Review

Progressive trends in prenatal genetic screening

Kirolos Eskandar a,*

a Faculty of Medicine and Surgery, Helwan University, Egypt

ARTICLE INFO

Article history:
Received 22 July 2022
Received in revised form 14 August 2022
Accepted 21 August 2022

Keywords:
Non-invasive prenatal tests
Cell free fetal DNA
Chromosomal microarray
Chorionic villus sampling
Maternal plasma
Fetal nucleated red blood cells
Next generation sequencing

ABSTRACT

According to the global report on birth defects in 2021, it is estimated that 8 million children are born with birth defects of genetic origin annually. These birth defects vary in their degree of severity; where some types are mild and do not require treatment but others may necessitate lifelong medications or even cause instant death just after birth. That is why prenatal screening is doubtless necessary to detect such genetic defects before birth aiming to drop the tragedy of these children off.

Recently, this approach has been developing towards non-invasive techniques that reduce the risk of miscarriage, which was common in the old-fashioned invasive ones. Non-invasive Prenatal Tests (NIPTs) like Chromosomal Microarray Analysis (CMA) and cell-free fetal DNA (cffDNA) caused a breakthrough in the screening methods of chromosomal aneuploidies. Thanks to their benefits, NIPTs are considered a fundamental clinical approach for pregnant women’ screening in multiple countries.

Thence, this paper gives prominence to the recentness of NIPTs along with each’s assets, liabilities, and prospective recommendations. In addition, it would demonstrate the importance of modern molecular technologies like next-generation sequencing (NGS) which are enforced for the appliance of NIPTs.

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Tendencias progresivas en el cribado genético prenatal

INFO. ARTÍCULO

Historia del artículo:
Recibido 22 Julio 2022
Recibido en forma revisada 14 Agosto 2022
Aceptado 21 Agosto 2022

Palabras clave:
Pruebas prenatales no invasivas
Micromatrices cromosómicas
Muestreo de vellosidades coriáceas
Plasma materno
Glóbulos rojos fetales nucleados
Secuenciación de última generación

RESUMEN

Según el informe mundial sobre anomalías congénitas de 2021, se estima que anualmente nacen 8 millones de niños con anomalías congénitas de origen genético. Estos defectos de nacimiento varían en su grado de severidad; donde algunos tipos son leves y no requieren tratamiento, pero otros pueden necesitar medicamentos de por vida o incluso causar la muerte instantánea justo después del nacimiento. Por eso es sin duda necesario el cribado prenatal para detectar tales defectos genéticos antes del nacimiento con el fin de acabar con la tragedia de estos niños.

Recientemente, este enfoque se ha ido desarrollando hacia técnicas no invasivas que reducen el riesgo de aborto espontáneo, que era común en las antiguas invasivas. Las pruebas prenatales no invasivas (NIPT) como el análisis de micromatrices cromosómicas (CMA) y el ADN fetal libre de células (cfDNA) provocaron un gran avance en los métodos de detección de aneuploidías cromosómicas. Gracias a sus beneficios, las NIPT se consideran un enfoque clínico fundamental para la detección de mujeres embarazadas en múltiples países.

Por lo tanto, este documento destaca la actualidad de los NIPT junto con los activos, pasivos y recomendaciones prospectivas de cada uno. Además, demostraría la importancia de las tecnologías moleculares modernas, como la secuenciación de próxima generación (NGS), que se aplican para la aplicación de NIPT.

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1. INTRODUCTION

Since about 37 years ago, diagnosing pregnant women with an increased risk for Down syndrome has been one of the main objectives for the discovery of prenatal screening techniques. These approaches are used to integrate between maternal age, levels of certain markers in the maternal serum, and ultrasound outcomes in order to estimate a possible risk for Down (T21) and Edward (T18) syndromes [1]. In 2016, prenatal screening achieved an accuracy of 92% for Down syndrome and up to 93% for Edward’s depending on whether the screening is carried out in the first, second trimester, or even during both. Alongside, there have been emerging screening procedures for detecting autosomal recessive disorders like cystic fibrosis to identify pinpoint parents with a 25% risk of having an affected child with those disorders [1, 2].

