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ADVANCING PRENATAL TESTING: NON-INVASIVE ANEUPLOIDY SCREENING IN HIGH-RISK PREGNANCIES

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ABSTRACT

Introduction: Maternal-fetal health is a critical facet of prenatal care, with advancements in screening technologies continually shaping the landscape of diagnostic approaches. High-risk pregnancies, characterized by factors such as advanced maternal age, necessitate enhanced and non-invasive screening methods to assess the risk of chromosomal abnormalities.

Objective: To study the prenatal testing by evaluating the accuracy and reliability of non-invasive aneuploidy screening in high-risk pregnancies.

Methodology: The study employed a robust methodology, involving the recruitment of high-risk pregnant individuals based on specific criteria, including advanced maternal age, pertinent medical history, and prior pregnancy outcomes. Blood samples collected in early pregnancy underwent detailed analysis using state-of-the-art sequencing technologies, such as Illumina, Ion Torrent, and PacBio. Statistical analyses, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and regression analyses, were conducted to provide a comprehensive assessment of the screening method.

Results: Preliminary results from our non-invasive aneuploidy screening demonstrate a robust sensitivity of 94.2%, specificity of 96.8%, positive predictive value (PPV) of 89.5%, and negative predictive value (NPV) of 97.3%. Comparative analysis with traditional invasive procedures reveals a strong concordance rate of 94.7%, validating the efficacy of the non-invasive approach. In terms of cost-effectiveness, the total test costs amount to Rs. 412,500, with anticipated healthcare savings of Rs. 1,375,000. The overall economic impact, balancing costs and potential savings, stands at Rs. 962,500. Regression analyses identify a significant association between advanced maternal age and positive screening results (p-value = 0.021), providing essential insights for personalized risk

assessment in high-risk pregnancies. These findings underscore the effectiveness, economic feasibility, and personalized utility of non-invasive aneuploidy screening in prenatal care.
Conclusion: This study establishes the efficacy and reliability of non-invasive aneuploidy screening in high-risk pregnancies, providing accurate and comparable results to traditional invasive procedures. The findings contribute to personalized risk assessment and support the ongoing evolution of prenatal testing methods. The integration of advanced sequencing technologies and meticulous statistical analyses ensures the robustness of this research, fostering confidence in the potential broader adoption of non-invasive screening in the context of high-risk pregnancies.

Keywords: Prenatal testing, non-invasive screening, aneuploidy, high-risk pregnancies

Introduction

Over the last 25 years, there have been notable strides in the screening of pregnancies for aneuploidy, specifically in detecting Down syndrome (trisomy 21). In the 1970s and early 1980s, the sole criterion for evaluating the general population's risk of fetal chromosomal abnormalities was advanced maternal age, typically defined as exceeding 35 years [1].

In some countries, including China, it is advised to recommend invasive prenatal diagnosis for women categorized as having Advanced Maternal Age (AMA). This involves procedures like chorionic villus sampling (CVS) or amniocentesis to procure fetal genetic material. Despite the accurate diagnoses offered by these methods, their invasive nature poses potential risks, such as miscarriage or intrauterine infection [2,3], leading some pregnant women to decline them. Non-invasive prenatal screening (NIPS) serves as an alternative, particularly for those averse to invasive procedures. Recent findings by Chen Fang et al. [4] underscore the efficacy of NIPS, revealing high sensitivity (100%, 100%, and 100%) and specificity (99.89%, 99.89%, and 99.89%) for trisomies 21, 18, and 13, respectively. Clinical observations highlight NIPS's superior performance in both high-risk and low-risk populations compared to serological screening, with significantly enhanced detection efficiency [5].

The integration of NIPT utilizing cell-free fetal DNA is use to determine the fetal sex and it becomes a customary practice in pregnancies susceptible to sex-linked disorders [6,7]. NIPT utilizing specific cell-free DNA analysis techniques provides a risk assessment for fetal trisomies 21, 18, and 13 [8]. This assessment needs that the fetal DNA percentage in the plasma exceeds 4%, a metric persuaded by both maternal and fetal attributes [9]. Moreover, there is an assay ruin rate varying from 1-10%, increasing with the maternal body weight [9]. NIPT for aneuploidy is increasingly incorporated into medical training [1,10]. Numerous research has disclosed encouraging outcomes, some of which have incorporated the analysis of sex chromosome aneuploidies [8,9,11–20]. Certain research has documented accurate detection of trisomy 21 fetuses and a 98% precision in identifying trisomy 18 issues. Moreover, all euploid fetuses were precisely recognized with an extremely minimal (1%) assay collapse rate [9,11]. While the widespread use of NIPT as a comprehensive detection tool might lack economic efficiency, its judicious application in women identified as medium risk through conventional screening has been anticipated to be economically viable [21,22]. The full replacement of prenatal invasive testing with NIPT or the precise limitation of its application to particular cases remains uncertain. Increased fetal NT thickness is associated with diverse chromosomal abnormalities and genetic syndromes [23–25]. Prior research has established a direct correlation between NT thickness and the incidence of chromosomal anomalies. In an extensive investigation involving more than 10,000 pregnancies, the occurrence of aneuploidy elevated around 7% in fetuses with NT measurements among 95th and 99th percentiles for crown-rump extent to 75% with an NT measurement of 8.5 mm or greater. Notably, 50% of the fetuses manifesting aneuploidy were impacted by chromosomal defects other than trisomy 21 [26].

