Non-invasive pre-natal testing vs amniocentesis and karyotyping: A David vs Goliath story

Varun Bajaj[1], Rony Chakravarty[2], Yaduvir Singh[3,*], Shankar Ganesh[4], Avnish Kumar[5]

[1]Graded Specialist, [2]Classified Specialist, [1]Dept. of Pathology, [2]Dept. of Obstetrics and Gynaecology, [1,2]Military Hospital, Wellington, Tamil Nadu, [3]Classified Specialist, Dept. of Community Medicine, Station Health Organisation, Bengaluru, Karnataka, [4,5]Classified Specialist, Dept. of Radiodiagnosis, Military Hospital, Wellington, Tamil Nadu, India

***Corresponding Author:**
Email: djbazi2@gmail.com

Received: 10th April, 2018 **Accepted:** 18th April, 2018

Abstract

Introduction: Chromosomal anomalies constitute a potentially burdening and distressing group of illnesses which if screened/diagnosed in time can offer respite to the expectant family. Currently detection is based on a host of screening tests followed by Chorionic villous sampling or amniocentesis which are invasive procedures associated with a mild risk of pregnancy loss and have a long turnaround time. Non-invasive pre-natal testing uses sensitive and specific techniques to overcome the issue and has the potential to replace the conventional diagnostic tests.

Materials and Methods: A total of 700 consecutive Ante natal cases reporting to Gynaecology OPD in two centres over a duration of two years were screened using protocols applicable as per gestational age. 39 screen positive high risk positive cases were further subjected to NIPT and CVS/amniocentesis and the detection rates compared.

Results: In the primary outcome it was found that the detection rates were comparable with NIPT and conventional diagnostic tests and both showed equivocal results.

Conclusion: The study suggests no significant difference in the detection rates on NIPT vs the conventional karyotype. However, the results need to be interpreted in the light of low incidence rates of chromosomal anomalies in general population. While a universal application of the test is desirable cost factor needs to be permissive for it to be beneficial in a holistic manner.

Keywords: Chromosomal anomalies, Non-invasive Pre-natal testing (NIPT), Chorionic villous sampling, Amniocentesis, karyotyping, Cell free DNA, Quantitative polymerase chain reaction.

Introduction

Chromosomal anomalies cause a significant disease burden for the affected as well as the care giving family, not to mention the cost of therapies, the risk of comorbidities and in some cases even debilitating malignancies.

One in two hundred live births are eclipsed by the presence of chromosomal defects. Most of these are balanced translocations and do no harm to their possessor.[1] Out of the most debilitating chromosomal defects the stand out is Down's syndrome with an incidence of 1 in 800 both due to a high rate of occurrence as well as the nature of the disease. Amongst other chromosomal defects XXX is found in 1 in 650 females, XYY and Klinefelter's syndrome (XXY) in 1 in 750 live births but are comparatively less morbid. Trisomy 13 and 18 are rarer at 1 in 10,000 live births.[1]

In the current century the life expectancy of people with chromosomal anomalies particularly Down's syndrome have increased. But they do not absolve the care giver and the medical fraternity of the recurrent admissions, management issues and crippling costs of care. Most of the issues encompass medical as well as social and societal aspects like mental retardation, congenital heart disease, gastrointestinal abnormalities and congenital hypothyroidism. These conditions are often recalcitrant to behavioural and speech therapies and intervention.[2] Studies by Tenebaum et al have shown that patients with Down's syndrome require longer and frequent admissions for a multitude of conditions mostly respiratory infections, hypothyroidism and seizure disorders at the fore front.[3] Klinefelter's syndrome on the other hand has its own plethora of morbidities ranging from hypogonadism, congenital malformations, psychiatric disorders and endocrinopathies.[4]

Modern society comes with the scourge of increasing maternal age at first pregnancy along with increasing use of In Vitro Fertilisation (IVF) techniques both of which have been shown to be significant risk factors in the conception of chromosomal anomalies.[5]

Pre-natal detection of chromosomal anomalies is traditionally done using screening tests like the double marker, Triple marker or the quadruple marker along with imaging studies and further confirmed with Chorionic Villous Sampling (CVS) or amniocentesis followed by karyotyping. (Fig. 1)

Whilst CVS and amniocentesis remain the gold standard procedures for diagnosis both are invasive and time consuming. (Fig. 2) several authors have found out that chromosomal defects might be found in patients without any significant risk factors.[6] Although Universal screening has been advocated, developing nations continue to address the problem in a cost-effective manner and applying the tests to a high-risk

population. This creates a need for a procedure which is non-invasive with a low turnaround time and can be applied to a large population irrespective of risk stratification.

