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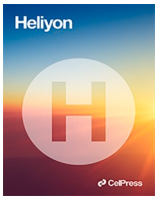

Review article

Research progress on ultrasound and molecular markers for prenatal diagnosis of neural tube defects

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ABSTRACT

Neural tube defects (NTDs) are severe congenital anomalies that result from the failure of early neural tube closure during fetal neurogenesis. They are the most common and severe congenital malformations of the central nervous system. Identifying reliable prenatal diagnostic ultrasound and molecular markers that can predict NTDs is of paramount importance. Early diagnosis of NTDs allows embryonic treatment and prevention strategies, which are crucial for reducing the disability rate associated with these malformations, reducing the burden on individuals and on society. The purpose of this comprehensive review was to summarize the ultrasound biomarkers between 11 and 13 weeks of gestation and the molecular biomarkers used in the diagnosis of NTDs, providing additional insights into early screening for NTDs.

1. Introduction

Neural tube defects (NTDs) are among the most serious birth defects and profoundly affect human health. There are different types of NTDs, including anencephaly, spina bifda, encephalocele, craniorachischisis, and iniencephaly. The estimated average global prevalence of NTDs is two cases per 1000 births, resulting in approximately 214,000 to 322,000 affected pregnancies worldwide each year [1]. The etiology of NTDs remains unclear. Research suggests that the pathogenesis of NTDs may involve complex interactions of multiple factors, including genetics and the environment [2]. NTDs can lead to miscarriages, perinatal fetal mortality, and other adverse outcomes [3]. Although prenatal folate supplementation reduces their incidence, NTDs remain a common and severe congenital anomaly. Therefore, NTDs are a challenging subject in international research, and early detection and appropriate intervention during pregnancy are crucial.

In current clinical practice, the primary methods for the prenatal diagnosis of NTDs include direct observation via ultrasonography and mid-pregnancy screening using the serum biomarker alpha-fetoprotein (AFP). In recent years, with improvements in ultrasound instrument resolution, an increasing number of scholars have turned their attention to the ultrasound image characteristics of NTDs in early pregnancy (11 + 0 to 13 + 6-week of gestation). Various diagnostic indicators for NTDs in early pregnancy have been proposed, which have contributed to early NTD diagnosis. Although ultrasound examination offers economic, non-invasive, and convenient operation advantages and has high acceptance among pregnant women, it is time-consuming and labor intensive. Moreover, the diagnostic accuracy relies heavily on the operator's skills and experience. The maternal serum AFP test was first introduced into

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Abbreviations

AFP	alpha-fetoprotein
AOS	aqueduct of Sylvius
AUC	area under the curve
AC	abdominal circumference
BPD	biparietal diameter
BS	brain stem diameter
BSOB	brain stem to occipital bone distance
BST	brainstem-tentorium
CI	confidence interval
CM	cisterna magna
CP	choroid plexus
CP-A	choroid plexus area
CP-L	choroid plexus length
CRL	crown–rump length
CSF	cerebrospinal fluid
DNM2	dynamin 2
DBP	dibutyl phthalate
GFAP	glial fibrillary acidic protein
HA	head area
HCY	homocysteine
IT	intracranial translucency
J-MB	junction between midbrain and brainstem
LMNA	lamin A
lncRNAs	long non-coding RNAs
MRI	magnetic resonance imaging
miRNA	microRNA
MMC	myelomeningocele
M − O line	maxilla-occipital line
MoMs	multiples of the median
MMA	methylmalonic acid
MBP	monobutyl phthalate
m6A	N6-methyladenosine
NTD	neural tube defect
OFD	occipitofrontal diameter
OSB	open spina bifida
PFF	posterior fossa fluid
PPV	positive-predictive value
SD	standard deviation
SVM	support vector machine

clinical practice to screen for fetal NTD in the 1970s [4]. However, its high false-positive rate poses a diagnostic challenge. According to a study conducted in China, the positive predictive value (PPV) of AFP for NTD diagnosis was only 16.2 % [5]. Consequently, numerous studies have explored new diagnostic biomarkers in diverse maternal biological fluids (serum and amniotic fluid), using various biological technologies, and some progress has been achieved in this area. This review summarizes the recent advancements in research on ultrasound and molecular markers for NTDs.

2. Ultrasound markers for NTDs

Currently, research has focused on ultrasound markers for predicting NTDs in early pregnancy (11–13 weeks), with particular emphasis on open spina bifida (OSB). Fetuses affected by OSB experience cerebrospinal fluid (CSF) leakage, leading to displacement of the brain tissue and exhibiting unique ultrasound features in different planes, which aids in early diagnosis. In contrast, in closed neural tube defects such as meningocele, closed anencephaly, and lipomyelomeningocele, the absence of cerebrospinal fluid leakage makes accurate diagnosis challenging during early pregnancy using typical ultrasound features. Sometimes, these conditions often require higher-resolution fetal cranial magnetic resonance imaging (MRI) for definitive diagnosis, as MRI techniques can provide detailed visualization of spinal cord and brain tissue abnormalities. The following sections outline the ultrasound markers for the prediction and screening of NTDs, primarily focusing on OSB, during early pregnancy.

2.1. Ultrasound markers for OSB

2.1.1. Intracranial translucency

Intracranial translucency (IT) (Table 1, Fig. 1a), also known as the fourth ventricle, is visible in the midsagittal view of the fetal face. It is situated between the brainstem and choroid plexus (CP). This structure is termed "intracranial translucency" due to its echogenicity, which resembles that of the nuchal translucency seen in the neck region. In normally developing fetuses between 11 and 13 weeks of gestation, the midsagittal view of the fetal face commonly exhibits a distinct and well-defined fourth ventricle. However, in fetuses with OSB, the loss of CSF leads to posterior displacement of the brain tissue, exerting pressure on the fourth ventricle, thereby resulting in a relatively constricted manifestation. Chaoui et al. [6] were the first to propose the use of IT for early prenatal diagnosis of OSB. Their study revealed that the IT was consistently visible in normal fetuses, whereas it was not observed in the four comparative cases of fetuses with OSB. In a study conducted in Mexico evaluating IT as an early pregnancy screening marker for NTDs, it was found that eight fetuses lacked visible intracranial translucency. Upon reassessment at 14 weeks of gestation, two of these fetuses showed normal IT. However, the remaining six were later diagnosed with NTDs, specifically OSB (n = 5) and encephalocele (n = 1) [7]. A recent retrospective study evaluated ultrasound markers for early pregnancy OSB. The study included 648 pregnancies between 11 weeks and 13 weeks 6 days. The results showed that among the 5 OSB cases, 4 had abnormal IT, with 1 case having an IT measurement below the 5th percentile and 4 cases with IT not visible. In contrast, in the 643 control cases without OSB, the normal range for IT measurements was between 1.51 and 2.63 mm [8].