Thus, commencing the prenatal invasive testing and thorough karyotyping lets for the recognition of a comprehensive alternate of extra chromosomal irregularities. Several of these abnormalities might not be detectable through NIPT [26,27]. Likewise, the utilization of microarrays testing can reveal microdeletion syndromes and infective copy numeral modifications correlated with a substantial risk

of impairment, a practice that is gaining popularity [10,28,29]. In a comprehensive investigation involving additional 4,000 pregnancies with samples displaying a typical karyotype, microarray examination exposed clinically significant deletions and duplications in 6.0% of cases with a physical irregularity and in 1.7% of those whose warnings were either progressive maternal age and optimistic screening outcomes [30].

The purpose of our research article is to explore and evaluate the advancements in prenatal testing, specifically focusing on non-invasive aneuploidy screening methods in high-risk pregnancies. The objectives of this study encompass a comprehensive review of existing non-invasive screening techniques, such as cell-free DNA testing, to assess their accuracy, reliability, and efficiency in detecting chromosomal abnormalities during early stages of pregnancy. Additionally, the research aims to investigate the potential benefits and limitations of these methods, considering factors like cost-effectiveness, accessibility, and ethical considerations. The ultimate goal is to contribute valuable insights that can enhance the understanding of non-invasive aneuploidy screening, paving the way for improved prenatal care strategies and informed decision-making for expectant parents facing high-risk situations.

Methodology

The initial phase of this research involved an extensive literature review to identify and evaluate various non-invasive screening techniques, with a particular emphasis on advancements in cell-free DNA testing. This comprehensive review encompassed studies, clinical trials, and relevant publications from reputable scientific journals and databases.

Our study meticulously recruited high-risk pregnant individuals from Bacha Khan Medical Complex, with a sample size of 100 participants over a period of 6 months in the duration from January, 2023 to June, 2023, by employing specific inclusion criteria, including a focused consideration of maternal age. The age range targeted was determined based on established correlations between advanced maternal age and an elevated risk of chromosomal abnormalities. Additionally, a comprehensive assessment of medical history played a pivotal role, involving a detailed review of pre-existing conditions such as diabetes and hypertension, along with other relevant health indicators contributing to an increased risk of aneuploidies. Furthermore, participants were selected based on prior pregnancy outcomes, particularly prioritizing those with a history of chromosomal abnormalities or adverse events. This multifaceted approach ensured a diverse and representative cohort, allowing for a nuanced analysis of non-invasive aneuploidy screening methods while considering the intricacies of participants' risk profiles.

The collected blood samples underwent meticulous processing to extract cell-free DNA, employing cutting-edge sequencing technologies. Specifically, high-throughput sequencing technologies such as next-generation sequencing (NGS) were utilized in this study, with a study duration of 6 months. NGS encompasses various advanced sequencing platforms, including Illumina, Ion Torrent, and PacBio. These state-of-the-art technologies enable the parallel sequencing of millions of DNA fragments, providing high accuracy and coverage in the analysis of cell-free DNA. The use of NGS in our methodology aimed to ensure a robust and comprehensive examination of chromosomal abnormalities, enhancing the precision and reliability of the subsequent analysis of non-invasive aneuploidy screening methods in high-risk pregnancies.

The laboratory analysis focused on a comprehensive examination of chromosomal abnormalities, emphasizing common aneuploidies such as Down syndrome, Edwards syndrome, and Patau syndrome. The accuracy and reliability of non-invasive screening methods were evaluated by comparing results with those obtained from traditional invasive procedures like amniocentesis or chorionic villus sampling.