Currently the protocols are based on imaging studies for nuchal translucency, nasal bone presence along with double marker in the first trimester and bi-parietal diameter along with triple or quadruple marker in the second trimester followed by an applicable invasive test as per gestational age.[6]

Non-Invasive Pre-natal testing (NIPT) is a recent procedure of extracting foetal DNA from either foetal cells or the free foetal DNA (cf DNA) in the maternal circulation and analysing it. Techniques such as Fluorescent In Situ Hybridisation (FISH) and Quantitative Polymerase Chain Reaction (Q-PCR) are utilised for DNA analysis.[7]

While the conventional NIPT using foetal cellular DNA estimates the risk at 1: 200, cell free DNA has been found to be more sensitive by up to 25%.[7] NIPT has the advantage of being highly sensitive and specific, can be offered at as early as 10 weeks and has a low turnaround time thus providing a recipe and scope for its elevation to a diagnostic procedure. Comparison of conventional tests vs NIPT highlights its advantages prima facie (Table 1).

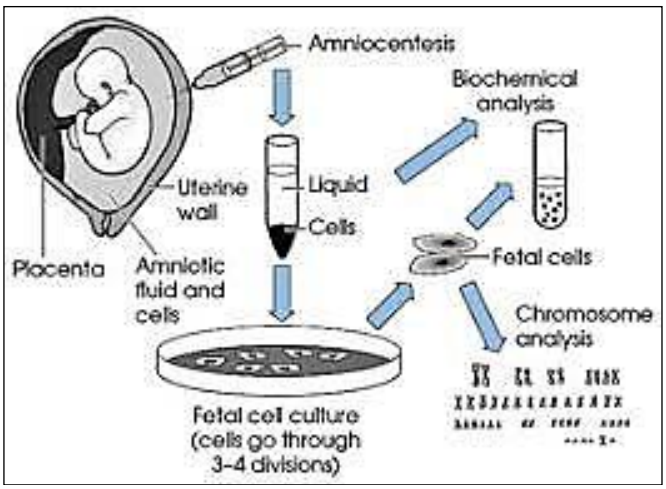

Fig. 1: Procedural flow of conventional prenatal testing

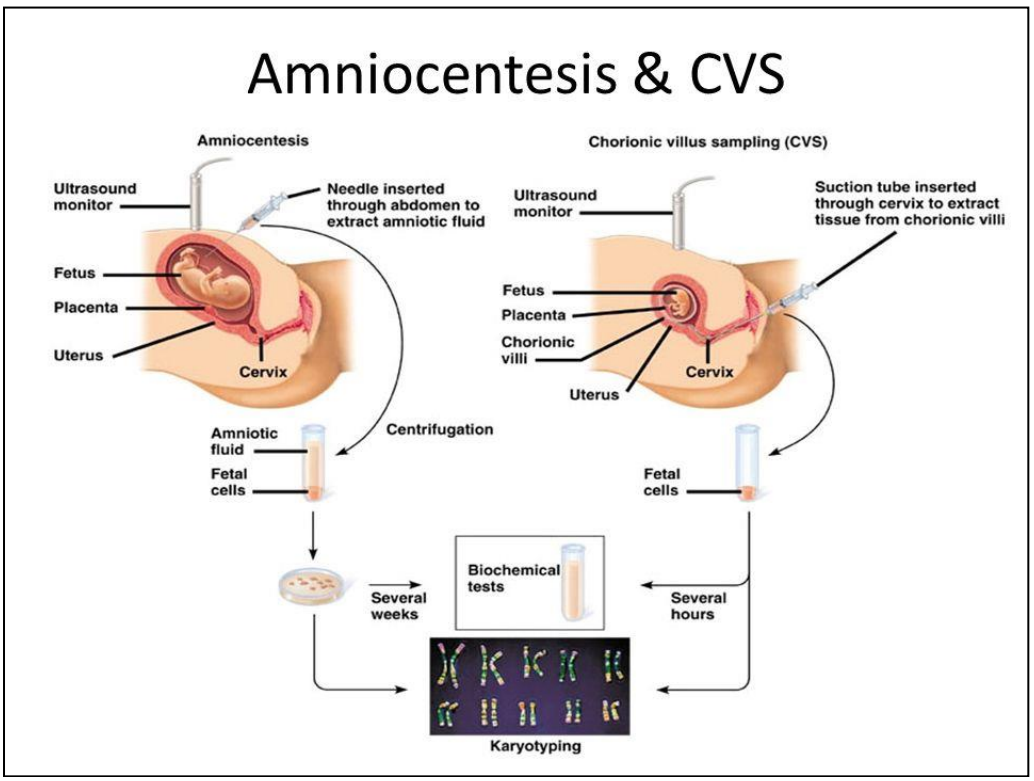

Fig. 2: Comparison of chrionic villus sampling and amniocentesis

Table 1: Comparison of various tests employed in prenatal diagnosis

Name of Test	Type of test	Principle	Advantages	Disadvantages
Double, Triple, Quadruple markers	Screening	Quantitative detection of developmental biomarkers	Non-Invasive	High false positives.
Nasal bone absence, Nuchal translucency	Screening	Visual confirmation of Nasal bone. Measurement of Nuchal translucency	Non-Invasive	Biased towards Down's syndrome. Requires technical expertise
CVS, Amniocentesis followed by Karyotyping	Confirmatory	Karyotyping of chromosomes obtained from Chorionic villi or Amniotic fluid	Invasive	Preanalytical contamination with maternal cells. Interpretational errors.
NIPT	Screening with merits of confirmatory	Analysis of cell free foetal DNA in maternal blood using Q-PCR	Non-Invasive	Can not interpret microdeletions and balanced translocations

Materials and Methods

All 700 consecutive Ante natal cases reporting to Gynaecology Out Patient Department of the two centres were screened using double marker/quadruple marker along with sonological evaluation for nuchal translucency and Presence or absence of nasal bone for a period of two years in two centres in Wellington and Coimbatore. A pre-natal counselling was carried out for all patients and relevant consents were taken. Chronology of the two tests allowed the authors to perform both and draw comparisons. The screened positive 39 pregnancies were further subjected to NIPT using cf DNA and Q-PCR method followed by CVS/amniocentesis for confirmation as per existing protocols and the detection rates compared, taking Karyotyping as gold standard.