On the ground floor of these screening techniques, Invasive prenatal screening tests were prevalent, where they require a needle to invade the cervix or the uterus of pregnant women, thus extracting amniotic fluid and placental tissues as in Amniocentesis and Chorionic Villus Sampling (CVS) respectively. Afterward, these samples have to be directed to labs to be tested for Rh incompatibility, genetic defects, and maternal infection. However, they increase the risk of miscarriage with a rate of one in every 100 women, the probability of mosaicism, and contamination with maternal cells. Thanks to the recent technological revolution, methods to single out structural and numerical chromosomal abnormalities along with point mutations is undergoing an unprecedented expeditious transformation. For example, Next generation sequencing (NGS) and chromosomal microarray analysis (CMA) which have the credit for discovering birth defects, intellectual disability, and discrete genomic disorders [2]. As a result, this has galvanized the development of wide-ranging carrier screens for a variety of genetic disorders and to establish cfDNA-based screens for fetal single-gene disorders and chromosomal aneuploidy [2].

2. HANDLING CHROMOSOMAL MICROARRAY ANALYSIS AS A PRENATAL DIAGNOSTIC TOOL

The primitive goal of prenatal genetic screening was chiefly to identify women at risk to have a child with Down syndrome –results from the addition of an extra chromosome 21- or what is known as trisomy 21. That is besides examining for Patau and Edwards syndromes which are trisomy 13 and trisomy 18 respectively. During that time, the two well-known diagnostic ways were CVS and amniocentesis which could be offered especially for those at
increased risk. Karyotyping, an analytical test for a set of chromosomes, has been the customary test for cultured cells extracted from amniotic fluid or placental tissues in amniocentesis and CVS correspondingly. A karyotype can detect structural abnormalities or chromosomal aneuploidy, which are only 5-10 megabases (Mb) in size. Moreover, it could be backed by fluorescence in situ hybridization (FISH) which has the capability to test for a few common aneuploidies if a hastened diagnosis is required [3]. Furthermore, FISH with locus-specific probes was also used in order to test for smaller structural chromosomal aneuploidies, but unfortunately, this method could only be applied for a few loci in a single assay.

This whole thing has been changed once chromosomal microarray analysis (CMA) has been invented. Where fluorescently-labeled DNA is being hybridized to slides that carry a millesimal of probes that spread across the genome. Accordingly, higher or lower fluorescence intensity from hybridized DNA towards specific probes categorizes regions that have extra or omitted copies of DNA respectively. In comparison with karyotyping, CMA has a much higher resolution spanning from the entire length of chromosomes to just multiple kilobases (Kb) or even a single exon [4, 5]. Additionally, it does not require cell culture, allowing results to be available at a faster pace. In addition, it is highly recommended for stillbirth samples. Nowadays, CMA is considered the pre-eminent genetic diagnostic test for adults and children anguish from genetic syndromes, several congenital anomalies, and developmental disabilities, where it has a diagnostic yield of about 17%. According to studies carried out by the National Institutes of Health, deep-rooted by other studies, CMA is demonstrated to detect clinically noteworthy and potentially significant copy number change in about 1.69% of pregnancies with pregnancies of normal karyotype and no apparent fetal anomalies [6]. Others found that it is a change of only 1% for significant copy number variations (CNVs).

Nonetheless, CMA is found to detect CNVs of indeterminate clinical significance, thus predisposing to later-onset disorders, which reach 1% up to 6% of cases when congenital anomalies are found in the fetus [7]. Although there were disadvantages to using CMA, most women decide to take this little risk of using CMA because of its high detection rate and specificity instead of CVS. On the other hand, developments in the cfDNA-based non-invasive screening of fetal aneuploidy or maternal plasma reversed this trend and cfDNA became widely known and desired by women in aim to avoid any potential risk in their pregnancies.