In a meta-analysis of systematic reviews, the diagnostic accuracy of IT in detecting spina bifida was evaluated. Results showed a sensitivity of 53.5 % (95 % confidence interval [CI] 42.4–64.3) and a high specificity of 99.7 % (95 % CI 99.6–99.8). The positive likelihood ratio was 62.1 (95 % CI 12.2–317), while the negative likelihood ratio was 0.55 (95 % CI 0.45–0.68). The diagnostic odds ratio was 223 (95 % CI 25–2039). This finding underscores the potential of IT as a diagnostic tool for spina bifida, particularly because of its high specificity. However, its relatively lower sensitivity implies that a proportion of individuals with spina bifida may not be accurately identified [9]. Furthermore, no international standard for the normal range of IT thickness measurements has been established to date, potentially leading to variations in results among different ultrasonographic practitioners. Future research with larger sample sizes is needed to establish precise boundaries for the normal range and formulate consistent measurement standards and assessment methods. This will facilitate the incorporation of IT thickness measurements into routine diagnostic protocols for the early diagnosis of fetal OSB, ultimately enhancing its accuracy and reliability in clinical practice.

2.1.2. Brain stem diameter and brain stem to occipital bone distance

The brain stem diameter (BS) represents a direct measurement of the diameter of the brainstem, while the brain stem to occipital bone distance (BSOB) refers to the measurement of the distance from the brainstem to the occiput. During early pregnancy, the ratio of the BS to BSOB (BS/BSOB) (Table 2; Fig. 1b) is used as a crucial reference index for screening OSB. Typically, the BS/BSOB ratio in normal fetuses is less than 1.0 [13]. Lachman et al. [14] introduced this ratio for the first time in a retrospective study that revealed that the values of BS and BSOB increased with crown-rump length (CRL), whereas their ratio (BS/BSOB) decreased with CRL in normal fetuses. In fetuses with OSB, the average BS increased significantly, the average BSOB decreased significantly, and the BS/BSOB ratio increased in all cases beyond the 95th percentile. The observed changes in the posterior brain were attributed to the tendency of the fourth ventricle to be compressed and reduced in size. A recent meta-analysis investigating the utility of BS/BSOB in predicting fetal OSB demonstrated a pooled sensitivity of 0.70 (95 % CI 0.47–0.87; $I^2 = 78.3$ %), specificity of 1.00 (95 % CI 0.99–1.0; $I^2 = 99.2$ %), positive and negative likelihood ratios of 51.44 (95 % CI 9.53–277.64; $I^2 = 85.5$ %) and 0.23 (95 % CI 0.04–1.17; $I^2 = 64.8$ %), respectively, and an area under the curve (AUC) of 0.9649 [15]. This approach has potential for clinical applications but requires further large-scale studies to ensure its accuracy and reliability.

2.1.3. Cisterna magna

Disappearance of the cisterna magna (CM) (Fig. 1a) is a critical early ultrasound manifestation in fetuses with OSB. Loss of CSF in fetuses with OSB leads to herniation of the vermis of the cerebellum, distortion and thinning of the fourth ventricle, posterior displacement of brain tissue, and changes in CSF dynamics and surrounding structures, ultimately resulting in obliteration of the posterior cranial fossa cisterns. Kavalakis et al. [16] observed the four-line view of the posterior fossa in 1330 fetuses in the first trimester, including the upper and lower boundaries of the brainstem, CP of the fourth ventricle, and occiput, and found that, in two

Table 1
Sensitivity, specificity, and AUC of IT for the diagnosis of NTD.

Samples	Ultrasound biomarker	Gestation(weeks)	Sensitivity	Specifcity	Sample size	References
The mid-sagittal view	Non-visualization of the IT	11–13	100.00 %	100.00 %	204	[6]
		11–13	100.00 %	99.97 %	7130	[7]
		11–13	53.50 %	99.70 %	21070	[9]
		11–13	28.80 %	88.50 %	260	[10]
			−48.10 %	−91.30 %		
	Non-visualization of the IT or IT < 5th percentile	11–13	80.00 %	100.00 %	648	[8]
	IT ≤5th percentile	11–13	50.00 %	90.90 %	1479	[11]
	IT < 5th percentile	11–13	59.30 %	–	1030	[12]

Note: IT, Intracranial translucency; -, no data.

