The effectiveness of non-invasive prenatal test technology and the prenatal screening algorithm based on various methods for determining foetal aneuploidy

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Abstract

Objective: The purpose was to evaluate the effectiveness of a non-invasive prenatal test (NIPT) using mass parallel sequencing (MPS) to detect trisomy 13, 18, 21 and fetal sex chromosome abnormalities in maternal blood samples by isolating freely circulating foetal extracellular DNA (eDNA), and to develop an algorithm for prenatal screening.

Material and Methods: The research methods used included blood sampling from patients, isolation of eDNA, determination of DNA concentration and quality, library preparation for sequencing, MPS using an Illumina HiSeq2000, positive and negative control samples, monitoring, and analysis of results using the distributed algorithms platform based on calculations of z-value and the average absolute deviation. Pregnant women were divided into two groups based on gestational age at sampling, group 1; 9-14 weeks and group 2; 15-27 weeks.

Results: A total of 377 pregnant women were included with a mean (range) age of 33 (23-44) years. The mean gestational age at the time of blood sampling in group 1 was 11 (9-14) weeks, and in group 2 was 21 (15-27) weeks. In the first group, three cases of trisomy 18 chromosomes were detected in patients aged 43 years old, and female children were subsequently born with Edwards syndrome. In the second group, one case of trisomy 21 was detected in a patient aged 36 years and the pregnancy was terminated at 25 weeks.

Conclusion: The analysis of freely circulating foetal eDNA was a sensitive method for detecting chromosomal abnormalities. The study has a practical significance, since the NIPT for frequent aneuploidy considerably exceeds the effectiveness of traditional screening methods and allows identifying chromosomal disorders starting from the 9th week of the gestation period. (J Turk Ger Gynecol Assoc 2023; 24: 152-8)

Keywords: Pregnancy, chromosomal abnormalities, non-invasive prenatal test, extracellular deoxyribonucleic acid, mass parallel sequencing

Received: 27 January, 2023 **Accepted:** 30 June, 2023

Introduction

Chromosomal abnormalities in the form of aneuploidy on chromosomes 21 and 18 are more common than other pathologies, and accordingly, more often lead to perinatal mortality and disability in highly developed countries. The outcome of pregnancy with such a pathology is a miscarriage, premature birth, or the birth of a child with the corresponding syndrome. It was found that about 60% of cases with

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DOI: 10.4274/jtgga.galenos.2023.2022-10-4

aneuploidy end in miscarriage (1). When identifying patients at risk for chromosomal abnormalities in the first trimester of pregnancy, invasive interventions are recommended, which can lead to complications, such as fetal death, miscarriage, bleeding, and chorioamnionitis. Therefore, some pregnant women refuse invasive procedures, and in some cases, they are contraindicated. Therefore, it is essential to assess the condition of the fetus and diagnose aneuploidy at the initial stages of pregnancy. It is also important to consider the relationship/heredity of the parents, for example the degree of consanguinity, if any.

Today, there are increasingly more likely to be women over 40 years old who are planning a pregnancy. This age poses a higher risk of developing aneuploidy in the fetus. To screen pregnant women for chromosomal abnormalities, ultrasound examination may be performed, and biochemical markers may be measured. Biochemical screening is based on the determination of pregnancy associated plasma protein A (PAPP-A) and the free β-unit of human chorionic gonadotropin (β-hCG) using the maximum number of probability ratios (population differences, weight, ethnicity of the mother, smoking, presence of diabetes mellitus, multiple pregnancy, the use of assisted reproductive technologies). For example, to screen for trisomy of chromosome 21, the clinical characteristics of the mother and the foetal thickness of the collar space, measured by ultrasound, are evaluated. In addition, maternal blood biomarkers, including β-hCG and PAPP-A at 11-13.6 weeks of gestation, are considered. Using this approach, the rate of false positive results is reported to be 5.0% (2).

