Original Research Article

False Positive Results in High-Risk Pregnancy for Chromosomal Anomalies - Psychological Considerations

ABSTRACT

Background: With a high sensitivity and specificity, non-invasive prenatal testing (NIPT) is an incomparable screening test for fetal aneuploidy. However, the method is rather newly introduced, and experiences with false positive results are few. Even rare cases of discordant results may cause psychological stress.

Aim of the Study: The aim of this study was to examine false positive cases of NIPT tests in high-risk pregnancies and review psychological consequences of discordant results.

Material and methods: A retrospective study was conducted between 2015 and 2022. The Natera Panorama test was used to analyse the risk of trisomies 21, 18, 13, X monosomy and other sex chromosome abnormalities. High risk result of NIPT for aneuploidy was confirmed by the invasive testing.

Results: 2000 women with a singleton pregnancy participated in the study. Out of 2000 NIPT tests 22 cases with high risk results for chromosomal anomalies were detected. Only one false positive case with high risk result for trisomy 21 was detected. The overall positive predictive value (PPV) of NIPT for trisomies 21, 13, 18, X monosomy and XYY syndrome was 95%.

Conclusion: Our study has showed only one case with false positive result for trisomy 21. However these cases with discordant results are very sensitive from bioethical point of view. So such couples need further follow up and sometimes even psychological counselling. NIPT due to its high PPV significantly reduces the need for an invasive testing, thereby reducing the risk of miscarriage.

Key words: non-invasive prenatal testing, bioethics, false positive results, chromosomes

Introduction

A high risk pregnancy for chromosomal anomalies is a pregnancy in which the possibility of the fetus having a chromosomal abnormality, such as trisomy 21 (Down Syndrome) or trisomy 18 (Edwards Syndrome), is increased [1]. The likelihood of such a pregnancy can be influenced by factors including maternal age, which is the most known risk (National Society of Genetic Counselors, 2016), as well as previous family history (American College of Obstetricians and Gynecologists, 2019). Testing such as chorionic villus sampling or amniocentesis are invasive prenatal diagnostic techniques used to determine whether a fetus has chromosomal abnormalities. In both techniques, it is required that an instrument is implemented close to the fetus itself which is why invasive techniques bear a higher risk and are less popular [2].

It is noted that even during the testing stage, psychological stress for the mother is already expected (Women's Ultrasound Specialists Melbourne). Due to the risk and associated stress, non-invasive prenatal testing (NIPT) techniques are more common [3,4]. A simple blood test is used to determine the chromosomal abnormalities which carries less risk for the fetus as well as less stress for the mother (Royal College of Pathologists of Australasia, 2017). Even though the NIPT causes less anxiety to the mother, the pure process of testing generally causes distress. After a positive result, the parents of the child should be educated on the consequences this result might bring, the future accommodations which might have to be taken into account ,as well as psychological counselling to deal with the fear, stress, and worries the diagnosis might cause. The importance of previous testing is not only to rule out possible disabilities and risks, but to possibly treat or prepare for an abnormality. This preparation is through being educated but mainly mentally since a child with a disability can cause a multitude of emotional strains [5] . However, what happens in case of a false positive, meaning the case in which a diagnosis was mistakenly made according to test results which wrongly appeared to be positive and a mother is emotionally prepared as well as strained?

Materials and Methods

Study subjects

We collected the data retrospectively on pregnant women with a singleton pregnancy who underwent NIPT in InMedica clinic. The study was authorised by the Ethics Committee of the Lithuanian University of Health Sciences, and a written informed consent was obtained from all participants.

Laboratory analysis

The Panorama Test (NIPT) was performed to all the subjects of our study. The medical personnel took two blood samples of 10 ml from each subject. All the blood samples were transported within 48 hours by plane to Natera laboratory in San Carlos, California (United States). The samples were analysed as previously described using validated methodologies for cfDNA isolation, polymerase chain reaction

amplification targeting 19,488 SNPs, high-throughput sequencing, and the analysis with the next-generation aneuploidy test using SNPs (NATUS) algorithm (15,19,20).

The Natera Panorama test was used to analyse a cffDNA from maternal blood for the detection of the following chromosomal abnormalities: 1) trisomies of 21, 18, 13; 2) X monosomy; 3) triploidy; 4) other sex chromosome abnormalities and fetal gender. All the samples with a risk score ≥ 9:10 were reported as a high risk for fetal aneuploidy and the samples with the risk scores < 1:10 000 were considered of low risk. The samples were processed and the results were obtained within 5–7 business days. The high risk results were confirmed by the invasive diagnostic procedures (amniocentesis or chorionic villus sampling). A follow-up was performed by a telephone call for a low-risk group in order to ascertain that the infants would be born without chromosomal abnormalities.