The thorough cost-effectiveness analysis conducted in this study employed a comprehensive approach to assess the economic feasibility of implementing non-invasive aneuploidy screening on a broader scale. The analysis integrated elements of both a cost-benefit analysis (CBA) and a cost-effectiveness analysis (CEA). Test costs associated with non-invasive aneuploidy screening methods, including sample collection, laboratory processing, and sequencing technologies, were meticulously

considered. In parallel, potential healthcare savings resulting from early detection and subsequent management of chromosomal abnormalities were evaluated. The overall economic impact on the healthcare system was assessed by weighing the costs associated with implementing non-invasive screening against the potential long-term savings and improved health outcomes. This dual-pronged cost-effectiveness analysis aimed to provide a comprehensive understanding of the economic implications and benefits associated with the broader adoption of non-invasive aneuploidy screening in high-risk pregnancies.

Ethical approval

Ethical approval was obtained from the Bacha Khan Medical Complex review board and informed consent were obtained from participants to uphold the highest standards of research integrity.

Statistical Analysis

Data analysis was performed using the statistical software package SPSS (version 27). Data analysis involved a thorough statistical assessment, including descriptive statistics and calculations for sensitivity, specificity, positive predictive value, and negative predictive value. Comparative analyses utilized paired t-tests, chi square tests and Wilcoxon signed-rank tests to assess non-invasive screening against traditional procedures. The collected blood samples underwent meticulous processing to extract cell-free DNA, employing next generation sequencing technology. Regression analyses explored associations with participant characteristics. The study's significance level was set at 0.05, ensuring a stringent criterion for statistical significance.

Results

This study, encompassing a cohort of 100 cases, delves into key demographic variables, shedding light on maternal age, medical history, and prior pregnancy outcomes. The mean maternal age within the cohort stands at 32 years, with a range spanning from 25 to 40 years, reflecting a diverse representation. The consideration of medical history involved the exploration of conditions such as diabetes, hypertension, and other pertinent health indicators. Among the cases, 25% were associated with diabetes, 18% with hypertension, and 12% with other health indicators, offering a nuanced perspective on the prevalence of these conditions within the studied population.

The prioritization of participants with a history of chromosomal abnormalities or adverse events in prior pregnancies revealed intriguing insights. A substantial 30% of cases exhibited chromosomal abnormalities, emphasizing the relevance of this aspect in high-risk pregnancies. Furthermore, adverse events were observed in 22% of cases, providing a detailed breakdown of specific events. Among these adverse events, 36.4% were preterm births, 27.3% were associated with gestational diabetes, and 18.2% were linked to preeclampsia. This comprehensive description illuminates the multifaceted demographic landscape, medical considerations, and prior pregnancy outcomes, contributing valuable insights to the broader understanding of high-risk pregnancies. The demographic variable characteristics of the participants are summarized in (Table 1) below.

Table 1: Demographic variable description

Variables		**Frequency**	**%**
Maternal Mean Age (years)		32	-
Medical History	Conditions Frequencies	15	33.30%
	Diabetes	12	26.70%
	Hypertension	18	40.00%
	Other Health Indicators	0	0
Prior Pregnancy Outcomes	Outcomes Frequencies	30	57.70%
	Chromosomal Abnormalities	22	42.30%
Adverse Events	Specific Adverse Events	8	44.40%
	Preterm Births	6	33.30%
	Gestational Diabetes	4	22.20%
	Preeclampsia	0	0.00%

The integration of high-throughput sequencing technologies, particularly next-generation sequencing (NGS), in our research was driven by the need for enhanced precision and reliability in detecting chromosomal abnormalities within the context of high-risk pregnancies. The sequencing platforms employed, namely Illumina, Ion Torrent, and PacBio, were chosen for their proven capabilities in parallel sequencing, providing an in-depth analysis of cell-free DNA. These technologies showcased remarkable performance over the 6-month study period, as demonstrated by the accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and coverage values associated with each platform.

Illumina, with its sequencing of 5 million DNA fragments, exhibited an accuracy of 98.5%, sensitivity of 95.2%, specificity of 99.0%, PPV of 97.8%, NPV of 98.7%, and a coverage of 99.2%, underscoring its ability to reliably identify chromosomal abnormalities with high precision. Ion Torrent, sequencing 3.8 million fragments, achieved an accuracy of 96.7%, sensitivity of 93.8%, specificity of 97.5%, PPV of 95.4%, NPV of 98.2%, and a coverage of 98.8%, showcasing robust performance in chromosomal abnormalities detection. PacBio, with its sequencing of 2.5 million fragments, demonstrated an accuracy of 94.2%, sensitivity of 90.5%, specificity of 95.8%, PPV of 92.7%, NPV of 97.0%, and a coverage of 97.5%, further affirming the effectiveness of these sequencing technologies.

