ECRONICON OPEN ACCESS

EC GYNAECOLOGY
Research Article

Non-Invasive Prenatal Testing (NIPT) by DNA Sequencing Method

Sandeep Thapa[1,2]*, Nilam Thakur[1,2], Govardhan Joshi[1,2], Santosh Khanal[1,3], Sumida Tiwari[1], Rajesh Deo[1], Manisha Sapkota[1,2], Pabitra Bista[1], Nurakant Neupane[1], Bishnu P Marasini[3,4], Ujwal Thakur[1] and Ajaya Jang Kunwar[1,2]

[1]Nova International Diagnostics Pvt. Ltd., Nepal

[2]Kathmandu Center for Genomics and Research Laboratory, Nepal

[3]Department of Biotechnology, National College, Tribhuvan University, Kathmandu, Nepal

[4]Nepal Health Research Council, Ramshah Path, Kathmandu, Nepal

***Corresponding Author:** Sandeep Thapa, Kathmandu Center for Genomics and Research Laboratory (KCGRL), Nova International Diagnostics Pvt. Ltd, Gwarko, Lalitpur, Nepal.

Received: January 12, 2024; **Published:** January 31, 2024

Abstract

Background: Non-invasive prenatal testing (NIPT) for fetal aneuploidies using cell-free DNA (cfDNA) which can be isolated from mothers' plasma has been widely adopted in clinical practice due to its improved accuracy.

Objective: The core goal of cell-free based prenatal testing is to provide minimally invasive, clinically accurate screening for fetal chromosomal aneuploidies in the early stages of pregnancy.

The purpose of this study was to establish a validated NIPT workflow for cell-free fetal DNA (cffDNA) sequencing from maternal plasma for the detection of trisomy 13, 18, 21 and sex chromosomal aneuploidies (SCA) on a semiconductor sequencing instrument.

Method: A total of ninety-one plasma samples from healthy pregnant provided by Yourgene Health; their cfDNA library was prepared and loaded on the Ion 540 chip for sequencing. The sequencing output data was analysed by using the bioinformatics pipeline of Yourgene Health.

Results: Eighty-one samples were successfully validated out of a total of 91 samples. However, 3% of samples did not meet quality, and 3% of sample libraries had low reads and failed validation. Moreover, building the validated workflow based on Ion Torrent Next-Generation Sequencing (NGS) would allow for fetal aneuploidy detection.

Conclusion: This research aims to introduce and set up probably Nepal's first NGS laboratory for NIPT, utilizing the Ion Torrent technology to provide in-house diagnostic solution for pregnant women.

Keywords: *Aneuploidies; Cell-Free DNA (cfDNA); Cell-Free Fetal DNA (cffDNA); Maternal Blood; Non-Invasive Prenatal Genetic Testing (NIPT); Prenatal Testing*

Abbreviations

NIPT: Non-Invasive Prenatal Testing; cfDNA: Cell-free DNA; cffDNA: cell-free fetal DNA; SCA: Sex Chromosomal Aneuploidies

Introduction

Non-invasive prenatal testing (NIPT) is a method of determining the risk of how likely a fetus is to be born with specific genetic abnormalities [1]. Recently, this test's development, implementation, and services have revolutionized prenatal screening for chromosomal aneuploidies globally. It is based on the sequencing of Circulating Cell-free fetal DNA (cffDNA) [2,3].

Prenatal testing has passed a long evolution from traditional invasive methods without experiencing the risk of miscarriage [4]. Eventually, which results in the non-invasive screening of pregnancies highly precise for the chromosome aneuploidies possible with sensitivity and specificity approximately 99% for trisomy 21, the most common chromosomal disorder [5].

NIPT is extensively studied, and only a few countries are incorporating it as a standard test, alongside combined screening methods. The objective of this study was to establish Nepal's first NGS laboratory for NIPT and make this service available locally.

Methods

The study was designed to validate Ion Torrent NGS of cffDNA from maternal plasma for fetal aneuploidy detection. Accordingly, 91 cfDNA plasma samples of singletons pregnancies were provided to the department of Clinical Genetics, Nova International Diagnostics, Lalitpur which was established by Yourgene Health, Singapore.

The received cfDNA was stored at -80°C until processing. The specimens were processed in different batches consisting of around 14 samples in a single set, as the maximum numbers of the sample that could be run in the sequencer in a single run were limited. Fourteen samples were pooled in equimolar concentration into the Ion 540 chip (Thermo Fisher Scientific Inc., Ref. No. A27765) with a maximum of 60 - 80 million reads per Chip.