Statistical analysis

The descriptive data of demographic information are presented as median and minimum/maximum values. Continuous variables for normal distribution were inspected using the Kolmogorov–Smirnov test. We used the Spearman and partial correlation tests to evaluate the correlation of various factors associated with the fetal fraction. The Mann–Whitney U test was used for comparing the continuous variables between the groups. PPV was calculated by the formula PPV= true positive (TP)/ (true positive (TP) + false positive (FP)). A p value < 0.05 was considered statistically significant. A statistical analysis was performed using the SPSS 23.0 program.

Results

The flow chart for the study is presented as Figure 1. We collected the data of 2000 women who received NIPT, the sample collection dates ranged between November 2015 and June 2022.

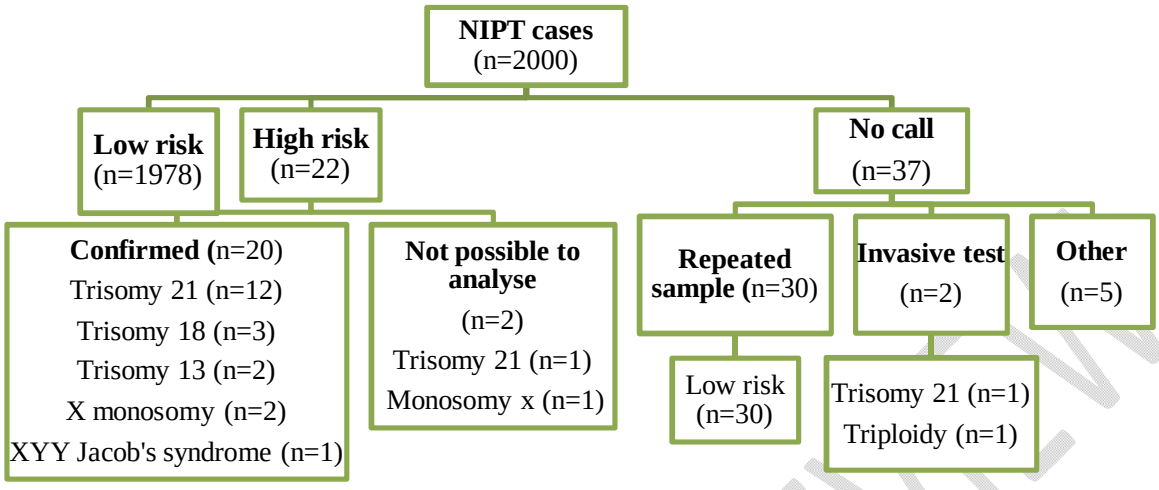

Figure 1. Flow chart of the study

The prevalence of high risk cases among the study participants was 1.1% (22/2000). Table 1 represents all high risk results of NIPT test for different chromosomal abnormalities as well weather the result was confirmed with invasive testing. Only one false positive case with high risk result for trisomy 21 was detected. So positive predictive value (PPV) for Down syndrome is 91.7 %. The overall PPV of NIPT for all abnormalities - trisomies 21, 13, 18, X monosomy and XYY syndrome was 95%.

Table 1 Positive predictive values of non-invasive prenatal screening

	n	Confirmatory test	FP	FF (%)	PPV
High risk	22	21	0	6.4	95.5%
• Trisomy 21	12	11	1	8.8	91.7
• Trisomy 18	3	3	0	7.4	100%
• Trisomy 13	2	2	0	5.1	100%
• Monosomy x	2	2	0	8.4	100%

- XYY (Jacob's syndrome) 1 1 0 6.4 100%

FP-false positive, FF-fetal fraction, PPV- positive predictive value

Discussion

NIPTs are gaining more popularity due to their reputation of being low risk and highly accurate. Despite the advancement in NIPTs technology, the accuracy of these tests is not perfect, leading to the possibility of false-positive and false-negative results. This inaccuracy can be attributed to a variety of factors, such as technical limitations, biological variation, and maternal factors, which may affect the reliability of the test.

Our study showed, that overall PPV of NIPT for all chromosomal abnormalities was very high 95%. We received only one discordant case for trisomy 21. Previous publications showed that PPV was 97.4% for trisomy 21 and 88.9% for trisomy 18 [6]. However, an Indian study estimated PPV of trisomy 21 and sex chromosome abnormalities XXX, XXY (80% and 50%, respectively) which was much lower compared with our results, but PPV of trisomy 18 was identical to the findings obtained from our research (100%) [7]. An important issue is, that any high-risk score in NIPT should be confirmed by invasive prenatal diagnosis before any decisions about pregnancy. So NIPT testing is much more precise that biochemical screening from maternal blood. According to recent data false positive rats in first trimester prenatal screening may be up to 4% [8].

Grobman et al. (2018) advocates for support and education for mothers who receive a high risk result [9]. Leung et al. (2016) found that false negatives can cause negative psychological responses such as anxiety, depression, and stress reactions as well as feelings of guilt towards themselves [10].