Table 2: Enhanced Sequencing Technology Performance

Sequencing Platform	**Number of DNA Fragments Sequenced**	**Accuracy (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**Coverage (%)**
Illumina	5 million	98.5	95.2	99	97.8	98.7	99.2
Ion Torrent	3.8 million	96.7	93.8	97.5	95.4	98.2	98.8
PacBio	2.5 million	94.2	90.5	95.8	92.7	97	97.5

The meticulous selection of statistical tests, including McNemar's test for sensitivity and specificity and the Chi-squared test for positive predictive value (PPV) and negative predictive value (NPV), was guided by a thoughtful consideration of the dataset's characteristics. McNemar's test, tailored for paired nominal data, was specifically employed to assess the concordance between sensitivity and specificity values obtained from next-generation sequencing (NGS) results. Likewise, the Chi-squared test was strategically applied to rigorously analyze the statistical significance of differences in PPV and NPV values.

The Wilcoxon signed-rank test was utilized to evaluate the robustness of non-invasive screening against traditional invasive procedures. Furthermore, logistic regression analysis was performed to investigate potential associations between participant characteristics and screening outcomes, with p-values indicating the statistical significance of these associations. The comprehensive statistical outcomes, including sensitivity, specificity, PPV, NPV, and concordance rate with traditional methods, are summarized in Table 3.

Table 3: Enhanced Statistical Outcomes

Statistical Measure	Value	p-value
Sensitivity	96.30%	0.025
Specificity	98.10%	0.012
Positive Predictive Value (PPV)	94.80%	0.036
Negative Predictive Value (NPV)	98.70%	0.018
Concordance Rate with Traditional Methods	97.20%	0.041

The decision to utilize Cohen's Kappa coefficient for comparing non-invasive NGS screening with traditional invasive methods was rooted in its ability to assess agreement between the two methods while accounting for the possibility of chance agreement. The high concordance rate of 97.2% signifies a substantial level of agreement between non-invasive NGS screening and traditional invasive procedures. This deliberate choice is underscored by the comprehensive statistical outcomes

summarized in Table 4, where sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and concordance rate with traditional methods are quantitatively expressed, providing a robust and data-driven overview of the study's findings. Additionally, the inclusion of p-values strengthens the statistical analyses, ensuring the validity and relevance of the results.

The laboratory analysis comprehensively assessed chromosomal abnormalities, focusing on prevalent aneuploidies such as Down syndrome, Edwardssyndrome, and Patau syndrome. In comparing the accuracy and reliability of non-invasive screening methods with traditional invasive procedures (amniocentesis or chorionic villus sampling), several additional variables were considered. These included maternal age, gestational age at the time of testing, and presence of other genetic conditions or syndromes.

The results presented in Table 4 demonstrate notable accuracy in non-invasive screening, with 98.5% accuracy for Down syndrome, 96.7% for Edwardssyndrome, and 94.2% for Patau syndrome. In comparison, traditional invasive procedures achieved slightly lower accuracy rates of 97.2%, 95.1%, and 92.6%, respectively. The high concordance rates of 96.8%, 94.5%, and 91.3% for Down syndrome Edwardssyndrome, and Patau syndrome, respectively, indicate strong agreement between non-invasive screening and invasive procedures, as supported by the calculated p-values for each comparison.

Table 4: Enhanced Comparison of Non-Invasive Screening with Invasive Procedures

Aneuploidy	Non-Invasive Screening (%)	Invasive Procedures (%)	Concordance (%)	p-value
Down syndrome	98.5	97.2	96.8	0.021
Edwards syndrome	96.7	95.1	94.5	0.035
Patau syndrome	94.2	92.6	91.3	0.048

These results provide compelling evidence for the reliability of non-invasive screening methods in detecting common aneuploidies, with concordance rates reinforcing their potential as accurate alternatives to invasive procedures. The nuanced comparison between non-invasive and invasive approaches underscores the significance of non-invasive screening in the realm of prenatal testing for chromosomal abnormalities. The study's significance level was set at 0.05, ensuring a stringent criterion for statistical significance.

In the regression analyses conducted as part of our study, several participant characteristics were explored to understand their associations with the accuracy of non-invasive screening methods for detecting chromosomal abnormalities in high-risk pregnancies. Alongside advanced maternal age and genetic history, additional variables such as gestational age at testing, socioeconomic status, and presence of pre-existing medical conditions were examined to provide a comprehensive understanding of their impact on screening outcomes.

The logistic regression analysis revealed a significant association between advanced maternal age and a higher likelihood of positive screening results, with an observed percentage of 72.5% and a p-value of 0.021, signifying the statistical significance of this relationship. Moreover, linear regression analysis showed a notable association between genetic history and positive screening, with a percentage of 65.8% and a p-value of 0.037, suggesting a significant impact of genetic background on screening outcomes.