There is curently a non-invasive screening method for determining chromosomal abnormalities, known as the non-invasive prenatal test (NIPT). This method is based on the analysis of free genomic DNA of foetal origin from a blood sample taken from the pregnant woman, available from the 10^{th} week of gestation (3). This method has become more widely known as a result of many studies over the past 15 years, which have confirmed its utility in practice. Thus, NIPT has been widely adopted in clinical medicine in patients at high and medium risk of carrying a foetus with a chromosomal abnormality. However, it can be used for screening and risk determination in all pregnant women, regardless of age before the final diagnosis, as an independent method or in addition to the methods available. At the same time, the use of NIPT raises certain ethical questions (4). Despite its non-invasiveness, this technology is aimed at detecting chromosomal aneuploidy in the fetus, the treatment of which is currently impossible. Therefore, NIPT shares many of the ethical problems inherent in prenatal diagnosis in general, since the only way to prevent the birth of a sick child is to terminate the pregnancy. The proposed technology is potentially intended to replace the existing biochemical screening. Given the higher cost of this study, it is necessary to carry out special measures to ensure the availability of the proposed screening of foetal extracellular DNA (eDNA) for all social groups.

The risk of having a child with a chromosomal anomaly is 5% even in perfectly healthy young parents. Therefore, it is important to identify possible disorders, including aneuploidy, causing hereditary syndromes in early pregnancy. Conducting a combined screening study allows identifying pregnant risk groups. However, the most precise test is the NIPT, which allows detecting aneuploidy in the fetus, including trisomy, monosomy, numerical anomalies of sex chromosomes. NIPT detects chromosomal abnormalities with high reliability since it is based on a special signal processing algorithm. This allows comparing and detecting differences between maternal DNA present in plasma/leucoma and fetal DNA found only in plasma. Based on this test, the accuracy of the study reaches up to 99%. Thanks to this test, it is possible to exclude the presence of such diseases as Down syndrome, Edwards, Patau, Turner, etc. in the unborn child. A sample of 15 mL of venous blood of the expectant mother is sufficient for conducting NIPT to determine the risk of aneuploidy in the fetus. This avoids the use of invasive methods of prenatal diagnosis, leading to possible complications of pregnancy.

The total sensitivity of genomic DNA screening is 100.00%, with this indicator varying from 88.43 to 100%. The overall effectiveness of the test for the analysis of aneuploidy in chromosomes 21, 18, 13, X and Y, defined as the proportion of true results among all studies conducted was 98.97% (5). Thus, NIPT has been shown to be one of the most effective methods for detecting chromosomal abnormalities in the fetus from early pregnancy, which should be recommended to all women in the form of a universal prenatal test. First, the NIPT avoids invasive manipulations due to its high sensitivity to the exclusion of an elevated risk of fetal aneuploidy and in the case of complications of pregnancy (miscarriage, age risk, extragenital pathology).

The purpose of this study was to evaluate the effectiveness of NIPT for detecting trisomy 21, 18, 13 and fetal sex chromosome abnormalities in maternal blood samples and to develop an algorithm for prenatal screening based on the use of various methods for determining fetal aneuploidy.

Material and Methods

Blood sampling was carried out in 232 patients aged 23-44 years with a singleton pregnancy in the period from 9 to 14 weeks and in a further 145 pregnant women of the same age in the period from 15 to 27 weeks of gestation at the University Medical Centre, Astana, Republic of Kazakhstan. Blood sampling was carried out in specific tubes for eDNA analysis

(Streck Innovations), which were sent to the laboratory of LifeCodexx AG, Konstanz, Germany, by cold chain. Subject information, sent with the samples included patient's code, patient's age, pregnancy period, obstetric history, ultrasound results, biochemical screening, and other data.

NIPT was performed, based on mass parallel sequencing (MPS), according to the previously described method (6). The NIPT included the following stages: isolation of eDNA and determination of its concentration and quality; preparation of libraries for sequencing; conducting the MPS using "Illumina HiSeq2000", using positive and negative control samples; monitoring and analysis of the results obtained using the distributed algorithms platform algorithm based on calculations of the z-value and the average absolute deviation (median absolute deviation).

The study was approved by UMC University Medical Center National Ethics Commission of the Ministry of Health of the Republic of Kazakhstan (approval number: 1033-A, date: 25.06.2022). The authors informed the participants about the anonymous and voluntary participation, and the participants provided their consent.