### 3. CELL-FREE FETAL DNA-BASED APPROACH

Prenatal testing has been developed unconventionally after implementing non-invasive procedures based on blood sampling. In 1959, it was found that intact fetal cells are present in maternal plasma, but 10 years later after more research, it is observed that this approach might have allegations for prenatal diagnosis. However, its main limitation is the presence of a low concentration of intact fetal cells in maternal circulation [8, 9]. Encountering cell-free fetal DNA (cfDNA) in maternal plasma, floated a new era of NIPT which has been integrated into daily clinical practice. It was established that a major fraction of cfDNA is being released in maternal circulation during trophoblast’s apoptosis in the placenta, which means that cfDNA is of placental origin and not isolated from circulating fetal cells. Moreover, cfDNA’s concentration is found to be 25 times more than that of fetal DNA excerpted from intact nucleated blood cells in a comparable volume of completely maternal blood. Additionally, this approach provides less labor-intensive and easier ways to interrelate with fetal DNA [10]. Cell-free fragments acquired from fetal DNA reach 150 base pairs in length which are relatively shorter than those of maternal cell-free DNA (cfDNA). Gestation of 11 to 13 weeks is enough period for fetal DNA fraction to range from 7.8 to 13% depending on the gestational age [11]. Therefore, starting from 11 weeks of gestation would be useful to obtain a useful result for an aneuploidy. Due to rapid clearance, cfDNA is no longer available after 24 hours of birth in most cases. It is worth mentioning that maternal body mass index is a crucial factor in determining cfDNA fraction, as there is an inverse relation between them. Studies have shown that the median fetal fraction decreased from 11.7% at 60kg to 3.9% at 160kg [12]. The reasonable explanation for this decrease is that in obese pregnant women, there is an increase in the level of maternal cfDNA emanating from active apoptosis and necrosis of adipose tissues. This means that obesity has a negative brunt on the capability of diagnosing genetic defects using NIPT, and thus it would be less likely to provide a gossipy result in obese women. Similarly, the levels of placental proteins like pregnancy-associated plasma protein A (PAPP-A), placental growth factor (PIGF), and free beta-human chorionic gonadotropin, are positively enforced with placental mass and fetal fraction. Besides, low PIGF and PAPP-A levels correlated with a high risk of an adverse pregnancy fallout. Therefore, a low fetal fraction might be a convenient guideline in detecting high-risk pregnancies [13, 14].

A new scope for the disclosure of trisomies and subchromosomal aberrations in the brand-new method of non-
invasive manner has been opened since NGS technology became available alongside cfDNA. At present, approaches are based on low-covering immensely parallel whole-genome sequencing analysis of maternal plasma DNA. Where the whole cfDNA is sequenced, human genome is referenced through the alignment of sequence reads, and these aligned reads are counted. That is how the corresponding exemplification of each chromosome can be calculated and thus chromosomal ploidy prominence could be determined [15]. Some studies have reported the usage of whole-genome sequencing plasma DNA for the uncovering of microdeletions and microduplications, which are generally known as sub-chromosomal copy, number variants. Because this procedure requires an exceptionally high number of byzantine interceptions and sequenced reads by cause of the apperception variants of unfamiliar clinical significance. In comparison to common aneuploidies, it is estimated that the whole-genome sequencing method has higher false-positive (79 – 100%) and lower positive predictive (0 – 21%) values for a set of selected microdeletion syndromes such as Prader–Willi/Angelman, 1p36 deletion, cri du chat/5p, and 22q11del/Di-George syndromes [16]. Accordingly, screening for microdeletions should not be dealt with as an accredited procedure to be used with patients until clinical authentication studies indicate the value for mainly low-risk patients.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>0.994 (95% CI) 0.983-0.998</td>
<td>0.999 (95% CI) 0.999-1.000</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>0.906 (95% CI) 0.823-0.958</td>
<td>1.00 (95% CI) 0.999-1.000</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>0.977 (95% CI) 0.952-0.989</td>
<td>0.999 (95% CI) 0.998-1.000</td>
</tr>
<tr>
<td>Rhesus D</td>
<td>0.993 (95% CI) 0.982-0.997</td>
<td>0.984 (95% CI) 0.964-0.993</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>0.929 (95% CI) 0.741-0.984</td>
<td>0.999 (95% CI) 0.995-0.999</td>
</tr>
<tr>
<td>Fetal sex</td>
<td>0.989 (95% CI) 0.980-0.994</td>
<td>0.996 (95% CI) 0.989-0.998</td>
</tr>
</tbody>
</table>

Adapted from Akolekar et al. [20].