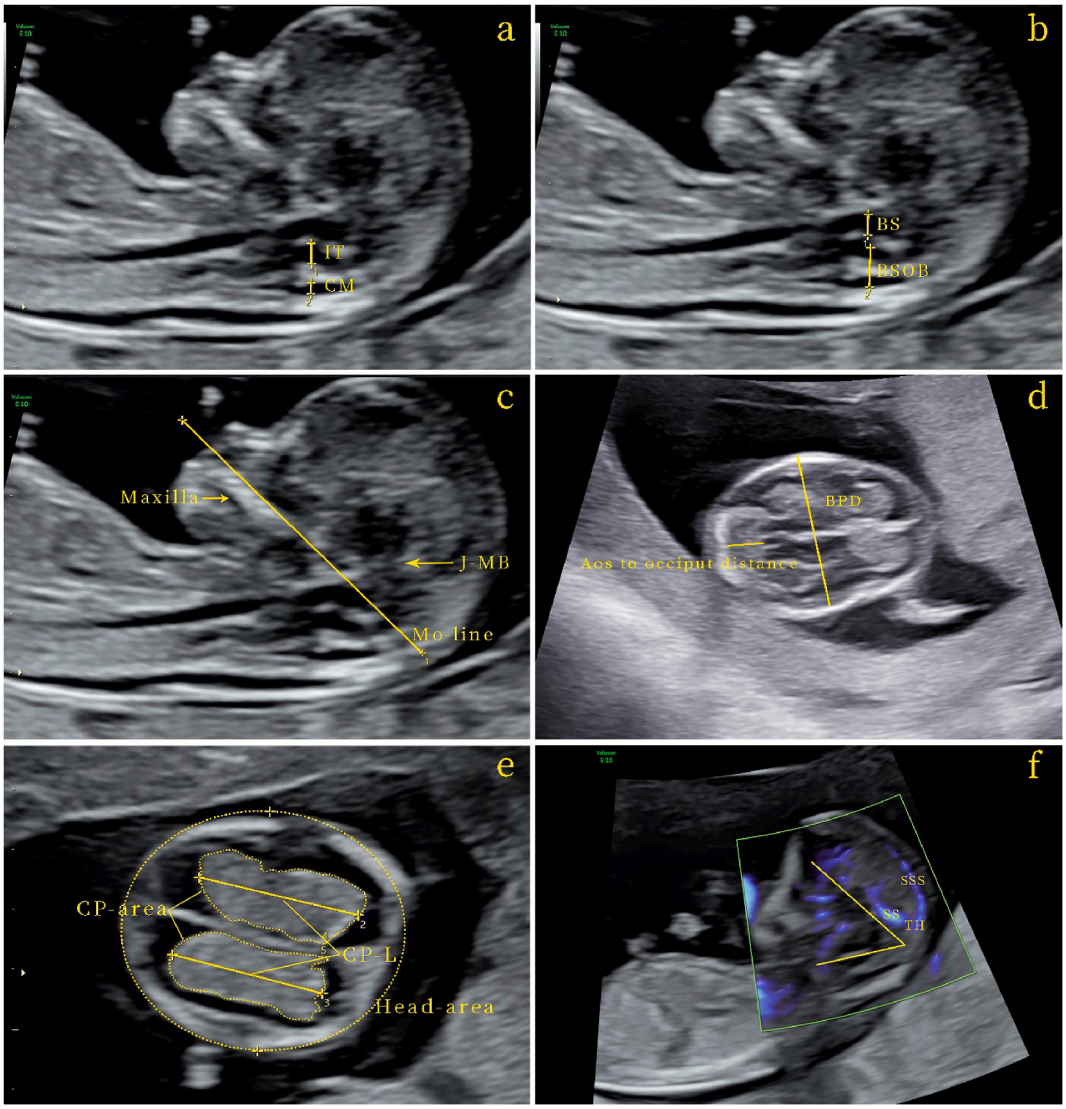

Fig. 1. Ultrasound markers for OSB. Note: OSB, open spina bifida; (a) IT, intracranial translucency; CM, cisterna magna; (b) BS, brain stem; BSOB, brain stem to occipital bone distance; (c)M-o line, Maxillo-occipital line; J-MB, the junction; (d) BPD, biparietal diameter; AOS, aqueduct of sylvius; (e) CP, choroid; CP-L, choroid plexus-length; OFD, occipitofrontal diameter; (f) TH, torcular herophili; BST, brainstem-tentorium; SS, straight sinus; SSS, superior sagittal sinus.

Table 2
Sensitivity, specificity, and AUC of BS, BSOB and BS/BSOB for the diagnosis of NTD.

Ultrasound marker	Cutoff value	Gestation (weeks)	Sensitivity	Specifcity	AUC	Sample size	References
BS	≥95th percentile	11–13	96.70 %	–	–	1030	[14]
		11–13	100.00 %	94.40 %	–	1479	[11]
	Caudal displacement	11–13	34.6%–82.7 %	86.1%–98.1 %	–	260	[10]
BSOB	≤5th percentile	11–13	86.70 %	–	–	1030	[14]
		11–13	100.00 %	94.80 %	–	1479	[11]
BS/BSOB	The ratio increased	11–14	70.00 %	100.00 %	96.49 %	17598	[15]
	≥95th percentile	11–13	–	–	–	2621	[13]
		11–13	100.00 %	–	–	1030	[14]
		11–13	100.00 %	94.40 %	–	1479	[11]
	>95th percentile	11–13	96.30 %	–	99.70 %	1030	[12]

Note: BS, Brain stem; BSOB, diameterbrain stem to occipital bone distance; -, no data.

fetuses with OSB, despite the IT value being within the normal range, the CM had disappeared. Similarly, research findings have indicated that the absence of CM visualization exhibits superior screening efficacy, with a sensitivity range of 50–73 %. Conversely, both non-visualization of the IT and posterior displacement of the brainstem toward the occipital bone showed lower detection rates, with sensitivities ranging from 29 to 48 % and 35–83 %, respectively [10]. Hence, when the CM disappears, it functions as a significant warning signal, prompting further measurements of other ultrasound indicators, such as IT, BS, and BSOB. Kose et al. identified the key characteristics of OSB as follows: increased BS diameter, elevated BS/BSOB ratio, and non-visualization of CM. Although all markers demonstrated high sensitivity and specificity, CM visibility showed the highest PPV of 66.7 % (100 % sensitivity and 99.9 % specificity). Conversely, PPVs for other markers were lower: BS diameter ≥95th percentile (2.5 %), IT ≤ 5th percentile (0.8 %), BSOB distance ≤5th percentile (2.7 %), and BS/BSOB ratio ≥95th percentile (2.8 %) [11]. In a study by Chen et al., non-visualization of the CM was associated with a 64 % detection rate for OSB.

Furthermore, based on extensive statistical analyses, researchers have determined a critical CM threshold of 1.6 mm, resulting in a 73 % detection rate for OSB when applying this threshold. Kapoor et al. observed an increase in the IT/CM ratio (referred to as the R ratio) in fetuses with OSB [17]. Furthermore, assessment of the CSF area in the fetal posterior fossa is considered to be an effective method to assist in early OSB diagnosis. This can be achieved by measuring the areas of the CM and IT in the midsagittal view of the fetal face and quantifying them in a two-dimensional plane. Some scholars have defined the sum of the areas of IT and CM as the posterior fossa fluid (PFF). From 11 to 13 weeks of gestation, the average PFF area in normal fetuses was noted to increase from 8.55 to 29.72 mm^2. However, in fetuses with OSB, the PFF values were significantly reduced, ranging from 2.39 to 5.08 mm^2, due to factors such as CSF leakage [18]. Therefore, a more comprehensive indication of the risk of OSB in the fetus can be achieved by integrating assessments of various structures within the fetal posterior cranial space.

