maternal mosaic tetrasomy 9p incidentally detected on non-invasive prenatal testing. Genes. 2021; 12: 370. doi: 10.3390/genes12030370.
35. McAuliffe F, Winsor EJT, Chitayat D. Tetrasomy 9p mosaicism associated with a normal phenotype. Fetal Diagn Ther. 2005; 20: 219e22. doi: 10.1159/000083909.
36. Nakamura-Pereira M, do Cima LC, Llerena Jr JC, Guerra FAR, Peixoto-Filho FM. Sonographic findings in a case of tetrasomy 9p associated with increased nuchal translucency and Dandy-Walker malformation. J Clin Ultrasound. 2009; 37: 471-4.
37. Ogino W, Takeshima Y, Nishiyama A, Yagi M, Oka N, Matsuo M. Mosaic tetrasomy 9p case with the phenotype mimicking Klinefelter syndrome and hyporesponse of gonadotropin-stimulated testosterone production. Kobe J Med Sci. 2007; 53: 143-50.
38. Baronchelli S, Conconi D, Panzeri E, Bentivegna A, Redaelli S, Lissoni S, *et al.* Cytogenetics of premature ovarian failure: An investigation on 269 affected women. J Biomed Biotechnol. 2011; 2011: 370195. doi: 10.1155/2011/370195.
39. Chen CP, Chang TY, Chern SR, Lee CC, Town DD, Lee MS, Wang W. Prenatal diagnosis of low-level mosaic tetrasomy 9p by amniocentesis. Prenat Diagn. 2007; 27: 383e5. doi: 10.1002/pd.1678.
40. Chen CP, Wang LK, Chern SR, Wu PS, Chen YT, Kuo YL, *et al.* Mosaic tetrasomy 9p at amniocentesis: prenatal diagnosis, molecular cytogenetic characterization, and literature review. Taiwan J Obstet Gynecol. 2014; 53(1): 79-85.