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Noninvasive prenatal Diagnosis (NIPD) Techniques of Down syndrome: A Review

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Abstract

A Down syndrome foetus is born with an additional copy of chromosome 21. (Trisomy 21). Down syndrome can cause intellectual disabilities, poor muscle tone, and unusual facial features in newborns.50% of patients having Down syndrome get heart problems. The condition, which is the most common hereditary cause of intellectual disability, is more common in children whose parents were 35 years old or older when they became pregnant. The most prevalent genetic basis for developmental abnormality and the main reason why women elect to get invasive prenatal diagnostics is Down's syndrome (DS) which affects 1 in 691 live babies. New non-invasive diagnostics, however, have long been sought after because intrusive testing entails a 1% risk of miscarriage. Trisomy 21 (DS), which accounts for 53% of all confirmed chromosomal abnormalities, is the most frequent aneuploidy that is compatible with living and significantly contributes to mental impairment. During pregnancy, Down syndrome could be detected non-invasively. Since its introduction in the 1970s, testing procedures, recommendations, and screening alternatives have grown to encompass things like anatomical ultrasonography, maternal serum testing, and noninvasive prenatal testing. This article offers a summary of all currently available non-invasive techniques for diagnosing Down syndrome. One of the elements in the advancement of the noninvasive prenatal aneuploidies diagnosis has been cited as the discrepancy between maternal entire blood and foetal (placental) DNA.

The advancement of next-generation sequence (NGS) technology has rendered non-invasive prenatal diagnosis (NIPD) the most promising method currently available. The lack of thorough large-scale validation trials in low-risk pregnancies prevents the clinical implementation of NIPD for DS identification at this time. Prenatal screening is currently the first-line technique for detecting foetal aneuploidy. Even because DS can't be diagnosed with screening, creating a more thorough screening approach can help to increase detection rates and consequently lower the number of women that undergo invasive treatments. This research first examined the progress in developing new screening markers before detailing how well the prenatal screening process has changed that since the adoption of maternal age as the primary "screening" test. Fetuses with Down syndrome are more likely to exhibit certain sonographic traits known as "soft indicators" for the disorder. Soft Down syndrome indications are typically found during second-trimester ultrasounds. These symptoms don't necessarily mean that a baby has Down syndrome. Small morphological changes, most frequently trisomy 21, that raise the probability that the foetus has a chromosomal issue are known as soft indicators (Down syndrome).

DNA methylation is one of the potential diagnostic markers for trisomy 21 and other aneuploidies. It has been discussed how effectively the methylation DNA immunoprecipitation method works for enriching overly methylated foetal DNA. Differentially Methylated Regions (DMRs) have been identified as a result of research on the methylation differences between the

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foetus and the mother. Patients with trisomy 21 who are normal may have their condition appropriately detected using the fetal-specific methylation ratio approach. Maternal plasma may contain foetal DNA, which may be used for non-invasive prenatal diagnosis. Due to the minimal number of fetal-origin nucleic acids present, Any method used to isolate or detect foetal genetic information in maternal plasma would face significant technical difficulties.

This review article provides an overview of the state-of-the-art in the domain of noninvasive prenatal analysis of foetuses with Down syndrome as well as an outlook on new foetal markers and cutting-edge molecular methods that may one day be employed in the medical setting as reliable and secure options for women who choose noninvasive precise prenatal diagnosis. With the right medical attention and family support, children with Down syndrome can enjoy happy and fulfilling lives.

Keywords: *Cell-free fetal DNA; Down syndrome; noninvasive prenatal diagnosis; trisomy 21, sonographic markers*

INTRODUCTION

Since Lejeune et al. first described Down syndrome in 1959, it has been about 50 years. The most comprehensive risk assessment is to be given to women using the least invasive procedure possible, despite the fact that prenatal Down syndrome screening technology has evolved significantly [1]. Human chromosomes (2n=46) are split into two categories using International System for Human Cytogenetic Nomenclature (ISCN) as a guide. These include the 44 non-sex chromosomes, sometimes known as autosomes, and the two sex chromosomes (X, Y). The latter group's chromosomes are numbered from 1 to 22 based on their progressively smaller sizes. Autosomes are composed of two homologous chromosomes in somatic cells. Down syndrome was thought to be caused by chromosome 21, the second-smallest chromosome in the sufferer's karyotype and the subject of three studies (DS). Later research found that the smallest chromosome in DS is trisomic. The two smallest chromosomes (chromosomes 21 and 22) were swapped from their original positions to minimise confusion between earlier and subsequent publications, which resulted in the identification of DS as trisomy 21. Chromosome 21 was sequenced in 2000 [2].

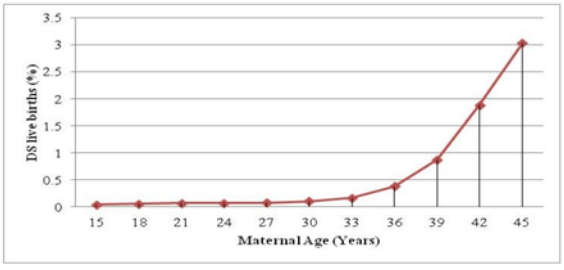

Figure 1: The predicted risk of DS based on the age of the mother (adapted from [3).