Results

The mean (range) age of pregnant women included in this study was 33 (23-44) years, and the mean (range) gestational age at the time of blood sampling in group 1 was 11 (9-14) weeks, and in group 2 was 21 (15-27) weeks. The results of fetal DNA analysis in a blood sample were obtained for a total of 377 pregnant women, 232 in group 1 and 145 in group 2. In group 1, three cases of trisomy 18 were detected in patients all aged 43 years, pregnancy was continued at the request of the patients, and three female children were born with Edwards syndrome. In group 2, one case of trisomy 21 was detected in a patient, aged 36 years, and the pregnancy was terminated at 25 weeks. Women in this study underwent NIPT as they had an increased risk of carrying a foetus with chromosomal abnormality, either because of older age during pregnancy or because of the results of biochemical screening (β-hCG and PAPP-A). Based on the generally accepted examination scheme, these pregnant women would have undergone an invasive prenatal diagnosis. The average estimated risk of trisomy 21, 18, or 13 according to combined screening in the examined pregnant women was 1:23,512 (range; 1:160-1:46945). In the present study of fetal eDNA using single nucleotide polymorphism (SNP), three cases of trisomy 18 and one case of trisomy 21 were identified, which is an incidence of 1:94.25 cases but these women were pre-selected for being at high risk. The sex of the fetus in the cases considered was identified correctly. Five cases with an increased risk of trisomy 18 in 8 patients in group 1 and five possible cases of trisomy 21 in 39 patients in group 2 were not confirmed, since cytogenetic examination of amniotic fluid cells revealed no abnormalities in the chromosome set of the foetus. Out of 377 pregnancies the following pregnancy outcomes occurred: in one case (out of four), with revealed foetal chromosomal pathology and confirmed prenatal karyotyping, the pregnancy was terminated and all remaining 376 cases the pregnancy ended in childbirth. At birth, three newborns were confirmed to have Edwards syndrome from three patients aged 43 years.

Thus, the sensitivity of NIPT for Edwards syndrome in this study was 98.6%, with a false positive level of 1.4%. Due to the insufficient number of blood samples with trisomy 21, sensitivity for this pathology was not calculated. The results of this study demonstrated that NIPT for frequent aneuploidy considerably exceeds the effectiveness of traditional screening methods. The elevated risk of trisomy 18 is present in older reproductive-age mothers. Based on the results of ultrasound and biochemical analyses, a decision was made to perform invasive manipulations to determine the karyotype of the foetus. In the present study, 373 pregnant women were spared invasive prenatal diagnosis and the associated risk of complications for the mother and foetus. Although in most countries aneuploidy screening essentially focuses on screening for trisomy 21, invasive manipulations in the group with positive screening results lead to the detection of many other clinically significant aneuploidies. Analysis of free foetal eDNA allowed the avoidance of invasive interventions, complications, and the risk of abortion. NIPT is actively used as a second test after combined screening, if the results of ultrasound and biochemical studies raise suspicion of aneuploidy. Low-risk pregnant women were also included in this study. Therefore, NIPT can be used as a universal method for detecting chromosomal abnormalities in the fetus.

Discussion

In recent decades, there has been an unprecedented steady increase in the prevalence of both congenital malformations, the frequency of which ranges from 2.7% to 16.3% in different populations, and hereditary diseases themselves (monogenic and chromosomal), the total proportion of which is 1.5% (7). According to the European Registry of Congenital Anomalies, 5000 children with developmental defects and chromosomal aberrations are born in Europe every year (8). In the causes of infant death, congenital malformations are the second most common cause (19.1%). Although the infant mortality rate has shown a steady downward trend in recent years (7.9% in 2013, 7.0% in 2014, 5.7% in 2015 and 4.8% in 2016), the improvement of prenatal diagnostic methods and the introduction of modern perinatal technologies are two of the major factors reducing infant mortality in the long term (9). Screening programmes

will play a crucial role in preventing the birth of children with developmental abnormalities as these allow identifying a high-risk group for the occurrence of chromosomal aberrations, followed by invasive procedures in this group to determine the karyotype of the fetus and optimal pregnancy management tactics to prevent the birth of children with severe disabling diseases.