[8] All the lab tests were outsourced to a NABL accredited Life cell lab for standardisation in the procedure. Results obtained were tabulated and analysed on Microsoft excel software.

Inclusion Criteria: All ANC cases reporting to Gynaecology OPD for two years duration.

Exclusion Criteria: Cases voluntarily opting out/ failure to get complete evaluation/loss to follow up.

Results

Total 39 cases were screen positive with mean age of 29.92 +/- 3.56 years (Table 2). The mean gestation period was 12.5 +/- 1.07 weeks (Table 3). Among the 39 cases subjected to both the diagnostic tests, total four came positive for trisomy 21 by both tests, there were no false positive or false negative results from NIPT as compared to Q-PCR (Gold standard) (Table 4). NIPT was found to be 100% sensitive and specific.

Table 2: Age distribution of patients

S. No	Age	No of patients
1	22-25	05
2	26-30	16
3	31-35	16
4	> 35	02

Mean = 29.92, S.D. = 3.56

Table 3: Distribution of patients by period of gestation

S. No	Period of Gestation	No of patients
1	< 12 weeks	05
2	12 - 14 weeks	32

Mean = 12.5 S.D. = 1.07

Table 4: Sensitivity, specificity and predictive values of test vs gold standard

Result of NIPT	Result of Karyotyping (Gold Std)		
	Positive	Negative	
Positive	4	0	4 =Total +ve by test
Negative	0	35	35 =Total - ve by test
Total	4	35	39 = Total subjects studied
	Total Truly Diseased	Total Truly Not - diseased	

Validity of NIPT
Sensitivity = 1 i.e. 100% sensitive
Specificity = 1 i.e. 100% specific
Positive predictive value (post-test probability) = 1
Negative predictive value = 1

Conclusion

In this study, among high risk pregnancies, free foetal DNA (cf DNA) in the maternal circulation detects all the cases of trisomy 21 with a zero-false positive rate. With further studies on larger sample sizes / meta-analysis, this strong evidence can support NIPT to reduce invasive procedures for diagnosis of chromosomal defects and prevent foetal losses. Studies from the United States show a sensitivity of 100 %and 99 % for trisomy 21 and 18 using NIPT. Similar results have been obtained in the studies conducted by Zhang H et al and literature reviews from world over by Costa et al.[9,10]