The most prevalent condition that stops children from developing normally on a physical and mental level is known as Down syndrome (DS). The most prevalent chromosomal aneuploidy as well as the main genetic contributor to developmental delay is Down syndrome (DS). However, there is a strong relationship between foetal trisomy risk and maternal age, with risk increasing gradually until age 33 and then rapidly thereafter (Figure 1). The down-syndrome risk is anticipated based on the mother's age. Women in their late 40s give birth to a live child about one in every 32 times [3]. According to current research, the percentage of women who fell Pregnancy after reaching 35 increased from 9% to 20% between 1989 and 2008, It caused a 71% rise in the incidence of pregnancies with Down syndrome. In Britain and Wales, the projected rate declined from 736 newborns in the year 1989 to 750 newborn babies in 2008 [5], a 1% decrease, despite an increase in the total number of live births of individuals with Down syndrome of almost 1.32 times. The National Down's Syndrome Cytogenetic Register (NDSCR) in the UK estimates that a 48% increase in live births of children with Down syndrome would have resulted from the constant rise in maternal age between 1989 and 2008. In western civilizations, the average age of mothers giving birth is rising continuously. From 12.5 per 10,000 in 1981–1985 to 21.7 per 10,000 (73.6%) in Europe and from 11.6 to 19.2 per 10,000 (65.5%) in the US, the predicted rate of Down syndrome (DS) deliveries in Western countries is steadily increasing [4]. This increase is largely attributable to older pregnant women.

Despite the apparent ethical problems surrounding prenatal diagnosis and the fact that most women terminate affected or premature pregnancies, The provided research convincingly shows the value of keeping an eye out for Down syndrome. Since 43% of Down syndrome (DS) abortions occur between 7 and 10 weeks, preceding genetic history and gestational age are additional risk factors in addition to the advanced maternal age. [6]. Since the early 1980s, new biochemical and sonographic indicators have continuously developed, increasing the sensitivity of contemporary screening techniques to above 95% [6]. In order to determine the percentage of newborn infants having chromosomal abnormalities who endured live births, irrespective of whether they would ordinarily have been identified in early infancy, cytogenetic studies of all newborns were carried out in the 1960s and 1970s [7]. Not all infants with chromosomal abnormalities are identified before they are born alive under routine medical care. In children with sex chromosomal anomalies, questions may not become relevant until later in life, if at all [8]. Children who appear healthy at birth remain unkaryotyped, and marker chromosomes are known to have a wide range of phenotypic differences [9].

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As a result, from the prevalence of cytogenetic surveys, the diagnosed incidence among babies cannot be determined. On the other hand, the introduction of prenatal screening has enhanced the diagnosis of chromosomal abnormalities that result in phenotypically normal infants as well as premature diagnosis of some infants with genetic defects who wouldn't survive to live births [9]. Currently, trisomy 21 is identified during pregnancy via cytogenetic or DNA analysis, which calls for the amniotic fluid, chorionic collection, or cordocentesis gathering of foetal genetic material. The aforementioned operations carry a significant risk of pregnancy death (1%) and are intrusive by nature, which makes them risky [7]. Therefore, the creation of Non-Invasive Prenatal Diagnostic (NIPD) methods is necessary. Alternative therapies for delaying the onset of NIPD have centred on the identification of liberated foetal DNA (ffDNA) in the mother during pregnancy. Cell-free DNA (cfDNA) from maternal plasma was used in two studies that were published in 2008 to demonstrate non-invasive prenatal care (NIPT) for trisomy 21.

The test was shown to be able to lower the frequency of pointless invasive operations and immune-mediated foetal loss in both trials. In the detection and treatment of pregnancies with chromosomal abnormalities, the test will remain to be a crucial first-line diagnostic, despite substantial recent breakthroughs in non-invasive prenatal diagnostics using next-generation sequencing (NGS) [10]. This study will examine how screening has changed over the past forty years and up to the present, as well as potential novel screening techniques that might be used ina therapeutic setting.

DEFINITIONS

Metrics that may be used to evaluate the effectiveness of a screening procedure include detection accuracy (DR), rates of false positives (FPR), screening positive predictive value (SPR), and the possibility of a satisfactory outcome (OAPR). The test's DR represents the proportion of afflicted cases that the testing programme successfully discovered (sensitivity). For example, a DR of 90% means that 9 out of 10 incidences of DS will be properly detected by the screening test. However, high sensitivity alone is insufficient for DS detection. Additionally, the FPR of the test—the percentage of positive results in unaffected cases—must be as low as possible. In reality, the SPR has indeed been utilised to replace the FPR more recently. The screen-positive rate identifies people whose test results are higher than the drop risk, including 1 in 150 [5]. The FPR/SPR must be maintained as low as is practical in order to decrease the number of women prescribed invasive procedures and, as a result, the number of successful pregnancies that miscarry. The OAPR measures the likelihood that a woman with a large screen risk will experience a DS pregnancy that is identified by CVS or amniocentesis. For every miscarriage caused by intrusive testing, more affected pregnancies will be successfully diagnosed when the OAPR of the test is high [11]. The risk threshold at which invasive screening is made available affects both the DR and the FPR/SPR. In a perfect screening test, the DR must be strong (>90%) and the SPR should be low (2%). The DR and SPR would, however, decrease if the threshold were raised (for instance, to 1 in 100), and they would both rise if it were lowered (for instance, to 1 in 300) [5].

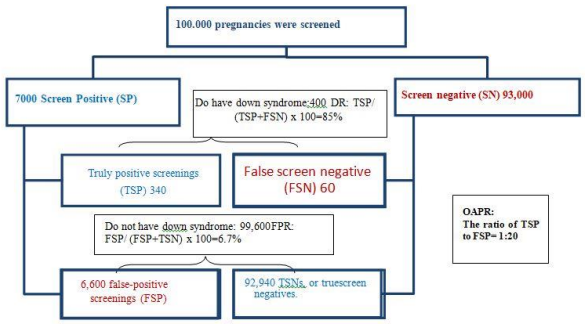

Figure 2: The screening procedure, potential results, and accuracy metrics [11]

The potential outcomes, screening process, and accuracy metrics are displayed in Figure 2. Detection rate: The proportion of instances in which the screening test correctly identified the condition
(TSP + FSN) / TSP = 85%.

The percentage of cases when the screening test incorrectly reported a positive result while not having the ailment is known as the false positive rate (FPR):
FPR = 6.7% of (FSP + TSN).

The likelihood of being affected by an OAPR (screen-positive outcome) is as follows:

True screen positives outnumber false screen positives by a factor of 1:20.