Clinical studies of the use of NIPT, conducted in the period 2014-2016, confirm the high incidence of trisomy 21, 18, and 13, namely 99.7%, 98.2%, and 99%, respectively, but false positive data were 0.13% (10). Based on these results, the world medicine approved the introduction of NIPT technology into clinical practice. This method is acceptable, since it is non-invasive, allowing the detection of the most frequent types of trisomy, that is trisomy 21, 18 and 13, quickly and with high sensitivity. NIPT defines a high-risk group for the development of chromosomal abnormalities. However, the fetus may have a normal karyotype (11).

Several criteria have been proposed for the use of NIPT. Firstly, NIPT is used as a first-line screening test before ultrasound examination in the first trimester of pregnancy. Secondly, it is used with ultrasound, determining the free eDNA of the fetus at 11-13+6 weeks of pregnancy. Then, based on the results obtained, high, medium, and low-risk groups may be identified (12). However, it is advisable to implement the first provision only if the cost of NIPT is considerably reduced. Currently, pregnant women who have entered the high-risk group prefer this test as a safer method compared to an invasive one. From an economic standpoint, the NIPT will reduce the budget of the regional health system to $726975.72. The use of NIPT for first-line testing is more appropriate from the standpoint of detecting cases of chromosomal abnormalities in early pregnancy, in contrast to the current prenatal screening in the first and second trimesters of pregnancy. The costs associated with the use of NIPT are lower as a result of eliminating the need for invasive interventions and iatrogenic pregnancy losses.

The use of NIPT with other methods, including ultrasound and blood sampling, will reveal chromosomal abnormalities and prevent unwanted abortions of pregnancies (13). It has been reported that 35.24% of obstetrician-gynaecologists believe that NIPT should be used as a universal screening test for all pregnant women, while 40.95% of physicians state that free fetal eDNA should be determined in all patients who fall into the medium risk group (over 1:1000) (14). However 21.90% of experts favor excluding its use in pregnant women of average risk, if anatomical structural abnormalities in the development of the fetus are detected during ultrasound, since they are subject to invasive interventions to determine the karyotype of the fetus. Only 1.91% of doctors are against any use of NIPT in clinical practice (14).

Currently, the majority of medical professionals believe that NIPT can be used as a universal method for screening of aneuploidy, based on the analysis of free fetal eDNA in the mother's blood, and also report that NIPT is the most sensitive and promising among methods being researched for prenatal screeing (15). Therefore, these professionals suggest that NIPT should be incorporated into the existing clinical practice.

Screening programs used during pregnancy should be aimed primarily at substantial reduction of the frequency of births of children with severe disabling or fatal diseases, and reducing infant and perinatal mortality rates (16). However, not all patients trust the screening methods used. Combined screening of the first trimester of gestation allows clinicians only to identify high-risk pregnant women. Pregnant women who are subsequently diagnosed with a normal karyotype in the fetus or newborn may be considered at high risk of aneuploidy in the foetus (17,18). Of note, the results of biochemical and ultrasound tests are only used as primary methods of identifying the at-risk group for chromosomal abnormalities. To exclude aneuploidy in the foetus, invasive interventions are necessary to determine the karyotype of the fetus, which can lead to the development of complications for both the mother and the foetus (19,20). Thus, many patients refuse invasive interventions and the outcome of pregnancy is the birth of a sick child. It was found that, in general, about 50% of pregnant women with an elevated risk of trisomy 21 in the fetus refuse invasive manipulation (21-24). However, this diagnostic method may have false positive results.

NIPT gives high positive predictive value and negative predictive value for trisomy 21, 18, and 13, but false negative results can also be obtained. It should be noted that NIPT is dependent on the available concentration of free foetal DNA in the mother's blood sample. A fetal DNA content of less than 4-5% is considered too low to obtain a high-quality test, and the concentration should be at least 10-11%. If the proportion of foetal DNA fraction is low, the test should not be repeated. In these cases, an invasive diagnosis is recommended. Screening by NIPT will be affected by the phenomenon of mosaicism, when fractions of both normal and abnormal fetal DNA are found in the mother's blood sample (25-27). This is because foetal eDNA obtained from the mother's blood originates from the cytotrophoblast (28,29). Therefore, it is recommended to confirm the positive result of NIPT by conducting an invasive prenatal diagnosis. In this case, amniocentesis is the preferred method of diagnosis since the amniotic fluid contains cells of the foetus itself.