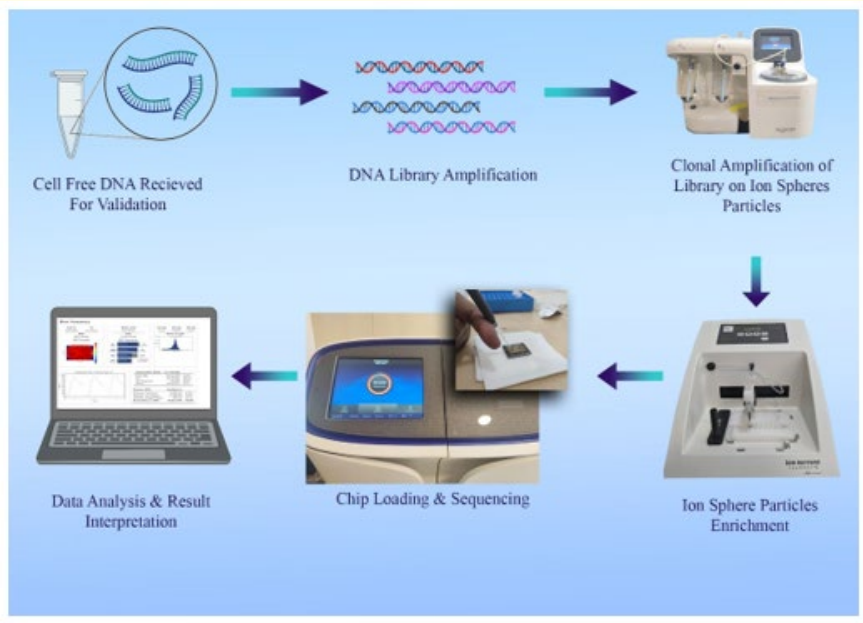

Figure 1: *Workflow of sequencing.*

The ion plus fragment library kit (Thermo Fisher Scientific Inc., Cat. No. 4471252) with ion xpress barcode adapters (ThermoFisher Scientific Inc., Cat. No. 4471250) were used to construct DNA libraries. The qubit dsDNA HS (high sensitivity) assay kit (Thermo Fisher Scientific Inc., Ref. No. Q33231) was used for the quantification of the prepared libraries of the sample accordingly in qubit fluorometer (Invitrogen, Ref No. Q33226). The quantified libraries were pooled, enriched, and loaded on Ion 540 chips. The chip was then subjected to sequencing on the ion GeneStudio S5 system (ThermoFisher Scientific Inc., Ref. No. 2774918100273).

The plan run was executed using the torrent suite software (Version 5.10). The 'FASTQ' files generated from the run reports were eventually uploaded to the Yourgene bioinformatics pipeline server (pipeline version b639506) for aneuploidies risks calculations, specifically chromosomes 13, 18, 21 and Sex chromosomal abnormalities (SCA).

Results

This validation was caried using ion GeneStudio S5 Technology which is semiconductor-based next-generation sequencing (NGS) system that offers easy targeted sequencing processes. It is designed to be economical for laboratories with smaller panels and lower sequencing throughput needs. It employs Ion semiconductor chips with high-throughput sequencing for diagnostics and research applications.

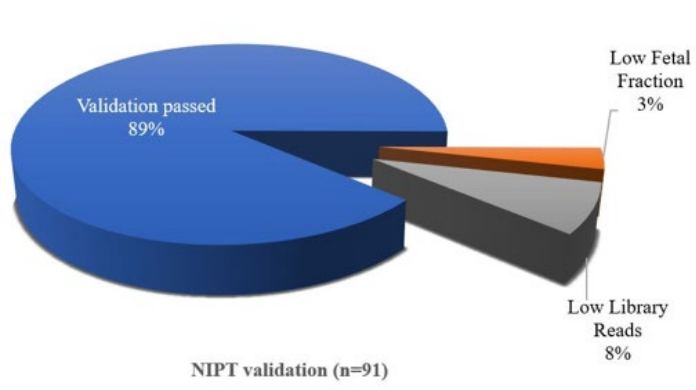

Figure 2: *Result of validation.*

We conducted a validation study of 91 samples provided by Yourgene Health, Taiwan in which all the samples have been sequenced and analysed. A total of 81 (89%) specimens passed the validation, whereas 7 (8%) specimens had low library reads. Likewise, 3 (3%) of the specimen had a low amount of fetal fraction.

Discussion

As a diagnostic screening test, NIPT has been validated clinically in this research. This is the first study to validate the use of benchtop NGS equipment for NIPT in Nepal. Prenatal testing aims to reduce invasive procedures and detect trisomics cell lines from maternal cffDNA. Ion fragment library kit used in the library preparation in this study is a targeted NGS that analyses the regions of clinical relevance in combination with a meticulously designed algorithm that detects precise fetal fraction. Targeted ion semiconductor sequencing can perform NIPT for both autosomal and sex chromosomal aneuploidies.