It is interesting to note that Leung et al. (2016) also found that women receiving a false positive displace higher levels of worry and stress than women receiving a true positive. These negative feelings can even persist throughout the pregnancy and can go as far as affecting the feelings of attachments the mother forms with her child once it is born. Furthermore, the relationship of the parents can also be damaged. Both studies showcase the importance of providing proper psychological support to not only the women but the entire family due to the negative impact this situation could have on the relationships among the family members. However, it also demonstrates the current lack of support provided

The adverse psychological reactions experienced by women are not solely attributable to the prospect of having an unhealthy child and the associated emotional burden. Rather, it is also attributed to the need for additional testing to validate the initial findings. Such testing is usually invasive and has the potential to harm the fetus, thereby increasing the anxiety and distress experienced by the mother. In severe cases, invasive procedures could result in the termination of the pregnancy.

Aite et al. (2019) conducted a research questioning a sample of 269 women who received a false positive result for either Down Syndrome, Edwards Syndrome or Patau Syndrome using NIPTs [11]. The women reported increased feelings of anxiety, stress, anger, as well as depression amongst others and expressed their disappointment with the lack of provided psychological health care.

NIPT is likely to be used in prenatal screening at the first trimester due to its high PPV. While reducing the invasive testing rates, NIPT saves life to a lot of fetuses who could potentially be miscarriaged as a result of the diagnostic testing with CVS or amniocentesis [12].

Conclusion

Our study detected only one case of false positive result, but generally discordant results in prenatal diagnostics may be big psychological stressor. Providing comprehensive education to families regarding the implications of a prenatal diagnosis and equipping them with the necessary tools to navigate the unique psychological and emotional challenges associated with such diagnoses is a critical but often overlooked aspect of healthcare. It is essential to recognize that the impact of a prenatal diagnosis extends beyond the medical implications and can have a profound effect on the psychological and emotional well-being of the family. Therefore, healthcare professionals must prioritize the provision of holistic care that addresses the multifaceted needs of the families affected by a high-risk pregnancy.

References

1 Shi X, Tang H, Lu J, Yang X, Ding H, Wu J. Prenatal genetic diagnosis of omphalocele by karyotyping, chromosomal microarray analysis and exome sequencing. Ann Med. 2021;53(1):1285-1291.

2. Caceres V, Murray T, Myers C, Parbhoo K. Prenatal Genetic Testing and Screening: A Focused Review. Semin Pediatr Neurol. 2022 Jul;42:100976.

3. Quezada MS, Gil MM, Francisco C, Oròsz G, Nicolaides KH. Screening for trisomies 21, 18 and 13 by cell-free DNA analysis of maternal blood at 10-11 weeks'' gestation and the combined test at 11-13 weeks'. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol 2015; 45(1): 36–41.

4 Gil MM, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol Off J Int Soc Ultrasound Obstet Gynecol 2015; 45(3): 249–66.

5. Szegda K, Bertone-Johnson ER, Pekow P, Powers S, Markenson G, Dole N, Chasan-Taber L. Prenatal Perceived Stress and Adverse Birth Outcomes Among Puerto Rican Women. J Womens Health (Larchmt). 2018 May;27(5):699-708. doi: 10.1089/jwh.2016.6118.

6. Eiben B, Krapp M, Borth H, Kutur N, Kreiselmaier P, Glaubitz R, et al. Single Nucleotide Polymorphism-Based Analysis of Cell-Free Fetal DNA in 3000 Cases from Germany and Austria. Ultrasound Int Open 2015; 1(1): E8–11.

7. Verma IC, Puri R, Venkataswamy E, Tayal T, Nampoorthiri S, Andrew C, et al. Single Nucleotide Polymorphism-Based Noninvasive Prenatal Testing: Experience in India. J Obstet Gynaecol India 2018; 68(6): 462–70.

8. Santorum M, Wright D, Syngelaki A, Karagioti N, Nicolaides KH. Accuracy of first-trimester combined test in screening for trisomies 21, 18 and 13. Ultrasound Obstet Gynecol. 2017 Jun;49(6):714-720.

9. Grobman W. A., Gilboa S. M., Iqbal S. N., Kline J., & Hogue C. J. (2018). Population-based screening for prenatal chromosomal abnormalities: A randomized trial of cell-free DNA versus maternal serum alpha-fetoprotein. American Journal of Obstetrics and Gynecology. 2018; 219(1): 71.e1-71.e10.

10. Leung W. C., Ding W., Sau T., & Leung, T. Y. Psychological consequences of false-positive prenatal screening results: A systematic review. Prenatal Diagnosis. 2016; 36(12): 1145-1153.

11. Aite L., Avagliano L., Marino D., & Pennesi M. Psychological impact of false positive results in non-invasive prenatal screening: A prospective study. Journal of Genetic Counseling. 2019; 28(2).

12. Bjerregaard L, Stenbakken AB, Andersen CS, Kristensen L, Jensen CV, Skovbo P, Sørensen AN. The rate of invasive testing for trisomy 21 is reduced after implementation of NIPT. Dan Med J. 2017; 64(4):A5359.