HISTORICAL OVERVIEW

The first malformation complex that could be categorised as a chromosomal defect was found to be DS in 1959. DS is still one of the genetic ID diseases with the lowest level of understanding despite having been studied for 155 years [12]. Beginning in the 1980s, prenatal diagnostics for high-risk populations were introduced. From the beginning of the 1980s, all women 35 years of age and older had access to invasive diagnostic testing, and maternal age was practically the only technique of screening for the diagnosis of DS [13]. However, this approach turned out to be ineffective and unsustainable for a number of reasons. First off, maternal age alone isn't a valid tool for screening because it has a DR of less than 35%, resulting in many women having unaffected pregnancies undergoing unnecessary invasive tests and the majority of DS-affected kids being undetected [5].

To increase the sensitivity of DS screening, sonographic and biochemical screening techniques that may be utilised in conjunction with maternal age to determine risk more precisely were developed. When numerous studies discovered a link between low levels of alpha-fetoprotein (AFP) in maternal blood (approximately a 25% reduction) and foetal aneuploidy in 1984, the first window of opportunity to improve screening appeared [14]. According to Gillespie (2000), AFP, a significant serum glycoprotein produced by

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the yolk sac and the foetal liver, functions similarly to albumin in adults. DiMaio et al. calculated that 25–30% of pregnancies where the foetus has DS will be detected with the AFP blood biomarker alone using a risk cut-off at which 5% of women under the age of 35 are offered invasive testing [15]. The link between low AFP levels and an increased risk of DS was only identified in this group. Initially, rising AFP levels were used to spot pregnancies affected by anencephaly and another foetal neural tube defects for the post-first trimester screening of DS, AFP is currently one of the biochemical blood indicators used in the triple test and is clinically used globally.

Since then, several maternal serum DS pregnancy-related markers have been studied. The screening process makes use of key markers. To eliminate trophoblast cells from the cervical canal, one can aspirate, use a cytobrush or cotton wool swab, rinse the endocervical cavity, or lavage the uterus. Early techniques for prenatal sex prediction that utilised endocervical materialsobtained by mucus aspiration or cytobrush had higher success rates [16].

The syncytiotrophoblast initially produces the hCG hormone after the embryo. A recent study looked into the possibility that foetal cells taken from the distal endocervical canal during the first trimester (as early as 5 weeks) may have the genetic material for NIPD of trisomy 21 [17]. Five out of five trisomy 21 pregnancies were successfully detected using the hybridization of foetal cells with chromosome 21-specific probes and analysed with an automated fluorescence microscope [18].

Examples

Its goal is to encourage the creation of progesterone, which supports the corpus luteum's upkeep [19]. Up until 12 weeks of gestation, hCG levels rise rapidly early in pregnancy before levelling off. Normal hCG levels during the second-trimester range from 4,060 to 165,400 IU/L.

A year after Bogart et al. linked a spike in blood levels of hCG and DS pregnancies (about double the typical values) in 1987 [20], the second-trimester double test was introduced in the UK.

This test assessed the maternal age and the levels of AFP and hCG in the mother's blood between 15 and 20 weeks of gestation.

With an SPR of 5% and a risk threshold of 1 in 250, the DR was almost 60% [5]. Shortly after the double test was made permissible in the UK, studies showed a 25% drop in unconjugated estriol in DS pregnancies (the typical value at 15 weeks of gestation is approximately 4 nmol/L) [21]. Estriol was added as a third marker, leading to the creation of the "Triple test" [22]. The triple test was altered in the early 1990s by substituting f-hCG for hCG because the free beta component of hCG (f-hCG), which is more obviously enhanced in DS pregnancies, is more substantially increased in DS pregnancies [23].

The SPR was not reduced, screening expenses increased, and even though the triple test had a higher sensitivity (67% DR), it was not considered to be a substantial improvement over the double test [24]. But in the early 1990s, it was discovered that inhibin A levels in DS pregnancies were considerably greater, which led to the development of the triple test, which has a better DR of 75% [25]. Although they can only be utilised during the second trimester, the DR for the double, triple, and quadruple tests are all higher than those for maternal age alone.

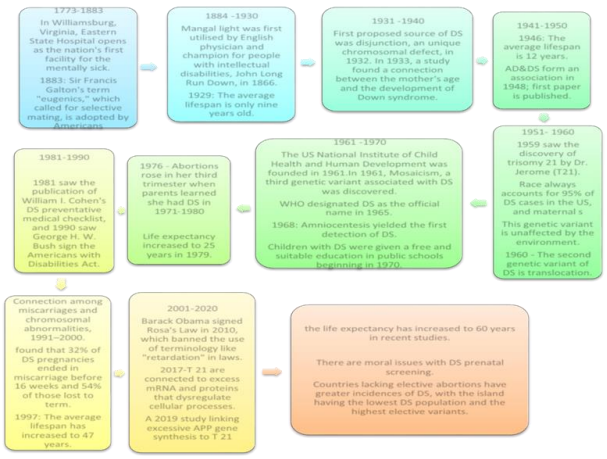

Figure 3: Shows the Timeline of Down Syndrome [28],

PAPP-A, a maternal serum-related plasma protein that was found to be decreased by around 50 per cent of the total in DS pregnancies in 1991, was also found to be detectable as early as eight weeks gestation [26]. The maternal blood PAPP-A values in DS-affected foetuses returned to those seen in unaffected pregnancies around 17 and 19 weeks of gestation [27]. The purpose of screening during the first trimester was to allow parents enough time to decide whether to consent to early, intrusive testing.

The 1990s were spent working toward this objective. In addition to these biochemical markers, sonographic imaging can be utilised to find physical signs to determine the risk of DS pregnancies. Between 11 and 13 weeks into the first trimester, a sonographic characteristic known as nuchal translucency (NT) can be seen behind the foetal neck. The ultrasonic screening test for NT was created in 1992 by Nicolaides et al. [29]. The foetal lymphatic maturation often occurs later in the second trimester in infants with Down syndrome and other chromosomal abnormalities, leading to an increase in fluid buildup.