NGS-based non-invasive prenatal testing relies on the detection of the fetal DNA fragments from the maternal plasma [6-9].

Preventing direct contact with the developing fetus and eliminating its risks, non-invasive testing has remarkably grasped the attention of modern society [8]. Furthermore, current research findings, non-invasive prenatal testing (NIPT) can be safely integrated into existing prenatal screening protocols to minimize invasive testing procedures during prenatal screening [6,10].

To ensure optimum levels of fetal DNA fragments that detect fetal aneuploidies precisely from the maternal plasma by the Ion semiconductor sequencing requires a validated workflow of the NGS procedure.

In Thailand, the first NGS laboratory for NIPT was validated in 2016 [11]. Similarly, in 2019 the first ion semiconductor platform-based NIPT laboratory was established in India [12]. However, the instrument used for NIPT in Thailand was performed using the ion torrent proton system on the ion torrent P1 chips [11].

On the contrary, the validation in India and this study were performed on the identical ion GeneStudio S5, the evolution of the ion proton system.

NIPT has also been validated on other NGS platforms. Illumina-based NGS has also been validated for NIPT. In a recent comparative analysis between the illumina and proton platforms, both have excellent performance for detecting fetal chromosomal aneuploidies [13].

In this study, Ion 540 chip was used, which could generate around 80 million reads. Additionally, this validation study required a minimum of 5 million base reads from a single library sample.

The amount of ISP loading influenced the number of reads into the chip. The higher the ISP loading, the higher the chances of key signal and higher-good quality reads. However, despite proper ISP loading, there were instances where more polyclonal reads also affected the quality of reads. However, the artificial intelligence-based software filtered the reads; still, the presence of polyclonal reads from a single well in the chip generated noise, overall affecting the quality reads.

So, it is vital that adequate ISP loading with less polyclonal reads, minimum adapter dimers are required to produce good quality reads. A study by Minarik G., *et al*. had demonstrated 100% sensitivity and specificity when standard filtering criteria were used for reading mapping, quality filtering, and GC-bias reduction [14].

NGS produced massive amounts of data that required multiple computational steps for appropriate analysis. As a result, the sequencing generated data are usually analyzed through the bioinformatics pipeline.

The bioinformatics pipeline involves treating raw 'FASTQ' files subjected to different steps such as quality check, indexing, alignment, sorting, duplication removal, variant calling, variant annotation, and final fetal aneuploidy detection [15].

The bioinformatics pipeline from Yourgene Health was used in this validation study based on their own patented algorithms for fetal aneuploidy identification [16]. Validation of the cffDNA sequencing from maternal plasma establishes the workflow based on ion S5 GeneStudio S5 machine.

Although being this robust technology for fetal aneuploidy detection, the cffDNA sequencing results does not precisely detect the possibility of other chromosomal anomalies tested like mosaicism, deletion, or duplication. In some rare cases, false-positive or false-negative results may occur due to mosaicism in the fetus, placenta or mother, or due to vanishing twin syndrome [17-19].

NIPT is less reliable if pregnancy is < 12 weeks [20]. This is because the NIPT test cannot be performed with less fetal fraction in maternal plasma. Likewise, in pregnancies where the mother has a prior bone marrow transplant, the test cannot be performed [21,22].

Furthermore, NIPT is not able to distinguish specific forms of aneuploidy. For instance, NIPT cannot determine if Down syndrome is due to high-level mosaicism. In cases where mosaicism is present, results may be inaccurate [23].

NGS-based NIPT is one of the fascinating research areas in molecular diagnosis. When it becomes possible to perform a whole fetal genome analysis in a clinical setting analysis routinely, it will open up the possibility that people will get more information regarding the fetal conditions. cffDNA sequencing can also be employed to detect monogenic disorders in fetal health apart from fetal aneuploidies. However, this validation hasn't established a workflow for monogenic disorders and fetal Rh incompatibilities. Therefore, future prenatal screening panels with monogenic disorders may be coupled with aneuploidy screening.

Conclusion

There is a need for clear criteria that apply across all health sectors, both public and private, to guarantee that tests are validated and robust and that pre-and post-test counselling is provided by specialists familiar with the tests available.

Acknowledgement

The authors would like to acknowledge all the staff of Nova International Diagnostics Pvt. Ltd for their steering support and cooperation.

Funding Support

The research project is entirely financed independently, without any contribution from external organizations or funding sources.

Conflicts of Interest

None.

Availability of Data and Materials

The manuscript has a data in the result section of the manuscript.