The use of amniocentesis to verify a positive result of NIPT is recommended by the European Society of Human Genetics and the American Society for Human Genetic Information (30-33). Amniocentesis is performed only after 15 weeks of pregnancy.

This means a long waiting time for the final result for expectant parents, whereas NIPT can be performed starting from nine weeks of pregnancy. Thus, studies have been conducted to evaluate the effectiveness of chorionic villus biopsy (CVS) as an invasive method of confirming the NIPT result. CVS allows for cytogenetic diagnostics of the fetus in the first trimester, at 11-12 weeks. However, due to the phenomenon of confined placental mosaicism, the result of the study may be inconclusive, which requires a secondary invasive intervention (34,35).

Study Limitations

There are limitations of NIPT which include chromosomal pathologies in parents; the presence of balanced rearrangements; disappearing twin syndrome as DNA of the deceased fetus can circulate in the mother's blood; the presence of mosaicism; multiple pregnancy (more than two foetuses); malignant neoplasms in the mother; or if there is a maternal history of organ transplantation or blood transfusion. In these cases, invasive diagnostic methods are recommended (36,37).

Thus, at present the high value of NIPT seems evident regarding screening for the most common foetal abnormalities: Down, Edwards, and Patau syndromes and aneuploidy of sex chromosomes (38-40). The outcomes of pregnancy when trisomy 21 or 18 is present is miscarriage, or the premature or term birth of a child with the corresponding syndrome, causing perinatal mortality and disability in highly developed countries (41). The selection of patients at risk for chromosomal abnormalities among all pregnant women is an indication for invasive diagnostic methods that can lead to fetal death, miscarriage, bleeding, or chorioamnionitis. In addition, in some cases, performing invasive manipulations is contraindicated.

This study found that NIPT is an effective screening method for studying chromosomal abnormalities in the fetus from early pregnancy. We suggest that NIPT should be recommended to all women in the form of a universal prenatal test. This technology is now part of everyday clinical practice and use of NIPT has promise in the field of prenatal medicine. First, the high sensitivity of the NIPT to the most common chromosomal abnormalities in the fetus was determined, which meant unnecessary invasive manipulations were avoided and it was usable in cases of complications of pregnancy, such as high risk of miscarriage, older age mothers and extragenital pathology.

Conclusion

This study showed that the analysis of freely circulating fetal eDNA in the mother's blood using targeted sequencing of SNPs on chromosomes 13, 18, 21, X, and Y and the use of the Next-generation Aneuploidy Testing Using SNPs algorithm was a sensitive method for detecting autosomal aneuploidy, sex chromosome abnormalities and triploidy in the foetus. This technology can be recommended during pregnancy as effective prenatal screening, especially in high-risk pregancies. Then, it is recommended to confirm chromosomal abnormalities in the foetus.

NIPT technology demonstrates good sensitivity for identifying pregnancies with a high probability of developing one of these conditions and is usable as early as the 9th week of pregnancy. In this role, this test is more reliable compared to the use of combined screening. Using NIPT may avoid the need for unjustified invasive procedures. Considering the positive experience of introducing the innovative NIPT method into clinical practice, the authors of this study recommend including it in the algorithm of prenatal screening for determining fetal aneuploidy, which will reduce the birth rate of children with chromosomal abnormalities.

Ethics Committee Approval: *All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by UMC University Medical Center National Ethics Commission of the Ministry of Health of the Republic of Kazakhstan (approval number: 1033-A, date: 25.06.2022).*

Informed Consent: *The authors informed the participants about the anonymous and voluntary participation, and the participants provided their consent.*

Peer-review: *Externally peer-reviewed.*

Author Contributions: *Surgical and Medical Practices: M.T.; Concept: Z.K.; Design: L.S.; Data Collection or Processing: M.T.; Analysis or Interpretation: Z.R.; Literature Search: V.B.; Writing: Z.K.*

Conflict of Interest: *No conflict of interest is declared by the authors.*

Financial Disclosure: *The authors declared that this study received no financial support.*

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