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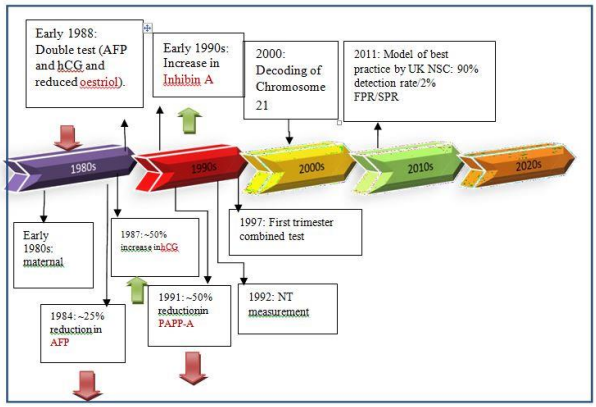

Figure 4: Timeline of the major UK DS screening developments

Studies from the early 1990s suggested a connection between DS and rising NT.

In a study including 1,273 pregnancies, Nicolaides et al. reported in 1994 that an NT value of 2.5 mm was discovered in 84% of DS foetuses and 4.5% of euploid foetuses [28].

The callipers must be precisely placed when assessing the NT thickness because a 0.4 mm inaccuracy could drastically change the risk.

According to estimates, the chances of producing a DS foetus are 1 in 1,394 and 1 in 563, respectively, when, for instance, NT measurements of 2.6 mm and 3.0 mm at 12 weeks of gestation are recorded [30-31].

The very first-trimester combination test was used to assess NT, maternal age, and early observable blood indicators (f-hCG and PAPP-A) [32]. According to studies, the first-trimester combination test has a 5% FPR and can catch 85–90% of instances of DS [32]. As demonstrated in Figure 4, significant advancements in DS screening have been made since the early 1980s and have been applied in a therapeutic environment. DS screening in the UK has seen significant modifications since the early 1980s, when maternal age was practically the sole approach used for screening, to the UK National Screening Committee's certification of the Model of Best Practice (MoBP) (Fig 4).

TYPES OF TRISOMY 21

Standard trisomy 21: The individual with this abnormality has 47 chromosomes, of which three are chromosomes 21, total. About 90% of DS cases are caused by it.

Robertsonian translocation: An unbalanced translocation of chromosome 21 to an acrocentric, satellite-bearing chromosome that might be heterologous or homologous is known as a Robertsonian translocation. The fusion products that are created may have a dormant centromere and be monocentric or dicentric. Chromosomes with monocentric translocations may have a hybrid centromere that derives from both partners or their respective centromeres.

Duplications: An aberrant crossing-over brought on by an uneven coupling of homologous pairs in the pachytene of meiosis might result in a duplicate in the cells of the carrier. The likelihood of duplication may be increased in the large version of a paternal chromosome 21 with a paracentric inversion.

Reciprocal translocations: created by a change in the euchromatin of many autosomes or gonosomes, which leads to swapping of the euchromatic's region of chromosome 21. Small duplications and reductions on chromosome 21 are the root cause of a wide range of diseases. Many times, carriers lack DS-like traits in their phenotypic makeup.

Mosaicism: Typically, mosaics form during the conceptus' initial postzygotic development as a result of abnormal mitosis. They may be the result of trisomy 21 in which a small percentage ofcells contain an abnormal zygote that lacked one of the three chromosomes. The zygote can still have a typical karyotype in this case, but postzygotic mitosis won't allow chromosome 21 to join the other chromosomes.

Tetrasomy 21: A specific type of hyperdiploid 21 is chromosome 21 tetrasomy, in which the chromosome is expressed four times. This anomaly may contain two healthy chromosomes and an additional isochromosome, or it may have four free chromosomes 21. Early in pregnancy, the condition frequently results in the death of the foetus.

SCREENING METHODS

Although it has been known for more than a century that foetal cells can appear in the mother's blood during pregnancy, it is now impossible to establish a diagnostic approach based oncounting these cells in the mother's plasma due to their rarity. Whether they are doing so for cytogenetic study using enriching or isolating these uncommon foetal cells is a substantial problem for researchers in this field [33]. According to multiple studies, moms who are carrying aneuploid babies have up to six times the typical amount of foetal cells in their blood [34]. Since the beginning of 1933, significant improvements in DS screening have been made, although work is still needed. All women who desire early pregnancy risk assessment should have access to a first-trimester ultrasound NT measurement and blood indicator combination. The ISPD admits that it is insufficient to evaluate the risk of foetal DS in pregnant women based solely on maternal age.

The ISPD advises women to do the triple test if they first seek maternity care after the first trimester (during 13 weeks and 6 days) of pregnancy [35]. Only the second trimester's quadruple testing, which has a higher FPR (6.9%) and a significantly reduced sensitivity (75% DR) than the first-trimester combo test, is available to women who forego first-trimester screening [5]. The two-stage integrated test is a second examination that certain hospitals offer [36]. The second-trimester biochemistry (quad test) is given after the combination test [35]. Due to the possibility possible that a mother who may have been at high risk after the combined test might be relatively safe as a consequence of the integration test's results, this test is utilised to assist in lowering the FPR. Integrated testing is not advised by the UK NSC for two main reasons. Emergency screening, which

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enables pregnant women with a noticeably significant risk during first-trimester screening to obtain invasive diagnostic therapies straight away, is one potential solution to this issue.