2.1.4. Maxillo-occipital line

The maxillo-occipital line (M − O line) (Fig. 1c) is a straight line extending from the upper margin of the maxilla to the occiput in the midsagittal view of the fetal face. In normal fetuses, the junction between the midbrain and brain stem (J-MB) is located above this line. Conversely, OSB results in the downward and posterior displacement of the J-MB below the M − O line. M − O line positivity was initially introduced as an assessment method by Ramkrishna et al. [19]. No instances of OSB were observed among 100 prospectively evaluated fetuses when the M − O line was positioned below the junction of the midbrain and brainstem. Conversely, all 14 patients diagnosed with OSB exhibited junctions between the midbrain and brainstem below the M − O line. Wertaschnigg et al. [12] found that, when applying the IT, BS/BSOB, and M − O line parameters, the detection rates for fetuses with OSB were 52.3 %, 96.3 %, and 96.3 %, respectively, and the false-positive rate for diagnosing OSB using the M − O line was only 0.1 %. Therefore, the M − O line and BS/BSOB ratio appear to be the most reliable indirect ultrasound indicators for diagnosing fetal OSB. However, as the originators of the M − O line, Ramkrishna et al. [19] were also cautious in indicating that the M − O line should serve as an adjunctive marker for OSB screening. The false-positive and false-negative rates of OSB diagnosis using the M − O line require further validation and research.

2.1.5. Biparietal diameter

A straightforward and repetitive method can be employed to measure biparietal diameter (BPD) in transverse section of the fetal thalamus (Fig. 1d). During early pregnancy, fetuses with OSB tend to exhibit lower BPD values. This phenomenon is attributed to CSF loss in fetuses with OSB, leading to a reduction in intracranial pressure and subsequent collapse of cranial bones [10]. However, studies have indicated that only approximately 50 % of fetuses with OSB have a BPD <5th percentile in early pregnancy [20–22]. Some researchers have suggested that combining BPD with other biometric measurements may enhance its efficacy for OSB diagnosis. Suresh et al. reported that optimal outcomes were achieved when the abdominal circumference (AC) was used to standardize BPD values in multiples of the median (MoMs). First-trimester OSB screening has demonstrated the capability to identify between half and two-thirds of cases [23]. This was similar to the study conducted by Simon et al. [24], whose report indicated that a ratio of BPD/transverse abdominal diameter ≤1.00 could detect 69.2 % of OSB cases, while using BPD ≤ the 5th percentile could screen out 46.2 % of fetuses with OSB during early pregnancy. A retrospective study by Wertaschnigg et al. indicated that using BPD and BPD/AC below the 5th percentile as cutoff values yielded OSB detection rates of 66.7 % and 70.4 %, respectively. At a false-positive rate of 5 %, the BPD detection rate was 66.7 %, and that for BPD/AC was 76.2 % [12].

2.1.6. The aqueduct of sylvius to the anterior border of the occiput distance

To obtain a cross-sectional view of the aqueduct of Sylvius (AOS) (Fig. 1d), a slight caudal movement on the thalamic section is necessary. The phenomenon of a shortened distance between the AOS and occiput is considered an indirect indicator for the early diagnosis of fetal OSB. This ultrasound indicator was first reported by Finn et al. They found that the lower threshold for the normal AOS-to-occiput distance (mean minus two standard deviations [SDs]) exhibited a range from 1.7 mm at a CRL of 45 mm–3.7 mm at a CRL of 84 mm, and the AOS-to-occiput distance in the nine fetuses with OSB in their study was below the established normal range [25]. This presentation is akin to the rear end of a car hitting a wall. Hence, some scholars have termed this ultrasound appearance the "crash sign" [26]. Wertaschnigg et al. previously reported that an AOS-to-occiput distance below the 5th percentile could achieve an OSB detection rate of 77.8 % [12]. Although the detection rates for the AOS-to-occiput distance were not as high as those for an elevated BS/BSOB ratio (96.3 %) or an abnormal M − O line (96.3 %) in the sagittal view, the AOS-to-occiput distance demonstrated the highest detection rate among the indicators in the axial view.

2.1.7. "Dry brain": the choroid-plexus-to-head-size ratio

At 11–13 weeks, most fetuses with OSB have reduced fluid in the lateral ventricles, leading to an apparent increase in the

proportion of CP within the cranium. Some scholars vividly describe this phenomenon as a "dry brain" and have conducted quantitative analyses of this phenomenon. Chaoui et al. established CRL-based reference ranges for the CP-area (CP-A)/head area (HA) and CP-length (CP-L)/occipitofrontal diameter (OFD) (Fig. 1e). From 11 to 13 weeks, CP-A increased with CRL (50 mm: 88.75 mm^2, 80 mm: 172.81 mm^2). Among the fetuses with OSB, 53 % had normal CP-A, 38 % exceeded two SDs above the mean, and 9 % were below two SDs. The CP-A/HA ratio increased with the CRL (50 mm: 31.2 %, 80 mm: 25.7 %), with 88.2 % of fetuses with OSB exceeding two SDs. The CP-L/OFD ratio increased with the CRL (50 mm: 56.7 %, 80 mm: 51.2 %), with 88.2 % of fetuses with OSB exceeding two SDs above the mean [27]. In a recent large-scale study, the mean CP-L/OFD ratio and its MoMs showed AUCs of 0.921 and 0.933, respectively. The optimal cutoffs for the CP/OFD and MoM ratios were 0.662 and 1.263, respectively. The optimal cutoffs for the mean CP/OFD and MoMs ratios yielded a PPV of 90.9 % and a negative predictive value of 99.6 % [28]. Therefore, the ratio of CPs to the head is a promising tool for prenatal detection of OSB.