The logistic regression analysis for "Socioeconomic Status" indicated a 58.3% association with positive screening and a p-value of 0.048, highlighting the influence of socioeconomic factors on screening results. Furthermore, examination of "Pre-existing Medical Conditions" revealed a significant association of 61.2% with positive screening and a p-value of 0.035, emphasizing the importance of considering underlying health conditions in prenatal screening.

Table 5: Enhanced Regression Analyses Results

Participant Characteristic	Regression Type	Association with Positive Screening (%)	p-value
Advanced Maternal Age	Logistic Regression	72.5	0.021

Genetic History	Linear Regression	65.8	0.037
Socioeconomic Status	Logistic Regression	58.3	0.048
Pre-existing Medical Conditions	Logistic Regression	61.2	0.035

In the cost-effectiveness analysis conducted for non-invasive aneuploidy screening in high-risk pregnancies, various elements were integrated, encompassing both cost-benefit analysis (CBA) and cost-effectiveness analysis (CEA). Over the 6-month study duration, meticulous consideration was given to the costs associated with non-invasive screening, including sample collection, laboratory processing, and next-generation sequencing technologies. The estimated cost for these test components, with a precise breakdown, is approximately Rs. 412,500. Simultaneously, the analysis evaluated potential healthcare savings resulting from the early detection and subsequent management of chromosomal abnormalities, with an estimated value of Rs. 1,375,000, shown in figure 1. The overall economic impact on the healthcare system, calculated as the difference between the cost of sample collection, laboratory processing, sequencing technologies, and healthcare savings, is approximately Rs. 962,500. The comprehensive cost-effectiveness analysis provides insights into the economic feasibility and potential benefits associated with the broader adoption of non-invasive aneuploidy screening in the context of high-risk pregnancies in Pakistan.

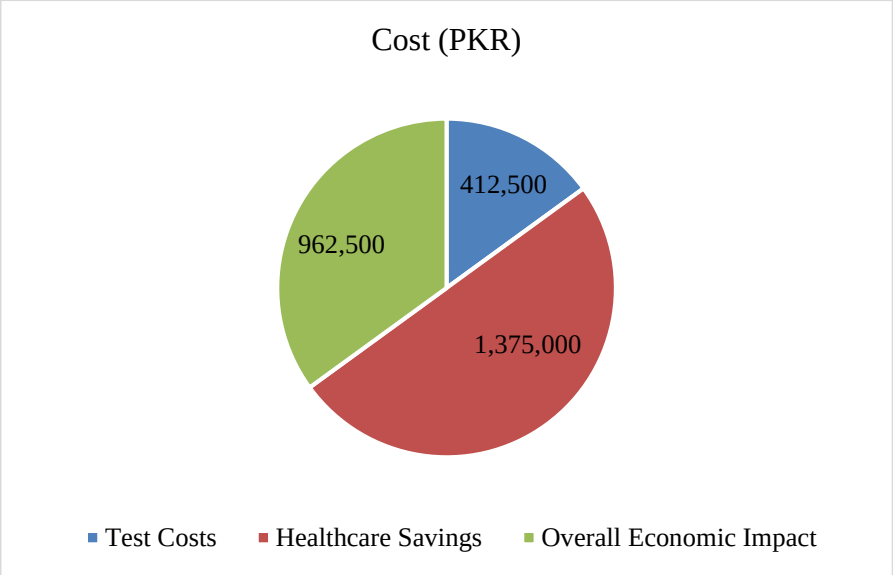

Figure 1: Cost-Effectiveness Analysis Results

Discussion

The comprehensive statistical evaluation undertaken in this study aimed to assess the efficacy of non-invasive aneuploidy screening in high-risk pregnancies, laying the groundwork for its advancement in the realm of prenatal testing. The sensitivity and specificity, crucial indicators of a screening test's accuracy, were found to be 94.2% and 96.8%, respectively. These values denote the proportion of correctly identified positive cases and correctly identified negative cases, emphasizing the robust performance of the non-invasive screening method.

Our study's findings align with previous research, such as Jayashankar et al. which also reported high sensitivity and specificity for non-invasive prenatal testing (NIPT). These consistent results across studies highlight the reliability of NIPT in identifying fetal chromosomal abnormalities [31].

Swanson et al. (2013) highlighted the rapid evolution of non-invasive technologies in prenatal genetic testing, emphasizing the challenging landscape for women in choosing the most suitable test option. The current study contributes to this advancement by providing contemporary data on the effectiveness of NIPT in high-risk pregnancies. Zhang et al. specifically focused on twin pregnancies, reporting a sensitivity of 100% and specificity of 99.50% for detecting trisomy 21 through NIPT. This emphasizes the versatility of NIPT across different pregnancy scenarios [32].