To identify 60–80% of kids with Down syndrome, current screening methods have the disadvantage of requiring invasive testing from 5% or more of tested mothers, which results in a high proportion of false-positive outcomes. Here, we examine novel screening approaches with the potential to improve the sensitivity of existing screening approaches (to >90% DR) and lowerthe FPR/SPR (to 2%), making it possible to identify more cases of DS and offer less invasive testing, which will reduce the rate of miscarriages in both influenced and unconcerned pregnancies. A pregnancy is deemed "high risk" as of 2007, according to the American Congress of Obstetricians and Gynecologists, if there is a family history of aneuploidy, the mother is older,there are abnormal blood test results, or there are abnormal ultrasound findings (ACOG, Bulletin No 77 and 88). The only diagnostic procedures offered for Down syndrome during pregnancy are amniocentesis and chorionic villus sampling, despite the fact that there are already a number of screening methods available. The risks, benefits, and drawbacks of all currently available prenatal screening methods for the finding of Down syndrome are compared in this review report(37).

SONOGRAPHIC MARKERS OF DOWN SYNDROME (TRISOMY 21)

Ultrasound is a critical component of the aneuploidy screening procedure. The importance of sonographic (ultrasound) indicators in the risk detection of Down syndrome has been majorly researched both at the time of the foetal anomaly scan in the middle of the third trimester and at the 11–14-week scan. During the 11–14 week ultrasound, abnormalities connected to the structure are found (exomphalos, cystic hygroma, etc.), the existence or lack of nostril bone, tricuspid regurgitation, and reversal flow in the ductus venosus, in addition to less obvious ones.

At the mid-trimester scan, anomalies that are structural in nature (congenital cardiovascular disease, anterior abdominal defects, ventriculomegaly, etc.) and a little less visible (choroid cysts, echogenic foci in the heart, expanded nuchal fold, etc.) are once more categorised as indicators. Ultrasonography can detect both large structural abnormalities and subtle "soft markers" in foetuses with aneuploidies. People with Down syndrome may have abnormalities of the digestive, musculoskeletal, cardiovascular, central nervous, craniofacial, and urinary tract systems. Septal defects, tetralogy of Fallot, heart atrioventricular canal abnormalities, and duodenal atresia are examples of major structural anomalies. These abnormalities are sometimes missed by prenatal ultrasound screening.

It is well established that aneuploidy detected during a mid- or first-trimester scan is linked to structural defects. For instance, there is a significant link between DS and Fallot's tetralogy or foetal exomphalos. Amniocentesis or CVS should be suggested if structural abnormalities are seen during 11–14 week scan or second–trimester scanning. An atrioventricular septal defect is a mid-trimester structural abnormality (AVSD). The prevalence of AVSD is 1 per 10,000 live births in pregnancy with a healthy foetal karyotype, but it considerably rises to 2 per 10,000 live births in pregnancy with DS (1 in 5 incidences) [38]. Nuchal translucency (NT) can be assessed during the first trimester.

NT displays the subcutaneous fluid-filled space between the skin and the foetal neck's back. With a 64–70% detection rate, elevated NT readings are linked to an increased risk of aneuploidies, including Down syndrome. Nuchal cystic hygroma, or pathologic nuchal oedema, is associated with aneuploidy, such as Down syndrome, in around 50% of cases. A nasal bonecan be discovered in 62–70% of Down syndrome foetuses in the first trimester, although only 1% of normal infants have one [1]. However, the interracial variation in this marker is significant. In African tribes, the prevalence of missing nasal bones in euploid infants during the late first trimester is over 10%, compared to 1-3% in Caucasian civilizations [5]. Numerous studies have shown that although 1 or more "soft indicators" can be identified in 50% or more of cases, severe structural abnormalities are seen in less than 25% of affected foetuses [39].

Additional helpful markers include tricuspid valve and ductus venosus Doppler flow measurements. When these markers are included in a first-trimester combination diagnostic method, the DR can increase to 93-96% with an FPR of 2.5% [40]. The soft markers that are most frequently discovered in the second trimester are echogenic intracardiac spots, pyelectasis, low femur lengths, choroid cysts, echogenic bowel, and ventriculomegaly.

The probability ratios for the first four illnesses are normally around 2, but in cases of the echogenic intestine, thicker nuchal fold, and ventriculomegaly, they are all more than 5. Even in isolated cases, extra genetic counselling, aneuploidy screening, or diagnostic testing is warranted due to the prognostic relevance of echogenic bowel, thickening nuchal fold, and ventriculomegaly. If aneuploidy screening hasn't already been performed. Numerous non-obvious signs increase the chance of Down syndrome and could suggest diagnostic testing. The most effective second-trimester ultrasonography sign for Down syndrome, with a likelihood ratio of 11 to 18, is an expanded nuchal skin fold, which also carries the highest risk of aneuploidy when observed alone.

Finding these unique signs takes a lot of time and effort, thus they haven't been incorporated into standard medical practice for universal screening. They might, however, be used in a contingent screening strategy, where women who, according to joint screening, have an intermediate risk and want more information before deciding whether to undertake intrusive testing, are given access to them [32]. Ultrasonography shouldn't be used to diagnose or exclude Down syndrome on its own. When ultrasound results are evaluated in conjunction with plasma analyte diagnostic tests, such as integration and progressive testing, first and second-trimester testing, and others, the sensitivity for diagnosing Down syndrome is increased. 99% of the time, Down syndromecan be detected

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using cell-free DNA. Diagnostic tests, such as chorionic villus sampling or amniocentesis, ought to be made available when screening test findings are favourable. More intense debate has surrounded the importance of the discovery of soft markers during the mid-trimester exam. The exceedingly low sensitivity and specificity of these soft markers for DS were confirmed in a 2001 analysis of their significance. The frequency of screening in the first and second trimesters during the past ten years is one factor contributing to the decline in the importance of soft markers. When the ailment being screened for is common, screening tests perform better.

The efficiency of testing with soft indicators has significantly decreased as efficient DS testing around 20 weeks has spread. The National Panel in the UK suggested in 2009 that the initial prior risk for DS not be changed in reaction to the presence or lack of a single soft indicator or a collection of soft markers because of the converging of these factors. (dilated cisterna magna, choroid plexus cysts, echogenic cardiac foci, and a 2-vessel chord).