2.1.8. Doppler ultrasound markers

In addition to traditional routine ultrasound examinations, some researchers have begun to explore the application of ultrasonic Doppler technology in diagnosing fetal OSB. Volpe et al. introduced the concept of the brainstem–tentorium (BST) angle. The torcular Herophili serves as the confluence of the straight sinus and superior sagittal sinus, acting as a proxy for the insertion point of the tentorium on the fetal skull. The BST angle is defined as the angle between the line tangential to the inferior margin of the BS and that tangential to the tentorium (Fig. 1f). Their study revealed that, in fetuses with OSB, the BST angle was notably more extensive than that observed in normally developing fetuses, with the BST angle approaching approximately 90°in OSB cases. Although current research on Doppler technology for the diagnosis of fetal OSB remains limited, the effectiveness and prevalence of using Doppler technology to diagnose fetal cranial abnormalities is gradually emerging [29]. Further investigations in this regard are anticipated, and these studies will contribute to a more comprehensive approach and provide a basis for the accurate diagnosis of early-pregnancy NTDs and other cranial abnormalities.

2.2. Ultrasound markers for other NTDs

Anencephaly is a lethal NTD characterized by the absence of the cranial vault (acrania), which leads to a lack of protection of the cerebral tissue. This condition results in gradual deterioration of the brain or its major structures due to exposure to amniotic fluid and mechanical injuries. Ossification of the frontal bones at 10 weeks of gestation is a critical period. During the 11–13-week period, abnormal head shapes may suggest the early stages of the acrania–exencephaly–anencephaly sequence. Three-dimensional ultrasonography serves as an adjunct diagnostic tool for various phenotypic presentations within the acrania–exencephaly–anencephaly

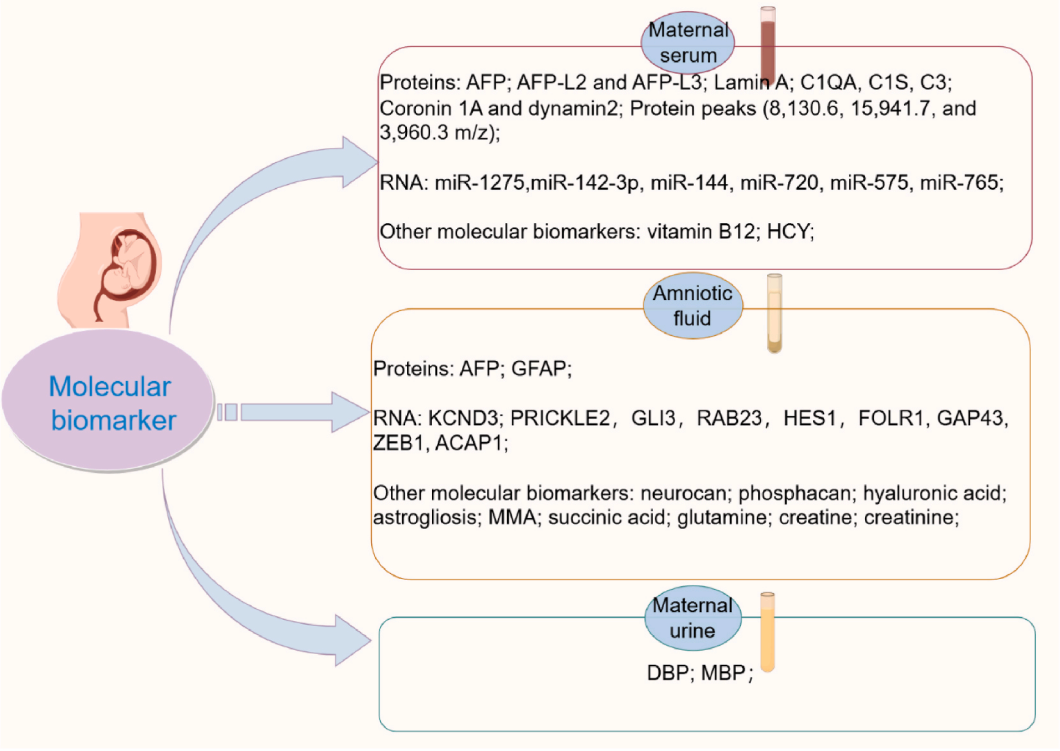

Fig. 2. Molecular biomarkers derived from various samples. Note: AFP, alpha-fetoprotein; miR, microRNA; HCY, homocysteine; GFAP, glial fibrillary acidic protein; MMA, methylmalonic acid; DBP, dibutyl phthalate; MBP, metabolite monobutyl phthalate.

sequence. The fetal head may exhibit characteristics such as a "Mickey Mouse" bilobular face, cystic formations, elongation, and irregular head shape [30]. Some researchers have categorized the head features of anencephalic fetuses into distinct subtypes. The frequencies of various subtypes classified as overhanging, elongated, bilobular, cystic, foreshortened, and irregular were 31 %, 25 %, 19 %, 11 %, 8 %, and 6 %, respectively [31]. Various studies have reported interesting findings. These features include the presence of a constriction ring around the external base of the developing skull and an enlarged globular head, reminiscent of a "Turkish turban," accompanied by large cystic spaces that replace the brain [32]. Szkodziak et al. proposed the "beret sign" to describe anencephaly. They observed that, in fetuses with anencephaly, cerebral structures were enclosed by an inertial rippled thin membrane, exhibiting a smooth outer contour. A thin anechoic space corresponding to the CSF was observed between the described membrane and brain structures. This observation led to the definition of the "beret" sign [33]. In addition, the amniotic fluid in anencephaly is often more echogenic than the extracelomic space, serving as a potential diagnostic indicator for developmental abnormalities in this sequence, and is observed in 89 % of cases [34,35].

3. Molecular biomarkers derived from various biological specimens

Exploring molecular biomarkers is of paramount importance for prenatal diagnosis. These biomarkers have extensive potential and can be extracted from various bodily fluids, including amniotic fluid, maternal serum, and urine. Especially when fetal neural tissue is exposed to amniotic fluid, it may cause changes in the concentrations of certain molecular biomarkers within these fluids. These biomarkers can serve as potent tools for the early detection, especially of open NTDs. In-depth exploration of these molecular markers in biological specimens helps to uncover rich information and provides a comprehensive foundation for prenatal screening. Below, we summarize the current diagnostic efficacies of molecular biomarkers for NTDs derived from various samples. (Fig. 2).