Although this technology has been extensively applied to aneuploidy, there has been relatively little connotation in the clinical application to diagnose monogenic disorders. Where diagnosing monogenic disorders has been arduous because of the background maternal cfDNA which hinders the direct observation of maternally inherited alleles [17]. Notwithstanding, it is noticed recently that it is possible to diagnose prenatal monogenic disorders non-invasively by merging targeted haplotyping of two parents with the intended sequencing of cfDNA which is extracted during pregnancy. Even though cfDNA-based NIPT has a variety of reimbursements in daily clinical practice as mentioned before, there are still some drawbacks that could be beneficial to be mentioned. Several samples could not be yet construed with certainty, which is due to the nature of statistical testing. The chance for a healthy sample to accomplish a greater z-score of 1.86%, could be achieved if the typical cutoff verge is a z-score of 2.5 for the consistent conclusion of healthy samples. In addition, biological reasons like fetoplacental mosaicism, non-identical vanishing twins or maternal malignancy might subsidize improper predictions of fetal conditions [18, 19]. However, according to the percentages provided in (Table 1), NIPT has proven its highly precise method of spotting common fetal chromosomal aneuploidies. As stated by the American College of Medical Genetics and Genomics (ACMG), it is guaranteed that NIPT can take over the unadventurous screening pathways for Down, Patau, and Edward syndromes. As well, it is recommended to use NIPT under the supervision of the ACMG’s updated guidelines in order to guarantee high-quality prenatal care. In the case of abnormal results, NIPT should not be considered a diagnostic tool and ought to be confirmed by invasive tests. Also, it is recommended by the International Society of Ultrasound in Obstetrics and Gynecology to contemplate using ultrasound scans during the first trimester as it allows physicians to identify additional chromosomal and structural abnormalities that may not be conspicuous in blood tests [20].

Some research scientists and physicians in the field of prenatal diagnosis have mentioned that NIPT has negative consequences since it was presented in the medical field. For example, it led to fewer cases with serious deletion syndromes being detected, resulting in an amplified number of infants being born with relentless disabilities. Likewise, it has significantly galvanized miscarriage rates due to the reduction in practice opportunities of invasive procedures performed by clinicians which by the way, increased the risk for side effects like fetal loss and infections, as a result of decreasing the quality of invasive procedures. During the period from 2000 to 2014, systemic reviews of reported clinical studies have approved that miscarriage risks have been appraised to be 0.22% (95% CI −0.71 to 1.16%) for CVS and 0.11% (95% confidence interval [CI] −0.04 to 0.26%) for amniocentesis. Afterward, later reviews in the period of 2014 to 2017, revealed higher miscarriage risks of 0.35% (95% CI −0.31 to 1.00%) for CVS and 0.35% (95%
CI 0.07 to 0.63%) for amniocentesis. This way, retaining and training skilful clinicians would be a major challenge in the field of prenatal care [16, 21].

4. CELL-BASED NON-INVASIVE PRENATAL DIAGNOSIS

Lately, multiple studies highlighted a non-invasive method to analyse fetal cells extracted from maternal circulation thanks to the continuous advancements in the field of single-cell genomics which increase opportunities for prenatal screening [20, 21]. Attention was mainly drawn to trophoblasts and nucleated red blood cells (nRBCs). However, trophoblasts gained more consideration because of fetal nRBCs’ low concentration and lo-specificity markers. Where the importance of cell-based non-invasive prenatal diagnosis (cbNIPD) emerges from overcoming the limitations of cfDNA-based NIPT. In an attempt to limit fetoplacental mosaicism’s complications, a silicon-based nanostructured microfluidic platform (Cell Reveal™) has been invented to apprehend extra-villous cytotrophoblasts and fetal circulating nRBCs for cbNIPD [22]. Where this procedure requires a microfluidic apparatus surrounded by antibodies that have the capability to attach with the consistent antigens on targeted cells. Using array comparative genomic hybridization, short tandem repeat analysis, NGS, and fluorescence in situ hybridization as well, confirmed that the extracted nRBCs are of fetal and not a placental origin. In spite of the limitations of this cell-based approach, it has proven that there is a possible scientific way to extract compatible fetal DNA from maternal circulation, and thus it could be examined for copy number abnormalities of slightly one Mb by using low-coverage NGS, which is a higher resolution technology. Accordingly, the predictive negative and positive values for the detection of microdeletion syndromes would be much better compared to the cfDNA-based approach [23].