ADVANCE RESEARCH: NEW SERUM BIOMARKERS

New avenues for NIPD were made possible by Lo et al (1997). first experimental proof that the mother plasma and blood of women giving birth to male children contain cffDNA. It has been proposed that cffDNA could be a useful biomarker to assess the placenta's health throughout pregnancy because of the connection between hypoxia and an increased release of DNA [6]. Thorough research has been done on new biochemical screening indicators to improve already-developed DRs and FPRs/SPRs [41]. In order to find new potential ultrasonic markers. Many improvements have been achieved in prenatal screening since the identification of cell-free fetal DNA (cffDNA) in maternal circulation. According to recent research looking at the proteomic profile of maternal blood [41]. The European Union (EU) developed the SAFE (Special Non-Invasive Advances in Fetal and Neonatal Evaluation) NoE (Network of Excellence) in 2004 with the goal of implementing regular, cheap NIPD and newborn screening by establishing long-term alliances internationally [42].

The programme made a substantial contribution to the standardisation of RhD genotyping and sought to identify a range of novel, more revealing biomarkers for foetal DS detection. New biochemical indicators are being researched right now. There are two ways to identify trisomy 21based on the use of cffDNA in maternal circulation.

The first methodology, known as the relative chromosomal dosage (RCD) method, compares the quantities of a reference DNA sequence derived from chromosome 21 and a DNA sequence derived from separate chromosomes in cffDNA. The RCD of chromosome 21 is 2:2 in a healthy pregnancy and is predicted to be 3:2 in trisomy 21. The allele ratio (AR) approach, sometimes referred to as the second method, measures the allelic proportion of single-nucleotide polymorphisms (SNPs) present in an embryo nucleic acid marker. The AR in cffDNA is projected to be 1:1 in a full-term pregnancy if the fetus is heterozygous for a specific gene sequence, while the AR of chromosome 21 would've been 2:1 in a trisomy 21 situation. The main limitation of this procedure is that it can only be applied to foetuses heterozygous for the detected SNP. Numerous studies have published results in recent years regarding potential novel indicators (both epigenetic and non-epigenetic), that may be used to boost the sensitivity of current screening systems. Since they are both essentially nonexistent by 10 weeks of gestation, PlGF and ADAM12 identification must occur earlier than that. Nevertheless, screening for DS atthis point in the pregnancy would be excellent. However, it has been found that adding PlGF to the combination test can assist to raise the DR by 4-7% [43] if early screening is available.

An illustration of an epigenetic marker is the phosphodiesterase gene [43]. Finding previously undiscovered aneuploidy biomarkers might be accomplished using a mix of bioinformatics and proteomic methods, according to the SAFE project [43]. Proteomics and bioinformatics would be used to provide a helpful tool for prenatal screening of Down syndrome as well as a method for the identification of additional birth abnormalities or diseases associated with pregnancy.

EPIGENETIC MARKERS IN Fast DOWN SYNDROME DETECTION

The term "epigenetics" refers to the molecular processes that regulate gene expression without modifying the Sequence of DNA or content. DNA methylation, which includes attaching a methyl group to a DNA sequence's cytosine residues, is the most studied epigenetic process and has an inhibitory influence on gene production when it happens in the promoters of genes. Other aneuploidies include epigenetic markers for cffDNA, Further research has proved the value of the allelic ratio for placentally produced hypomethylated SERPINB5 molecules in maternal plasma in the non-invasive identification of trisomy 18 [44]. In order to create a comparable technique for the NIPD of Down syndrome, there is now a great deal of interest in finding differentially methylated DNA patterns on chromosome 21 between the placenta and maternal blood cells. Such epigenetic markers may be helpful when compared directly to a DNA methylation marker obtained from a placenta on a reference chromosome or when the epigenetic allelic ratios are analysed [44].

DNA Methylation

The most well-known epigenetic change presently being researched for potential prenatal diagnosis of DS is the difference in DNA methylation between the mother and baby [45]. DNA methylation causes histone deacetylase activity to be recruited, which results in a restrictive chromatin structure. Gene-rich areas of the human genome replicate early during the S phase, dwell in open chromatin fibres, and located outside of their chromosomal domain in the interphase nucleus, according to recent genomic investigations utilizing microarrays with BAC-sized probes. Therefore, there is substantial DNA methylation in the same

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areas that have these euchromatic characteristics. This seeming paradox suggests that early replication timing, euchromatic fibre architecture, or nuclear localization are unaffected by the local restrictive chromatin structure controlled by DNA methylation. When seen in this light, cytosine methylation appears to be an epigenetic marker that only locally inhibits access to DNA and is not always associated with heterochromatic structures [46]. There are some restrictions on the current ffDNA-based NIPD techniques. The two main methods under investigation are the use ofsodium bisulfite transformation to enable the difference of differential methylation among maternal and foetal DNA, and the application of methylation-sensitive limitation enzymes to eliminate hypomethylated maternal DNA in order to directly analyse free fatal DNA (ffDNA) by polymerase chain reaction (PCR).

The need that sections of DNA with varying levels of methylation to have a constraint site for identification by methylation-sensitive restriction enzymes12 restricts the number of regions that can be examined [46]. However, employing sodium bisulfite conversions along with epigenetic alteration PCR, methylation-sensitive single nucleotide prime expansion, and/or bisulfite sequencing has two key drawbacks [10–13]. To accurately quantify the methylation status following bisulfite conversion, it is critical to get the whole transition of unmethylated cytosines to uracils, which is quite unique. Furthermore, it is more difficult to analyse and quantify very minute amounts of foetal DNA due to the degradation of DNA acquired after bisulfite treatment 15. We have circumvented the aforementioned limitations and enabled chromosome-wide identification of methylation patterns using a high throughput technique by combining a recently developed technique called methylated DNA immunoprecipitation (MeDiP) 16 with greater tiling oligonucleotide array analysis. In female whole blood and placental DNA, we carefully examined the methylation patterns of chromosomes 13, 18, 21, X, and Y. Since the uterus is the origin of ffDNA in human plasma, placental tissue was chosen for testing [8,9].