3.1. Molecular biomarkers derived from maternal serum

Currently, molecular-level testing for NTDs predominantly involves screening for the serum biomarker, AFP, during mid-pregnancy. Clinically, elevated maternal serum AFP level ($\geq$2.5 MoMs) is utilized for predicting fetal NTDs. However, the specificity of maternal serum AFP levels in diagnosing NTDs may be influenced by factors such as fetal abdominal wall defects, multiple anomalies, hypertensive disorders of pregnancy, placental injury, multiple gestations, and fetal demise [36]. It is imperative to investigate additional early pregnancy maternal serum biomarkers associated with NTDs; thus, various types of molecular biomarkers are pertinent.

3.1.1. Proteins

Combined application of AFP variants L2 and L3 (AFP-L2 and AFP-L3) from the maternal serum outperforms AFP alone in the predictive diagnosis of fetal OSB. Chen et al. [37] assessed the effectiveness of using AFP-L2 and AFP-L3 for screening fetal open NTDs at 15–20 weeks of gestation. When employing AFP-L2 and AFP-L3 independently for open NTD fetal screening, the AUC, sensitivity, and specificity values were 0.822, 0.667, and 1.000 and 0.882, 0.762, and 0.895, respectively. The combined application of AFP-L2 and AFP-L3 resulted in an AUC, sensitivity, and specificity of 0.955, 0.905, and 0.895, respectively. It is evident that joint utilization enhances sensitivity and marginally reduces specificity as compared to that with individual utilization. Nevertheless, both methodologies exhibited superior diagnostic efficacy compared to that of AFP alone, for which the AUC, sensitivity, and specificity for open NTD fetal screening were 0.777, 0.714, and 0.868, respectively. Therefore, AFP-L2 and AFP-L3 may serve as novel biomarkers for the detection of open NTDs in fetuses.

Lamin A (LMNA) is a nuclear intermediate filament protein that is critical for nuclear architecture and mechanics [38]. In a recent study, Chen et al. [39] investigated the value of maternal serum LMNA concentrations in predicting various adverse pregnancy outcomes in singleton pregnancies at 15–18 weeks and found a correlation between reduced LMNA levels and the occurrence of fetal NTDs. Finally, LMNA demonstrated superior diagnostic accuracy for NTDs as compared to that of AFP, with an AUC of 0.890, surpassing the AUC of AFP of 0.829. The combined use of LMNA and AFP exhibited an enhanced diagnostic efficiency, yielding an impressive AUC of 0.990. Utilizing LMNA in early- or mid-pregnancy serum screening, particularly in conjunction with AFP, may significantly improve the accuracy of NTD diagnosis. However, the specific predictive value of LMNA for fetal NTDs in early pregnancy warrants further confirmation in multicenter studies with larger sample sizes.

The classical complement cascade plays a significant role in neurodevelopmental processes, including cell proliferation, cellular differentiation, cellular migration, and synaptic pruning [40]. Dong et al. [41] explored the potential of certain complement factors in maternal serum as biomarkers for NTD diagnosis. In their study, 113 proteins were identified by proteomics analysis with differential expression in the maternal serum of 37 fetuses with NTDs (spina bifida aperta 18, exencephaly 5, anencephalus 2, hydrocephalus 12); among these, 23 exhibited a 1.5-fold greater upregulation or downregulation, including five complement proteins (C1QA, C1S, C1R, C9, and C3). Notably, C3 and C9 were significantly downregulated in the maternal serum of fetuses with spina bifida and hydrocephalus during weeks 19–40 of gestation. Another study employed a support vector machine (SVM) classifier to establish a predictive model for NTDs using complement proteins and AFP. The accuracy of the SVM model for complement factors (C1QA, C1S, and C3) was 62.5 %, with 60 % sensitivity and 67 % specificity. In comparison, the accuracy of the SVM model for AFP was 62.5 %, with 75 % sensitivity and 50 % specificity. Combining complement factor and AFP data increased the accuracy of the SVM model to 75 %, with the corresponding sensitivity and specificity both reaching 75 %. These findings suggest that a predictive model integrating complement factor and AFP data holds promise as a more accurate approach for noninvasive prenatal NTD diagnosis.

Wang et al. [42] employed proteomic analysis to identify novel NTD-specific proteins. They discovered that coronin 1A and

dynamin 2 (DNM2) exhibited exosome-specific expression and were associated with the embryonic development of spina bifida. Notably, the study revealed a significant reduction in DNM2 expression in the maternal serum of NTD fetuses, with a diagnostic specificity of 78.95 %, sensitivity of 73.68 %, and an AUC of 0.806. Subanalyses across different gestational periods indicated a more pronounced downregulation of DNM2 at weeks 12–18, with diagnostic specificity, sensitivity, and AUC reaching 100 %, 100 %, and 1.000, respectively. Similarly, coronin 1A expression was markedly decreased in the maternal serum of fetuses compared to that in normal fetuses, with a diagnostic specificity, sensitivity, and AUC of 89.47 %, 68.42 %, and 0.817, respectively. A highly significant reduction in its expression was observed during weeks 12–18, with diagnostic specificity, sensitivity, and AUC reaching 85.71 %, 85.71 %, and 0.857, respectively. Further analysis demonstrated that the combined assessment of DNM2 and coronin 1A enhanced the diagnostic performance for NTDs, with a specificity of 78.95 %, sensitivity of 94.74 %, and an AUC of 0.889. During weeks 12–18, the specificity, sensitivity, and AUC reached 100 %, 100 %, and 1.000, respectively.

Shen et al. [43] conducted a proteomic analysis of maternal serum to identify NTD-specific protein peaks as biomarkers for NTDs. In their final model, they identified three protein peaks linked to NTDs (8130.6, 15,941.7, and 3960.3 m/z). They developed a diagnostic model incorporating these three peaks that showed 100 % sensitivity and 75 % specificity, surpassing those of AFP in NTD screening. However, before applying this model for clinical screening of NTDs, extensive double-blind testing should be conducted on a large sample of individuals.