5. INFLUENTIAL BIOINFORMATICS AND EPIGENETICS IN NON-INVASIVE PRENATAL TESTS

The repetitive usage of NGS technology gives the whole world a chance of having huge genomic data, which could be stored, processed, and analyzed. The fact that bioinformatics is continuously struggling to keep the quality and quantity of genomic data that gigantic, is the main reason behind its usefulness in modern clinical laboratories. Shotgun sequencing and targeted sequencing are the preeminent two sequencing techniques that best fit with the acquisition of genetic data from NIPT, where they sequence a whole genome and peculiar genomic regions respectively [24, 25]. Stages like generating a sequence, alignment, and generation of genomic variations are those related to NGS data analysis where bioinformatics materialize. Bioinformatics’ algorithms are entrenched in quantification and disclosure for a combination of size distribution, allelic count, and regional genomic representation. Besides, it became possible to increase the specificity and accuracy of genetic tests substantially without the need for further investment in labware, thanks to the brand-new bioinformatic “in silico” approach. Fetal fraction – the calculation of cfDNA proportion present in the pool of maternal plasma cfDNA- and detecting structural variations are the two interrelated steps that bioinformatic analysis is consisted of. Where the abundance of sequenced DNA fragments of the concerned chromosome is the main column that traditional methods depend on [26]. On the other hand, refining the standard method of z-score is based on amelioration of lab-induced bias, predominantly eliminating long fragments of maternal origin, and accurate selection of the required chromosomes. In addition to the emanating technical methods that locate structural peculiarities depending on the distribution of fragment’s lengths and distribution. In addition, the combination of length and count-based scores may endanger improvements in the reduction of uninformative and false-positive predictions, further in aneuploid and euploid samples’ separation [26, 27].

Although chromosomal counts were of high accuracy in routine testing, lonely, count-based methods are not accurate enough to resolve fetal fraction in pregnancies characterized by the presence of the same karyotype as the mother within the fetus. On the contrary, other characteristics that distinguish fetal and maternal DNA as patchy fetal fragments’ distribution over the genome are found in general methods [27]. However, when supplementary patient-related attributes like body mass index and gestational age of the mother are combined with general methods – which do not have a high precision like that of the count-based ones, may lead to further improvements in their predictions [27]. Where fragment length distributions could be used with lower accuracy, as an alternative for specific deviation, which has been observed in the areas inveigled by DNA packaging in nucleosomes. Furthermore, testing could be further revolutionized in the upcoming era of genomic bio-banking with the help of new approaches like single-nucleotide polymorphism (SNP) which ascertains the source of each fragment depending on the known genotypes of each
parent [28].

It is well known that there are several epigenetic methods like nucleosome positioning, gene activation, splicing regulation, gene repression, and recruitment of transcription factors by which DNA methylation can regulate the functions and development of the placenta. Since the placenta plays a crucial role in the development of the fetus during pregnancy, developmental defects and abnormalities in the expression of the affected genes may result due to aberrations in placental DNA methylation. Because of these features, the ongoing NIPT research is focused on the analysis of placental DNA methylation dignity [28]. That could be done nowadays by using Whole-genome massively parallel bisulfite sequencing technique, which sets clinicians up to investigate placental methylation just through maternal circulation. Once sodium bisulfite interacts with DNA, non-methylated cytosine is converted into uracil while methylated one remains unchanged, thus allowing the determination of methylation status [23, 28]. Since different tissues are defined with specific methylation patterns in the plasma DNA tissue mapping-based approach, it would be beneficial in the determination of cfDNA fragments’ origin. That is how NIPT limitations by maternal malignancy could be overridden, where adopting bisulfite sequencing can discriminate between the origin of tumor-derived cfDNA and that of fetal-derived cfDNA in an attempt to evade false-positive results of NIPT analysis [29].