In this section, we discuss the development of novel foetal epigenetic molecular markers for the detection of the most common aneuploidies1, such as trisomy 13, 18, and 21 (associated with Patau, Edwards, and Down syndromes (DS-/Trisomy-21), respectively), as well as sex chromosome anomalies such as the XXY-Klinefelter, XYY, XXX, and X-Turner syndromes. We also discuss potential genetic targets with differentially methylated areas for non-invasive prenatal diagnostics [40]. Trisomy 21 can be detected noninvasively during pregnancy by combining a newly developed approach called MeDiP with real-time qPCR using maternal peripheral blood. After methylation enrichment of fetal-specific methylated chromosome 21 sites, the direct assessment of foetal DNA in maternal circulation forms the foundation of this strategy's main enablers [46]. To provide information on chromosomal dosage, the ffDNA must be hypermethylated in contrast to maternal DNA. A few epigenetically silenced genes could indicate that aberrant hypermethylation is either exceedingly selective or a rare, random occurrence.

SHORT TANDEM REPEAT–STR

Microsatellite regions, particularly short repetitive DNA sequences (Short Tandem Repeat- STR), have been thoroughly examined and extensively used, and this has led to the promotion of these markers as potential solutions as quick, precise, affordable, and easy genetic tools that could be used in DS detection. Short tandem repeats (STRs) are widely distributed, polymorphic loci with high mutation rates that are simple to type. The propensity for STR markers to be employed broadly in population, forensic, and medical genetics has clearly characterized them as molecular polymorphisms. According to some sources, STRs are short DNA sequences that are repeated several times at the specific locus and are typically base pairs in length. The number of repeats varies depending on the individual. The simplicity, speed, and ability to test several STR markers at once in so-called multiplex STR systems, which allows for a very high level of individualization in recognizing biological evidence, are what really make the use of these markers valuable [42]. It is crucial that bigger studies be carried out utilizing more modern equipment, such as liquid chromatography-mass spectrometers, which can identify more peptides in one study with high sensitivity because very few researchers have sought to find novel biomarkers for DS [43].

NEXT-GENERATION SEQUENCING (NGS) AND PCR

Genome-wide sequencing, also known as next-generation sequencing, has made it feasible to characterize whole genomes quickly and cheaply. DS and other aneuploidy diseases, including trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome), and monosomy X, are to be recognised using NIPD (Turner syndrome), following the discovery of cell-free fetal DNA (cffDNA) in maternal plasma [8]. Instead of identifying the risk of DS like screening does, NIPDenables a firm diagnosis. Currently, cffDNA is accessible for research purposes for several single gene illnesses such as sickle cell anaemia and has made it possible for effective NIPD to determine gender [38] and RhD condition [39]. Chromosome aneuploidy can be detected in maternal plasma by modern, advanced analytical techniques including digital PCR and massively parallel sequencing (MPS), which is sometimes referred to as next-generation sequencing (NGS) [16]. For the labour-intensive and Fetal aneuploidy allele-dependent non-invasive pregnancy testing (NIPT), several molecular methods have been developed [40]. Given that maternal serum only carries up to 10% cffDNA, a DS foetus would be required to be there for the diagnostic test to be capable of detecting a 5% change in plasma DNA concentrations for a region on chromosome 21. Better precision and accuracy are possible with microfluidic digital PCR because it does not depend on information gathered during the exponential PCR phase and because, unlike RT-PCR, It does not demand the use of an absolute quantification standard [11]. In 2007, Lo et al. discovered a technique using electronic PCR for the non-invasive diagnosis of DS. In comparison to the RNA-SNP

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technique, the RCD method has the benefit that it does not need polymorphisms for analysis; instead, it only compares copy number variation between chromosomes to identify over- or underrepresented alleles. However, DS was only able to be found in samples that have 25% fetal DNA [8].

Due to the high level of sensitivity reached (99% DR), given efficient prior-fetal enrichment, digital PCR is expected to replace current screening procedures. Although it is possible that digital PCR is less expensive than NGS-NIPT, large investigations to study are still required to determine the included here and costs of digital PCR. Prenatal screening has gained a lot of new potential with the introduction of non-invasive assays based on cffDNA in maternal circulation. The MPS technique has generated the strongest supporting data to date for a generally applicable test for cffDNA aneuploidy detection. This technique allows for the production of millions of quick labels that can be linked and precisely mapped to a standard genetic code, which is by necessity associated with a certain chromosome [41]. Using this method, the DR for foetal aneuploidy is determined by the depth of the sequencing and the subsequent counting data.

SHOTGUN SEQUENCING DNA

Fan et al. were the first to propose employing moderate parallelized shotgun sequencing (MPSS) equipment to count chromosomes. Simply dividing the entire number of sequence tags used in the sequencing run by the overall number of sequence tags on the targeted chromosome is what this method. However, it has been discovered that when using MPS, intra-run and inter-run variation can alter how the sequence reads are distributed across the chromosomes for every sample. MPS technology has been used to successfully treat foetal chromosomal aneuploidies. Shotgun sequencing, which detects all free DNA, is the opposite tailored approaches have been developed that only count certain sequences Multiplexed maternal plasma sequencing can detect and quantify millions of DNA fragments in biological samples in a matter of days, overcoming the challenge posed by the modest fraction of fetal DNA in maternal circulation [40]. In comparison to whole genome sequencing, targeted sequencing can multiplex more samples at once, making it more affordable (WGS). But this approach has the drawback of just allowing for the study of the targeted area. Smaller bench-top systems, such as the MiSeq and Ion Torrent, might be employed for speedier testing because to shorter sample preparation times and shorter run durations, but their throughput will be lower because of the lower number of single ends reads per run. A multiplex MPS test called "Digital Analysis of Selected Areas" (DANSR), created by Aria Diagnostics (41), sequences specific regions from target chromosomes. DANSR was utilized in a study by Sparks et al. to create the Fetal-fraction Optimized Risk of Trisomy Evaluation (FORTE), an algorithm that integrates the proportion of cffDNA in the samples with age-related hazards to provide a unique risk score for trisomy. Given that it is presently not cost-effective, the ISPD recommended that this NIPT be given to high-risk pregnancies and not given as the first test in MPS screening for all pregnancies [Benn, 2012]. It is essential to provide prenatal counselling to all women having MPS-based testing in order to explain the advantages and restrictions of the test.