3.1.2. MicroRNA

MicroRNAs (miRNAs) are a class of non-coding small RNA molecules that post-transcriptionally regulate gene expression. They serve as crucial regulators of gene expression and are important in cellular regulatory mechanisms, disease development, and potential therapeutic approaches [44]. Some miRNAs have the potential to be considered as markers for the prenatal diagnosis of NTDs [45]. In a study conducted by Gu et al. [46], 17 miRNAs with significant expression differences in the serum of pregnant women carrying NTD-affected fetuses compared to that of those with normal pregnancies were identified. Using quantitative reverse transcription polymerase chain reaction validation, six pregnancy-related miRNAs (miR-1275, miR-142-3p, miR-144, miR-720, miR-575, and miR-765) were identified. Further analysis revealed diagnostic AUC values of 0.702, 0.680, 0.676, and 0.646 for miR-144, miR-720, miR-142-3p, and miR-765, respectively. Integrating all the miRNAs using logistic regression increased the AUC to 0.803. In the context of anencephaly and spina bifida, miR-1275 (AUC: 0.759) and miR-720 (AUC: 0.722) were the most effective discriminators. Combining all miRNAs with significant expression changes increased the AUC to 0.894 for anencephaly and 0.788 for spina bifida. These findings underscore the potential of these miRNAs as diagnostic and prognostic biomarkers of fetal NTDs.

3.1.3. Other molecular biomarkers

Numerous studies have confirmed the preventative effects of folic acid, vitamin B12, and other nutrients on NTDs. A significant relationship has been observed between low maternal serum concentrations of vitamin B12, elevated homocysteine (HCY) levels, and the incidence of NTDs [47].

3.2. Molecular biomarkers derived from the amniotic fluid

Although amniocentesis is an invasive examination method, its role in prenatal diagnosis remains irreplaceable. In recent years, many studies have focused on exploring NTD molecular markers in the amniotic fluid.

3.2.1. Proteins

AFP, a biomarker for serological screening of NTDs during mid-pregnancy, is also upregulated in the amniotic fluid of fetuses with NTDs. However, other factors, such as gestational age, multiple pregnancies, abdominal wall defects, and fetal kidney issues, can also increase AFP levels in the amniotic fluid. Glial fibrillary acidic protein (GFAP) has been identified as a novel biomarker for brain-specific protein groups. Amniotic fluid GFAP demonstrates specificity for central nervous system injuries and is not elevated in cases of abdominal wall defects. In the context of open NTDs, 99.1 % of samples displayed amniotic fluid-GFAP levels exceeding 0.2 ng/mL, while 100 % of meningoceles exhibited amniotic fluid-GFAP levels below 0.2 ng/mL. This distinction is crucial because of the variations in the prognosis and management of NTDs. Additionally, a noteworthy aspect is that the sensitivity of amniotic fluid-GFAP remains uncompromised in advanced pregnancies [48].

3.2.2. RNA

Research suggests that RNA molecules in the amniotic fluid, particularly miRNAs, may play a significant regulatory role in the occurrence and development of NTDs. Li et al. [49] revealed an miRNA–mRNA regulatory network involving six miRNAs and 39 mRNAs related to spina bifida, including genes such as *TSPAN1*, *YOD3*, and *KCND3*. By analyzing cell-free RNA, Tomo et al. identified 284 differentially regulated genes in the amniotic fluid supernatant of mid-pregnancy fetuses with myelomeningoceles (MMC). This set of genes included 176 upregulated genes and 108 downregulated genes. Among these differentially regulated genes, a series of genes associated with spinal neural tube defects was identified, including *PRICKLE2*, *GLI3*, *RAB23*, *HES1*, and *FOLR1*. In addition, novel aberrantly regulated genes were identified. These aberrantly regulated genes were linked to neural development, neuronal regeneration (upregulated genes, such as *GAP43* and *ZEB1*), and axonal growth and guidance (downregulated genes, such as *ACAP1*) [50]. These findings provide a foundation for prenatal NTD diagnosis and the identification of new therapeutic targets.

3.2.3. Other molecular biomarkers

A recent study found a substantial increase in the levels of neurocan and phosphacan, which are chondroitin sulfate proteoglycans secreted by the developing nervous system in the amniotic fluid of early-pregnancy MMC rats [51]. Additionally, there was a significant reduction in the levels of hyaluronic acid in the amniotic fluid of MMC rat fetuses [52]. Furthermore, astrogliosis was observed in the exposed spinal cords of MMC-affected fetuses of the same age [53]. However, population-based experimental data demonstrating the effectiveness of these biomarkers in prenatal diagnosis are currently insufficient. Methylmalonic acid (MMA) serves as a specific and sensitive indicator of intracellular vitamin B12 deficiency. It also proves to be a sensitive marker for early NTD diagnosis through measurement of amniotic fluid MMA concentrations during the second trimester. In previous studies, researchers quantified proton-containing metabolites using in vitro 1H NMR spectroscopy and found that amniotic fluid levels of succinic acid and glutamine were significantly higher, while creatine and creatinine levels were significantly lower in the spina bifida group compared to the control group [54,55].

3.3. Molecular biomarkers derived from maternal urine

Currently, the research on NTD biomarkers in maternal urine is limited. A previous study revealed a higher detection rate of dibutyl phthalate (DBP) and its metabolite monobutyl phthalate (MBP) in the urine of pregnant women with NTD including spina bifida or anencephaly fetuses than in the control group. The positive detection rates for DBP and MBP in the NTD population were approximately 48.78 % and 21.95 %, respectively. These findings suggest a potential association between DBP exposure and NTDs. Although the exact pathogenic mechanisms remain unclear, this correlation has been substantiated in animal experiments, indicating a plausible link to oxidative stress [56]. These discoveries have not only enhanced our understanding of the pathogenic mechanisms of NTDs but have also provided valuable insights into the molecular biology underpinnings and pathophysiological mechanisms of NTDs.

3.4. Molecular biomarkers derived from other samples

In a recent study by Yang et al. investigating differentially N6-methyladenosine (m6A)-modified long non-coding RNAs (lncRNAs) and genes involved in NTD development, pregnant Kunming mice (9–10 weeks old) were treated with retinoic acid to create NTD models. The research found that METTL3-mediated m6A modifications may play a role in the development of NTDs. Specifically, reduced expression levels of Mir100hg, Gm19265, Gm10544, Malat1, Zfp236, Erc2, and Hmg20a may contribute to NTD formation. However, this study is still at the animal experimentation stage, and the samples were derived from brain tissue. There remains a significant gap in its applicability to prenatal diagnosis in humans. As prenatal molecular biomarkers for NTDs, these findings still require further research [57].