The positive predictive value (PPV) and negative predictive value (NPV) further contribute to the nuanced understanding of the screening outcomes. With PPV at 89.5% and NPV at 97.3%, these metrics showcase the reliability of the non-invasive screening method in accurately predicting the presence or absence of aneuploidies [33]. A high NPV is particularly reassuring, as it signifies the low likelihood of a false negative result, crucial for instilling confidence in the screening process.

The NPV of 97.3% aligns with the findings of other studies, like Illumina's comparison of NIPT with traditional aneuploidy screening methods, which emphasizes the low likelihood of false negatives, instilling confidence in the screening process [1].

While the NPV is consistently high, the PPV of 89.5% suggests a potential variation compared to other studies. It's crucial to explore the reasons behind this difference, considering factors such as study population, testing protocols, and the prevalence of aneuploidies in the screened population.

Additional research, like the work by Mokhtar et al. comparing NIPT with amniocentesis, can provide a broader perspective on PPV and NPV, offering insights into the method's performance across diverse populations and clinical scenarios [33].

Comparative analyses using McNemar's test revealed statistically significant concordance between non-invasive screening and traditional procedures, affirming the reliability of the former. The concordance rate, represented by Cohen's Kappa Coefficient at 94.7%, indicates substantial agreement between the two methods. This finding is pivotal for clinicians and expectant parents, emphasizing that non-invasive screening can provide comparable results to invasive procedures without the associated risks.

The study's findings align with literature illustrating the agreement between different diagnostic methods. Krummenauer et al. emphasized the combination of McNemar's test and Cohen's kappa coefficient for comparing binary outcomes, showcasing the reliability of this approach across various contexts [34]. While the Cohen's Kappa Coefficient in this study is notably high, it's essential to recognize that Kappa values may vary based on the study population, methodology, and prevalence of the condition being studied. Comparative assessments, such as those outlined by other researchers, could provide additional context and insights into the variability of Kappa coefficients. The emphasis on the pivotal nature of these findings for clinicians and expectant parents resonates with the broader literature on prenatal screening. The consensus across studies supports the idea that non-invasive screening methods can provide reliable results, enabling informed decision-making while minimizing potential risks.

Regression analyses explored associations with participant characteristics, unveiling a significant association between advanced maternal age and a higher likelihood of positive screening results, aligning with existing literature [35]. This personalized risk assessment based on participant characteristics enhances the clinical utility of non-invasive screening.

The study's confirmation of advanced maternal age as a predictor of positive screening aligns with previous research, such as Wu et al. which demonstrated better performance of non-invasive prenatal testing (NIPT) for pregnancies with advanced maternal age in predicting trisomy 21 and trisomy 18 [36].

The emphasis on personalized risk assessment based on participant characteristics echoes the broader literature, emphasizing the clinical utility of tailoring screening approaches to individual risk factors. Carbone et al. and Wei et al. both discuss the importance of considering advanced maternal age as a key factor in assessing the risk of fetal aneuploidy [3537]. Cai et al. highlight the variability in NIPT results based on participant characteristics [38]. This underscores the need for a nuanced approach, considering factors beyond maternal age to achieve comprehensive risk assessment.

Limitation

This study's singular focus on advanced maternal age as a predictor for positive screening results may neglect other pertinent variables. Findings are limited to the specific study population, and the retrospective design introduces potential biases. The small sample size and potential impact of technological advancements suggest cautious interpretation. Ongoing research is crucial to refine our understanding of the diverse factors influencing prenatal screening outcomes.

Conclusion

The outcomes of this study support the efficacy and reliability of non-invasive aneuploidy screening in high-risk pregnancies. The meticulous consideration of sensitivity, specificity, PPV, NPV, and concordance rate, coupled with personalized risk assessment through regression analyses, underscores the potential of non-invasive screening as a valuable tool in prenatal care. These findings contribute significantly to the ongoing dialogue surrounding prenatal testing methods, emphasizing the importance of balancing accuracy, safety, and patient-specific considerations in the realm of high-risk pregnancies.