Discussion

The study does suggest that there is no significant difference between the detection rates of the two tests. The screening tests currently in vogue using biochemical markers for Down's syndrome and trisomy 18 show a sensitivity of 75-78 % with a false positive rate of 7.5 % which is not ideal in the face of the mere weightage of considering a medical termination or continuing with a risk of a potentially burdening child care particularly in countries without the tools and means to perform conventional karyotyping.[11] With the advancement of techniques in terms of extracting cf DNA and its evaluation using Q-PCR little is left to err in terms of analysis of the genome. However, NIPT does come with the flipside of not being able to detect microdeletions and balanced translocations of which only balanced translocations may be interpreted using fluorescent tags on a karyotype still leaving the lacunae in terms of microdeletion analysis.[12] Conventional karyotyping techniques suffer from various pre analytical errors like contamination from maternal cells and extraembryonic tissue and analytical problems like interpreting false insertions, misclassified small insertions, rearrangements of pericentric regions and co-amplification from non homologous chromosomes to name a few.[13] Q-PCR approach on the other hand has been studied as a substitute for karyotyping per say in chorionic villus samples and has been found to be having the same detection rates.[14] So, procedurally speaking the idea of employing the PCR technique to a sample obtained through a non invasive means holds its merit in a holistic sense. In terms of preventing morbidity from the commoner and more debilitating chromosomal aberrations with Down's syndrome in its driver's seat, NIPT may prove to be a boon with the advantage of being non-invasive, sensitive, specific and with a turnaround time of 24 hours. Cost being permissive for universal application, the test is as the useful conventional methods.

Funding: No funding sources

Conflict of Interest: None declared

Ethical Approval: The study was approved by the Institutional Ethics Committee

References

1. Valentine GH. "Incidence of Chromosome Disorders", *Canadian Family Physician*, 1979;25:937-939.
2. Jaruratanasirikul S, Limpitikul W. Comorbidities in Down syndrome livebirths and health care intervention: an initial experience from the birth defects registry in Southern Thailand. *World J Pediatr.* 2017 Apr;13(2):152-157.
3. Tenenbaum A, Chavkin M, Wexler ID, Korem M, Merrick J. Morbidity and hospitalizations of adults with Down syndrome. *Res Dev Disabil*. 2012 Mar-Apr;33(2):435-41.
4. Bojesen A, Juul S, Birkebæk N, Gravholt C. Morbidity in Klinefelter Syndrome: A Danish Register Study Based on Hospital Discharge Diagnoses. *J Clin Endocrinol Metab*, 91 (4), 1 April 2006, 1254–1260.
5. Plachot M, Veiga A, Montegut J. Are clinical and biological IVF parameters correlated with chromosomal disorders in early life: a multicentric study. *Hum reproduction*. 1988 Jul;3(5):627-635.
6. Todros T, Capuzzo E, Gaglioti P." Prenatal diagnosis of congenital anomalies", *Images in Paediatric Cardiology*. 2001;3(2):3-18.
7. Norwitz ER, Levy B. "Noninvasive Prenatal Testing: The Future Is Now". *Reviews in Obstetrics and Gynecology*. 2013;6(2):48-62.
8. ACOG practice bulletin number 163," New pre natal testing guidelines", 2016.
9. Zhang H, Gao Y, Jiang F, Fu M, Yuan Y, Guo Y, Zhu Z, Lin M, Liu Q, Tian Z, Zhang H, Chen F, Lau TK, Zhao L, Yi X, Yin Y, Wang W, "Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146,958 pregnancies". *Ultrasound Obstet Gynecol.* 2015 May;45(5):530-8.
10. Costa C,"Non-invasive prenatal screening for chromosomal abnormalities using circulating cell-free fetal DNA in maternal plasma: Current applications, limitations and prospects," *The Egyptian Journal of Medical Human Genetics*, 2016 Jun;18(1):1-7.
11. Benn PA, Ying J, Beazoglou T, Egan JF. Estimates for the sensitivity and false-positive rates for second trimester serum screening for Down syndrome and trisomy 18 with adjustment for cross-identification and double-positive results. *Prenat Diagn.* 2001 Jan;21(1):46-51.
12. Swanson A, et al, "Non-invasive Prenatal Testing: Technologies, Clinical Assays and Implementation Strategies for Women's Healthcare Practitioners". *Curr Genet Med Rep.* 2013 Jun;1(2):113-121.
13. Bianchi D, Parker L, Wentworth J. DNA sequencing versus Standard Aneuploidy screening. *N Engl J Med*. 2014;370:799-808.
14. Grati F, Malvestiti F, Grimi B. QF-PCR as a substitute for karyotyping of cytotrophoblast for the analysis of chorionic villi: advantages and limitations from a cytogenetic retrospective audit of 44,727 first trimester pre-natal diagnosis. *Prenat Diagn* 2013 Mar;33(5):502-508.

How to cite this article: Bajaj V, Chakraverty R, Singh Y, Ganesh S, Kumar A. Non-invasive pre-natal testing vs amniocentesis and karyotyping: A David vs Goliath story. Ind J Pathol Oncol, 2018;5(3):366-369.