4. Combined diagnosis through ultrasound and biomolecular markers

Given the limitations of molecular biomarkers and ultrasound detection, their combination is expected to enhance the NTD diagnostic efficiency. In the 1980s, studies suggested the effectiveness of combining serum and amniotic fluid AFP measurements with ultrasonography for the prenatal diagnosis of NTDs, especially in cases with a positive family history. This integrated diagnostic approach appeared to be the most efficient method for detecting severe conditions. Early pregnancy ultrasound screening of the general population, followed by further assessment using serum or amniotic fluid AFP testing, can be applied in high-risk mothers [58]. Bernard et al. [59] assessed the combined use of BPD, AFP, and other serum markers for screening fetal open spina bifida cases during the 11–13-week period. They found higher median AFP levels in affected pregnancies (1.201 MoMs) than in unaffected pregnancies. The median free beta-human chorionic gonadotropin (β-hCG) level was reduced to 0.820 MoMs, while pregnancy-associated plasma protein levels were similar in cases and controls. Modeling predicted that using BPD alone can detect 50 % of cases at a 5 % false-positive rate or 63 % of cases at a 10 % false-positive rate. Adding AFP increases detection by 2 %; the combined application of BPD, AFP, and free β-hCG can detect 58 % of cases at a 5 % false-positive rate or 70 % of cases at a 10 % false-positive rate. Therefore, they proposed that the combined use of AFP, BPD, and free β-hCG in first-trimester aneuploidy screening enables early detection of approximately two-thirds of open spina bifida cases.

5. Conclusions and outstanding questions

We conducted a comprehensive review of early-pregnancy ultrasound markers and molecular biomarkers from various maternal specimens (including maternal serum, urine, and amniotic fluid) associated with prenatal NTDs, particularly OSB. Among the early-pregnancy fetal assessments, ultrasound examinations are the most promising because of their accessibility and ability to provide intuitive observations of structural fetal abnormalities with high sensitivity and specificity. However, factors such as fetal position and maternal body mass index can limit the accuracy of these evaluations, and early ultrasound markers are often limited in their association with OSB. The success of ultrasonography is largely hampered by variations in equipment quality, operator proficiency, and standardization. Therefore, identifying potential biomarkers in maternal fluids for accurate and operator-independent screening of fetal NTDs would contribute significantly to prenatal diagnosis.

The current research is limited to specific types of biomarkers, with most studies deriving results from a single healthcare institution. Organic integration of diverse biomarkers from various sources has the potential to enhance diagnostic accuracy. However, research on the combination of ultrasound markers and biomarkers remains scarce. Future studies should aim to establish more

effective multimodal models for the prenatal diagnosis of NTDs through multicenter, large-sample investigations integrating biomarkers from diverse sources. By harnessing the power of bioinformatics and rapidly evolving artificial intelligence technologies, multidimensional research designs can enhance our understanding of the diagnostic mechanisms underlying NTDs. This approach holds promise for clinical applications and facilitate the development of precise, early diagnosis and treatment strategies for NTDs.

Currently, early diagnosis of NTDs primarily focuses on open types such as OSB, MMC, and anencephaly. These conditions are typically diagnosed and monitored effectively using ultrasound markers and molecular biomarkers. However, closed neural tube defects present significant challenges in early diagnosis. Due to the absence of cerebrospinal fluid leakage, closed NTDs often do not exhibit typical ultrasound features early on, and the concentration changes of biochemical markers are extremely limited, thereby restricting accuracy and reliability. This complexity makes early diagnosis more complicated and uncertain. Future research should emphasize improving early diagnosis of closed NTDs. Exploration of novel biomarkers is recommended, alongside further advancements and application of advanced ultrasound technologies such as 3D and 4D ultrasound, which promise to enhance sensitivity and specificity for closed NTDs. Methodologically, broader multicenter studies integrating data from various biomarker sources should be promoted to establish more precise multimodal diagnostic models. These efforts will provide scientific foundations for enhancing early prenatal diagnosis and intervention for closed NTDs.

The literature to date indicates that research on the early diagnosis of NTDs has largely been conducted after the 11th week of pregnancy. Early ultrasound biomarkers are typically assessed between 11 and 13 weeks of gestation, whereas molecular biomarkers are predominantly investigated during the mid-to-late gestational period. However, at this stage, neural tube development is largely complete, and the optimal window for prevention and treatment has been missed. Future research should focus on the application of more reliable ultrasound indicators and molecular biomarkers before the 11th week of pregnancy and should propose standardized diagnostic protocols for early pregnancy. For example, first, select ultrasound indicators that can be observed and measured before the 11th week of pregnancy, such as fetal brain vesicles, choroid plexus, and the fourth ventricle, which provide crucial information on fetal neural tube development. Second, prioritize molecular biomarkers with established research support, such as specific miRNAs, lncRNAs, etc., which can early demonstrate features associated with neural tube defects. Furthermore, recommend the development and optimization of integrated analysis algorithms that combine ultrasound measurements and biomarker detection results to significantly enhance the accuracy and reliability of neural tube defect diagnosis. This would provide a new diagnostic approach for early screening and diagnosis of fetal anatomical defects, with significant implications for optimal prenatal care.

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Ethics declarations

Review and/or approval by an ethics committee was not needed for this study because this is a review article that primarily summarizes and analyzes existing research and does not involve the collection of original data or any human/animal experiments.

Data availability statement

This article does not involve data and code availability. This article is a review article that does not include raw data. It primarily provides a summary and analysis of research status and progress.

CRediT authorship contribution statement

Jiao Yin: Writing – review & editing, Writing – original draft. **Yan Wang:** Investigation, Formal analysis, Data curation. **Sihong Wang:** Methodology, Investigation, Formal analysis, Data curation. **Gang Li:** Visualization, Validation, Supervision. **Hui Gu:** Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lizhu Chen:** Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors do not possess any financial or other competing interests that require disclosure.

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