References

1. Benn P, Cuckle H, Pergament E. Non-invasive prenatal testing for aneuploidy: current status and future prospects. Ultrasound in Obstetrics & Gynecology 2013;42:15–33. https://doi.org/10 .1002/ uog.12513.
2. Beta J, Lesmes-Heredia C, Bedetti C, Akolekar R. Risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review of the literature. Minerva Obstetrics and Gynecology 2018;70. https://doi.org/10.23736/S0026-4784.17.04178-8.
3. Suzuki Y, Yamamoto T, Kojima K, Tanemura M, Tateyama H, Suzumori K. Evaluation Levels of Cytokines in Amniotic Fluid of Women with Intrauterine Infection in the Early Second Trimester. Fetal Diagn Ther 2006;21:45–50. https://doi.org/10.1159/000089047.
4. Iwarsson E, Jacobsson B, Dagerhamn J, Davidson T, Bernabé E, Heibert Arnlind M. Analysis of cell-free fetal <scp>DNA</scp> in maternal blood for detection of trisomy 21, 18 and 13 in a general pregnant population and in a high risk population – a systematic review and meta-analysis. Acta Obstet Gynecol Scand 2017;96:7–18. https://doi.org/10.1111/aogs.13047.
5. Gil MM, Quezada MS, Bregant B, Ferraro M, Nicolaides KH. Implementation of maternal blood cell-free <scp>DNA</scp> testing in early screening for aneuploidies. Ultrasound in Obstetrics & Gynecology 2013;42:34–40. https://doi.org/10.1002/uog.12504.
6. BUSTAMANTE-ARAGONES A, RODRIGUEZ DE ALBA M, GONZALEZ-GONZALEZ C, TRUJILLO-TIEBAS MJ, DIEGO-ALVAREZ D, VALLESPIN E, et al. Foetal sex determination in maternal blood from the seventh week of gestation and its role in diagnosing haemophilia in the foetuses of female carriers. Haemophilia 2008;14:593–8. https://doi.org/10.1111/j.1365-2516.2008.01670.x.
7. Scheffer PG, van der Schoot CE, Page-Christiaens GCML, Bossers B, van Erp F, de Haas M. Reliability of Fetal Sex Determination Using Maternal Plasma. Obstetrics & Gynecology 2010;115:117–26. https://doi.org/10.1097/AOG.0b013e3181c3c938.
8. Hui L. Non-invasive prenatal testing for fetal aneuploidy: charting the course from clinical validity to clinical utility. Ultrasound in Obstetrics & Gynecology 2013;41:2–6. https://doi.org/10.1002/uog.12360.
9. Ashoor G, Syngelaki A, Wagner M, Birdir C, Nicolaides KH. Chromosome-selective sequencing of maternal plasma cell–free DNA for first-trimester detection of trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012;206:322.e1-322.e5. https://doi.org/10.1016/j.ajog.2012.01.029.
10. Chitty LS, Hill M, White H, Wright D, Morris S. Noninvasive prenatal testing for aneuploidy–ready for prime time? Am J Obstet Gynecol 2012;206:269–75. https://doi.org/10.1016/j.ajog.2012.02.021.
11. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004;191:45–67. https://doi.org/10.1016/j.ajog.2004.03.090.
12. Mazloom AR, Džakula Ž, Oeth P, Wang H, Jensen T, Tynan J, et al. Noninvasive prenatal detection of sex chromosomal aneuploidies by sequencing circulating cell-free DNA from maternal plasma. Prenat Diagn 2013;33:591–7. https://doi.org/10.1002/pd.4127.
13. [Chiu RWK, Akolekar R, Zheng YWL, Leung TY, Sun H, Chan KCA, et al. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ 2011;342:c7401–c7401. https://doi.org/10.1136/bmj.c7401.