Bioinformatic software like Methy-Pipe is one of the main requirements in order to carry out whole-genome NGS methylomic bisulfite sequencing data analysis. Methy-Pipe is an amalgamated bioinformatic pipeline that is characterized by the ability for sequence alignment, data preprocessing, and downstream methylation data analysis such as computation of methylation level, sequencing quality report and basic statistics, recognition of distinctively methylated regions for paired samples, visualization and conception of the methylated data for easy elucidation and data mining [29, 30]. For the purpose of reconstructing the whole placental genome—which is a challenging objective- in a non-invasive manner, fetal methylome reconstructor (FEMER), an unusual algorithm that has been proposed after a great effort in intensive research by a group of scientists. FEMER may expedite promising clinical applications, as it provides a first-rate view of placental methylome from maternal plasma. Furthermore, quantitative polymerase chain reaction besides methylated DNA immunoprecipitation in combination with each other could be used as an effective approach for NIPT of Down syndrome in pregnant women [31]. This approach, which is equivalent to NGS, relies on the exception of maternal-fetal distinctly methylated regions to weigh the fetal DNA ratio. According to diagnostic productivity and statistical assessment for Down syndrome, it is proven that 100% sensitivity and 100% specificity have been attained. In addition, other testifying studies showed approximate results of 100% sensitivity (95% CI 92.89 to 100.00%) and 99.2% specificity (95% CI 95.62 to 99.98%) [32]. Where the interesting point in this approach in comparison with NGS is that it is technically easier, not overpriced, and requires available equipment in most genetic diagnostic laboratories around the world.

6. CONCLUDING REMARKS FOR THE FUTURE OF PRENATAL TESTS

It is obvious that the ordinary practice of prenatal genetic screening has changed once non-invasive prenatal screening for chromosomal abnormalities was rapidly introduced in the medical field. Unfortunately, this approach doesn’t have the same accuracy or diagnostic capability as CVS or amniocentesis, as well, its resolution or coverage is not high enough to replace those of a karyotype or CMA, however, cfDNA screening specificity and sensitivity for Trisomy 21 and other usual aneuploidies are respectable [33, 34]. Although more screening methods are being added by various laboratories for other aneuploidies like duplications and microdeletions, other concerns have to think about, whereas rare conditions are classified among high amassed false-positive rates and tolerable clinical validation, which would be conducive to nonessential diagnostic procedures and missed genetic diagnoses resulting from the high false-negative rates [35]. On the other hand, the awareness and understanding of those mentioned issues by patients and providers could be incomplete, where marketing for cfDNA screening is geared towards the avoidance of diagnostic procedures’ multiple risks. As a result, patients may choose cfDNA screening especially when its diagnostic testing is more optimal as in the identification of fetal abnormalities, and at the same time, CMA discerns clinical chromosomal aberrations in 6% of pregnancies elaborated with fetal abnormalities and in 1 – 1.7% in those without fetal abnormalities, together with chromosomal anomalies perceived by karyotyping [36]. Therefore, missing prenatal genetic diagnosis would be the cost of the common trend for replacing diagnostic testing with cfDNA screening until NIPT becomes more comprehensive and exact. Moreover, pregnant women should be admonished about cfDNA screening’ noted limitations in comparison with the lower risks of diagnostic procedures of about 1:600 and 1:909 for amniocentesis and CVS respectively according to a recent
meta-analysis. In addition, diagnostic NGS technology, which locates single genetic disorders, is anticipated to twofold the number of exposed genetic causes of fetal anomalies. In fact, the proof that principle studies have brought to light about the feasibility of non-invasively sequencing an entire fetal genome is not practicable especially in the meantime as it is not cost or time-effective [37, 38]. Up till NIPT improves to the meant point of the same precision as that of karyotyping and CMA, all the pros, cons, and limitations of all current prenatal screening approaches, should be objectively listed within the context of women’s “priori risk”, and according to the patient desire and after taking clinician’s advice, the appropriate approach should be used.

In conclusion, it is worth mentioning that the continuous amelioration in genomics medicine, impresses prenatal screening and diagnosis like other medical fields, however, these modernizations provide new opportunities that should be exploited to empower pregnant women with informative knowledge in an ethically responsible and evidence-based manner about screening risks, they have the right to decide after their decision-making sovereignty. Taking into account that profit-seeking companies, professional medical societies, drive mostly all of these, innovative approaches should play a crucial role in providing unbiased guidance to pregnant women and patients to be monitored after implementation.

7. CONFLICT OF INTERESTS

The authors have no conflict of interest to declare. The authors declared that this study has received no financial support.

8. REFERENCES