REAL-TIME POLYMERASE CHAIN REACTION (PCR) TECHNOLOGY

The plasma of women bearing male babies might be shown to hold mRNA that was transcribed from the Y chromosome, as demonstrated by Poon et al. in 2000 [42]. Since then, a number of studies have shown that cell-free fetal m RNA (cffRNA), which is primarily of placental origin and circulates in the maternal plasma in a reasonably protected condition, may be useful in NIPDfor Down syndrome [9]. The use of innovative molecular methods like digital PCR, in which specific target molecules are amplified, may enhance the procedures for processing and extracting plasma RNA and boost diagnostic output. Other m RNA species produced from fetuses may also be found using this technique in maternal plasma. These methods' primary drawback is that they need counting a staggeringly high volume of molecules for markers that are not fetal-specific (random sequences from chromosome 21).

DIGITAL PCR

After using this technique to calculate the RNA-SNP allelic fraction for the non-invasive detection of foetal aneuploidy in microwell cells, Lo et al. used digital PCR to distinguish trisomy 21 maternal Samples of DNA from euploid ones [8]. Using the PLAC4 m RNA SNP method, researchers were able to differentiate four aneuploid human fetuses from nine normal ones.

The same research team's second trial involved comparing the dosage of a locus on chromosome 21 to a locus on reference chromosome 1 in an effort to identify foetal aneuploidy in illusionary combinations of euploid and aneuploidy DNA including as little as 25% trisomic material. Digital PCR is distinct in that it allows for the simultaneous execution of several PCRs,

The number of affirmative responses at the end of the amplification will then be counted to determine the input pictured number.

FUTURE DIRECTIONS

The creation of a noninvasive test that may identify aneuploidy and do away with the necessity for invasive procedures like chorionic villus collection or amniocentesis has long been an aim of prenatal genetic diagnosis. The first steps in this approach have been made possible by advancements in noninvasive prenatal diagnostics, however, it is still not currently possible.

While other aneuploidy disorders are outside the purview of this review. Studies to validate noninvasive prenatal diagnostics for the identification of multiple gestations and fetal aneuploidies in the general population are presently being conducted. Aside from the typical aneuploidies, additional chromosomal disorders including microdeletions and microduplications may also be included in noninvasive

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prenatal diagnostics in the future. Physicians must stay current on new technologies and have a thorough awareness of their dangers, advantages, and limits given the constantly growing diagnostic choices currently accessible to pregnant mothers. Doctors may provide their patients with the knowledge they need to make an informed decision about medical care by thoroughly comprehending each technology and the potential alternatives [43]. Target assays will be created, but it is important to keep in mind that next-generation sequencing will make it possible to quickly and accurately describe both balanced and unbalanced genomic disruptions, as well as breakpoints in the case of structural rearrangements.

CONCLUSION

Prenatal diagnosis will continue to be an actively explored field with the goal of creating a non-invasive genetic test for Down syndrome that would offer accurate genetic information without endangering the development of the fetus. Since its inception in the 1930s, testing methods, recommendations, and screening alternatives have grown to encompass things like anatomical ultrasonography, maternal serum screening, and non-invasive prenatal testing. This review attests to the advancements made in DS screening since the early 1980s when maternal age was the only "instrument" at hand. It also sheds light on how novel physical and biochemical indicators can be used in regular screening in the future to improve test sensitivity and reduce false-positive results. These biochemical indicators could assist in enhancing the present screening tests if more validation studies are carried out. It could indicate that these processes arenot synchronized, but in order to draw a clear conclusion, a more extensive analysis including non-CpG island promoters may be required.

The results show that STR markers offer a straightforward, quick, and affordable molecular method for the detection of DS. Mosaicism becomes less likely to be identified when using the molecular approach alone. As a result, it could first offer typical cytogenetic assays supplemental, but also significant additional confirmation. The combined application of these techniques would considerably improve the cytogenetic laboratories' ability to diagnose chromosomopathy. Through non-invasive methods like digital PCR and NGS, the primary goal is to deliver a conclusive diagnosis. Prior to even assessing replacing IPD with NIPD, excessively large studies and the results of low-risk populations are required to ensure that NGS test sensitivity is similar to current invasive testing, even though NGS test sensitivity currently provides DRS comparable to those provided by CVS. An overview of all presently used noninvasive techniques for the detection of Down syndrome is given in this article. The development of innovative sequencing methods using maternal plasma DNA will simultaneously lead to the creation of a universal diagnostic for foetal aneuploidy. This test will depend more on the enrichment and measurement of cffDNA in maternal peripheral blood than it would on the presence of certain genetic polymorphisms at specific loci. Whole genome MPS for fetal aneuploidy testing is currently rather expensive, hence focused techniques are being explored to reduce costs. Although it will probably show a drop in sensitivity, the detection of numerous new biomarkers might offer a less expensive screening option for NGS. New biomarkers, on the other hand, can only be used for screening, whereas MPS directly detects fetal DNA and offers an NIPD approach that could eventually replace present IPD methods. An interesting field of study that may soon become a clinical reality for all pregnancies is NIPD of fetal aneuploidy, which is made possible by the ongoing decrease in MPS prices. The development of laboratory procedures using bioinformatics algorithms that will enable their use with many samples is another significant aim of the continuing study. However, in order to introduce empirically verified procedures into the clinical practice of fetal medicine, it will be necessary to conduct extensive research to verify the diagnostic effectiveness of these approaches.

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