Ethics Approval

Not Applicable.

Bibliography

1. Bedei I., *et al*. "Chances and challenges of new genetic screening technologies (NIPT) in prenatal medicine from a clinical perspective: A narrative review". *Genes (Basel)* 12.4 (2021): 501.

2. McLennan A., *et al*. "Noninvasive prenatal testing in routine clinical practice - An audit of NIPT and combined first-trimester screening in an unselected Australian population". *Australian and New Zealand Journal of Obstetrics and Gynaecology* 56.1 (2016): 22-28.

3. Taglauer ES., *et al*. "Review: Cell-free fetal DNA in the maternal circulation as an indication of placental health and disease". *Placenta* 35 (2014): 64-68.

4. Pös O., *et al*. "Recent trends in prenatal genetic screening and testing". *F1000 Research* 8 (2019): 1-10.

5. Carlson LM and Vora NL. "Prenatal diagnosis: Screening and diagnostic tools". *Obstetrics and Gynecology Clinics of North America* 44.2 (2017): 245-256.

6. Manegold-Brauer G and Lapaire O. "Clinical implementation of next-generation sequencing in the field of prenatal diagnostics". Next Generation Sequencing - Advances, Applications and Challenges. InTech (2016).

7. Xu XP., *et al*. "A method to quantify cell-free fetal DNA fraction in maternal plasma using next generation sequencing: Its application in non-invasive prenatal chromosomal aneuploidy detection". *PLoS One* 11.1 (2016): e0146997.

8. Norwitz ER and Levy B. "Noninvasive prenatal testing: the future is now". *Reviews in Obstetrics and Gynecology* 6.2 (2013): 48-62.

9. Taylor-Phillips S., *et al*. "Accuracy of non-invasive prenatal testing using cell-free DNA for detection of Down, Edwards and Patau syndromes: A systematic review and meta-analysis". *BMJ Open* 6 (2016): e010002.

10. Swanson A., *et al*. "Non-invasive prenatal testing: technologies, clinical assays and implementation strategies for women's healthcare practitioners". *Current Genetic Medicine Reports* 1.2 (2013): 113-121.

11. Chang BC., *et al*. "Setting up of first NIPT service laboratory in a Thailand hospital". *Journal of Next Generation Sequencing and Applications* 03 (2016): 3.

12. Pappachen DM. "54. Implementation of first NIPT service laboratory in an Indian hospital". *Reproductive BioMedicine Online* 39.1 (2019): e59-e60.

13. Xue Y., *et al*. "Non-invasive prenatal testing to detect chromosome aneuploidies in 57,204 pregnancies". *Molecular Cytogenetics* 12 (2019): 29.

14. Minarik G., *et al*. "Utilization of benchtop next generation sequencing platforms ion torrent PGM and miseq in noninvasive prenatal testing for chromosome 21 trisomy and testing of impact of in silico and physical size selection on its analytical performance". *PLoS One* 10.12 (2015): e0144811.

15. Meena N., *et al*. "A bioinformatics pipeline for whole exome sequencing: Overview of the processing and steps from raw data to downstream analysis". *Bio-Protocol* 8 (2018).

16. Chia-Han Chan. "Detecting chromosomal aneuploidy united states patent application 20160026759". Taiwan: United States Patent (2016).

17. Hartwig TS., *et al*. "Non-invasive prenatal testing (NIPT) in pregnancies with trisomy 21, 18 and 13 performed in a public setting - factors of importance for correct interpretation of results". *European Journal of Obstetrics and Gynecology and Reproductive Biology* 226 (2018): 35-39.

18. Andari VCM., *et al*. "Noninvasive prenatal testing: Benefits and limitations of the available tests". *Ceska Gynekologie* 85.1 (2020): 41-48.

19. Hartwig TS., *et al*. "Discordant non-invasive prenatal testing (NIPT) - a systematic review". *Prenatal Diagnosis* 37.6 (2017): 527-539.

20. Allahbadia GN. "Has noninvasive prenatal testing (NIPT) come of age?" *Journal of Obstetrics and Gynecology of India* 65.3 (2015): 141-145.

21. Hui L. "Noninvasive prenatal testing for aneuploidy using cell-free DNA - New implications for maternal health". *Obstetric Medicine* 9.4 (2016): 148-152.

22. Idler J., *et al*. "Pregnancy after bone marrow transplantation for aplastic anemia [37Q]". *Obstetrics and Gynecology* 131 (2018): 194S-194S.

23. Kotsopoulou I., *et al*. "Non-invasive prenatal testing (NIPT): Limitations on the way to become diagnosis". *Diagnosis* 2 (2015): 141-158.

Volume 13 Issue 2 February 2024