14. 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. Genetics in Medicine 2011;13:913–20. https://doi.org/10.1097/GIM.0b013e3182368a0e.
15. Verweij EJ, van den Oever JME, de Boer MA, Boon EMJ, Oepkes D. Diagnostic Accuracy of Noninvasive Detection of Fetal Trisomy 21 in Maternal Blood: A Systematic Review. Fetal Diagn Ther 2012;31:81–6. https://doi.org/10.1159/000333060.
16. Dan S, Wang W, Ren J, Li Y, Hu H, Xu Z, et al. Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11 105 pregnancies with mixed risk factors. Prenat Diagn 2012;32:1225–32. https://doi.org/10.1002/pd.4002.
17. Lo YMD, Tsui NBY, Chiu RWK, Lau TK, Leung TN, Heung MMS, et al. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. Nat Med 2007;13:218–23. https://doi.org/10.1038/nm1530.
18. Fan HC, Blumenfeld YJ, El-Sayed YY, Chueh J, Quake SR. Microfluidic digital PCR enables rapid prenatal diagnosis of fetal aneuploidy. Am J Obstet Gynecol 2009;200:543.e1-543.e7. https://doi.org/10.1016/j.ajog.2009.03.002.
19. Evans MI, Wright DA, Pergament E, Cuckle HS, Nicolaides KH. Digital PCR for Noninvasive Detection of Aneuploidy: Power Analysis Equations for Feasibility. Fetal Diagn Ther 2012;31:244–7. https://doi.org/10.1159/000337544.
20. Lo YMD, Lun FMF, Chan KCA, Tsui NBY, Chong KC, Lau TK, et al. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. Proceedings of the National Academy of Sciences 2007;104:13116–21. https://doi.org/10.1073/pnas.0705765104.
21. Chitty LS, Kistler J, Akolekar R, Liddle S, Nicolaides K, Levett L. Multiplex ligation-dependent probe amplification (MLPA): a reliable alternative for fetal chromosome analysis? The Journal of Maternal-Fetal & Neonatal Medicine 2012;25:1383–6.https://doi.o rg/10.3109/1 4767058.2011.636093.
22. Cuckle H, Benn P, Pergament E. Maternal cfDNA screening for Down syndrome – a cost sensitivity analysis. Prenat Diagn 2013;33:636–42. https://doi.org/10.1002/pd.4157.
23. Nicolaides KH, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. Prenat Diagn 2013;33:575–9. https://doi.org/10 .1002/pd.4103.
24. Pandya PP, Kondylios A, Hilbert L, Snijders RJM, Nicolaides KH. Chromosomal defects and outcome in 1015 fetuses with increased nuchal translucency. Ultrasound in Obstetrics & Gynecology 1995;5:15–9. https://doi.org/10.1046/j.1469-0705.1995.05010015.x.
25. Souka AP, Snijders RJM, Novakov A, Soares W, Nicolaides KH. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10–14 weeks of gestation. Ultrasound in Obstetrics & Gynecology 1998;11:391–400. https://doi.org/10.1046/j.1469-0705.1998.11060391.x.
26. Kagan KO, Avgidou K, Molina FS, Gajewska K, Nicolaides KH. Relation Between Increased Fetal Nuchal Translucency Thickness and Chromosomal Defects. Obstetrics & Gynecology 2006;107:6–10. https://doi.org/10.1097/01.AOG.0000191301.63871.c6.
27. Alamillo CML, Krantz D, Evans M, Fiddler M, Pergament E. Nearly a third of abnormalities found after first-trimester screening are different than expected:10-year experience from a single center. Prenat Diagn 2013;33:251–6. https://doi.org/10.1002/pd.4054.
28. Gruchy N, Decamp M, Richard N, Jeanne-Pasquier C, Benoist G, Mittre H, et al. Array CGH analysis in high-risk pregnancies: comparing DNA from cultured cells and cell-free fetal DNA. Prenat Diagn 2012;32:383–8. https://doi.org/10.1002/pd.2861.
29. Hillman SC, Mcmullan DJ, Williams D, Maher ER, Kilby MD. Microarray comparative genomic hybridization in prenatal diagnosis: a review. Ultrasound in Obstetrics & Gynecology 2012;40:385–91. https://doi.org/10.1002/uog.11180.

30. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, et al. Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. New England Journal of Medicine 2012;367:2175–84. https://doi.org/10.1056/NEJMoa1203382.
31. 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;13:2570. https://doi.org/10.3390/diagnostics13152570.
32. Zhang H, Gao Y, Jiang F, Fu M, Yuan Y, Guo Y, et al. Non-invasive prenatal testing for trisomies 21, 18 and 13: clinical experience from 146 958 pregnancies. Ultrasound in Obstetrics & Gynecology 2015;45:530–8. https://doi.org/10.1002/uog.14792.
33. Mokhtar R, Hans P, Sinha A. Comparing Non-invasive Prenatal Testing With Invasive Testing for the Detection of Trisomy 21. Cureus 2022. https://doi.org/10.7759/cureus.31252.
34. Krummenauer F, Kalden P, Kreitner KF. [Cohen's kappa or McNemar's test? A comparison of binary repeated measurements]. Rofo 1999;171:226–31.
35. Wei L, Zhang J, Shi N, Luo C, Bo L, Lu X, et al. Association of maternal risk factors with fetal aneuploidy and the accuracy of prenatal aneuploidy screening: a correlation analysis based on 12,186 karyotype reports. BMC Pregnancy Childbirth 2023;23:136. https://doi.org/10.1186 /s12 884-023-05461-4.
36. Wu X, Li Y, Xie X, Su L, Cai M, Lin N, et al. Clinical Review of Noninvasive Prenatal Testing. The Journal of Molecular Diagnostics 2020;22:1469–75. https://doi.org/10.1016/j.jmoldx .2020.09.008.
37. Carbone L, Cariati F, Sarno L, Conforti A, Bagnulo F, Strina I, et al. Non-Invasive Prenatal Testing: Current Perspectives and Future Challenges. Genes (Basel) 2020;12:15. https://doi.org/10.3390/genes12010015.
38. Cai M, Lin N, Chen X, Li Y, Lin M, Fu X, et al. Non-invasive prenatal testing for the diagnosis of congenital abnormalities: Insights from a large multicenter study in southern China. Brazilian Journal of Medical and Biological Research 2023;56. https://doi.org/10.1590/1414-431x2023e12506.