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Role of Fetal Second Trimester 2D Ultrasound Facial Parameters in Down Syndrome Detection

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Abstract

Introduction Down syndrome (DS) is the most common genetic cause of intellectual disability in humans. Various screening techniques are available, including the detection of facial characteristics of DS fetuses by prenatal ultrasonographic markers. Very few studies have tested the predictive validity of the markers in the Indian population.

Objective This article determines the role of second trimester ultrasound in predicting DS using facial markers, namely, nasal bone length (NBL), prenasal thickness (PNT), NBL/PNT ratio, and PNT/NBL ratio in the Indian population

Materials and Methods This prospective observational study recruited singleton pregnant women at 14 to 28 weeks of gestation with no comorbidities. Women with maternal disorders, abnormal amniotic fluid, and fetal structural anomalies were excluded. Three images of the midsagittal plane were obtained during a routine anomaly or well-being scan. The single "best" image was used for analysis. Scatter plots with regression lines and percentile curves for each gestation were created. Developed nomograms and scattered plots were validated by recruiting DS fetuses (diagnosed by amniocentesis and fetal karyotype).

Results This study included 450 normal fetuses for developing nomograms, which were verified by matching 45 DS fetuses. The diagnostic accuracy of NBL, PNT, NBL/PNT ratios and PNT/NBL ratios was found to be 94, 99, 95, and 94.8, respectively. Sensitivity and specificity were found to be 51.11, 42.42, 48.89, 65.4 and 99, 96, 99.56, and 96.7 for NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio, respectively. False negative rate and false positive rate were 38.89, 57.58, 51.11, 34.6 and 1, 4, 0.44, 3.3 for NBL, PNT, NBL/PNT ratio, PNT/NBL ratio, respectively.

Conclusion High diagnostic accuracy was found for PNT, followed by NBL/PNT ratio, PNT/NBL ratio, and NBL. However, considering sensitivity and specificity markers together, we found the NBL/PNT ratio as a good diagnostic marker in predicting DS. Furthermore, the NBL/PNT ratio performs slightly better than its inverse counterpart (PNT/NBL ratio) for detecting DS fetuses, primarily because it produced less false positive cases.

Keywords
- Down syndrome
- ultrasound facial markers
- nasal bone length
- prenatal thickness
- NBL to PNT ratio
- PNT to NBL ratio

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Introduction

Down syndrome (DS) is a genetic disorder caused by the presence of an entire or a part of extra third chromosome 21. John Langdon Down, an English physician, first accurately described the constellation of clinical features of DS (phenotype) in 1866.[1] DS is often presented clinically with a brachycephalic head, hypotonia, epicanthic folds, upward slanting palpebral fissure, flat nasal bridge, mottled spots on the iris (Brushfield spots), excess skin at the nape of the neck, small ears, large appearing tongue, single transverse palmar crease, large space between first and second toes, and shorter fifth finger with clinodactyly (incurring of the finger).[2] DS is the most common genetic cause of intellectual disability in humans.[3] The incidence of DS is approximately 1 in 1,100 to 1 in 1,000 live births globally. Around 3,000 to 5,000 children are born with DS per annum.[4] Due to the association of DS with intellectual disability, physical deficits, and the fact that every pregnancy carries a small probability of a fetus with a chromosomal disorder, screening of pregnant women for suspected DS fetuses is recommended universally, irrespective of maternal age.[5] DS detection can be done in two ways: prenatal screening tests and prenatal diagnostic tests. Screening tests predict the probability of having a DS child, while diagnostic tests confirm the same. Screening tests include ultrasound (US) scanning or maternal blood tests or both. Prenatal US is used to screen for the presence of various morphological markers associated with DS.[6] Diagnostic testing of DS involves the collection of fetal samples of genetic material and looking for the presence of the extra chromosome 21.[7] Prenatal diagnostic tests carry a risk of miscarriage and are expensive and are, therefore, offered to all patients but usually resorted to only for screen positive cases.

Prenatal detection of DS remains a challenge. Although multiple US markers in first and second trimester scans are available for DS screening, unfortunately, none of them are highly sensitive. Due to cost constraints associated with noninvasive prenatal test, combined first trimester screening is the most widely used method for fetal aneuploidy risk calculation, with about a 90% detection rate.[8] Ten percent of DS cases remain undetected using this method, strengthening the need for evaluation of second trimester markers.[9] Second trimester markers also help mothers who have missed first trimester aneuploidy screening. Evidence suggests that among the second trimester US markers for DS screening, presence/detection of ventriculomegaly, nuchal fold thickness, and aberrant right subclavian artery (ARSA) predict a threefold to fourfold risk and with hypoplastic nasal bone (NB), a sixfold to sevenfold increase in the risk of DS.[10] Since this evaluation is subjective, efforts are made to objectify these parameters. Since DS fetuses present distinctive facial features, various facial parameters have been identified as second trimester markers. Both facial parameters (NB length [NBL] and prenasal thickness [PNT]) were identified and confirmed as second trimester DS markers.[11] The diagnostic value of each measurement alone appears to be moderate, but their combination can improve the detection of DS in the second trimester.[12] One such combination is the NBL/PNT ratio and the PNT/NBL ratio.

The current study aims to determine the role of second trimester US in detecting DS using facial markers, namely, PNT, NBL, NBL/PNT ratio, and PNT/NBL ratio in the Indian population. The efficacy of their ratio in the detection of DS has not been studied in the Indian population to the best of our knowledge. This study is the first of this kind till date.

Objectives

The primary objective of the study was to determine the diagnostic accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR), false negative rate (FNR), and false positive rate (FPR) of PNT, NBL, NBL/PNT ratio, and PNT/NBL ratio in the detection of DS. The secondary objective is to compare the diagnostic accuracy between the parameters.

Materials and Methods

This prospective observational study was performed from May 2018 to January 2022. The study was approved by the Institutional Ethical Committee (IEC ref No.: 38_2018). All pregnant women between 14 and 28 weeks of gestational age (GA), with a singleton pregnancy, no comorbid conditions, and normal fetal growth, were included in the study. Pregnant women with maternal medical disorders, multiple pregnancies, abnormal amniotic fluid, and fetal structural anomalies were excluded. Every pregnancy presenting to the outpatient obstetrics department who met our inclusion and exclusion criteria was explained the study objectives and their queries were cleared. Those who accepted participation and signed the informed consent form were recruited.

The sample size was calculated by taking a study by Szabó et al[13] as a reference and assuming the mean ratio of 1.5 and standard deviation (SD) of 0.375 and a true mean difference of 0.05. 441, subjects were required to formulate a nomogram and 450 subjects were included in our study for developing nomograms which were validated by matching 45 DS fetuses.

Fetal scans were done between 14 and 28 weeks of GA with routine anomaly scans between 18 and 24 weeks using a high-resolution two-dimensional (2D) US probe (GE Voluson E8, E10). The 2D midsagittal fetal facial image was obtained, and three images were acquired, the "best" of which was used for analysis. NBL and PNT were recorded. The settings used for imaging were low gain, medium dynamic contrast, and maximum magnification so that the fetal face occupied the entire screen. The diencephalon, NB, lips, maxilla, and mandible were used as reference points for correct measurements. PNT was measured as the shortest distance from the lower margin of the frontal bone to the outer surface of the overlying skin. NBL was measured between the margins of the proximal and distal ends of the ossification line on the NB. ►**Fig. 1** shows the midsagittal view of the fetal facial plane and the measurement of NBL

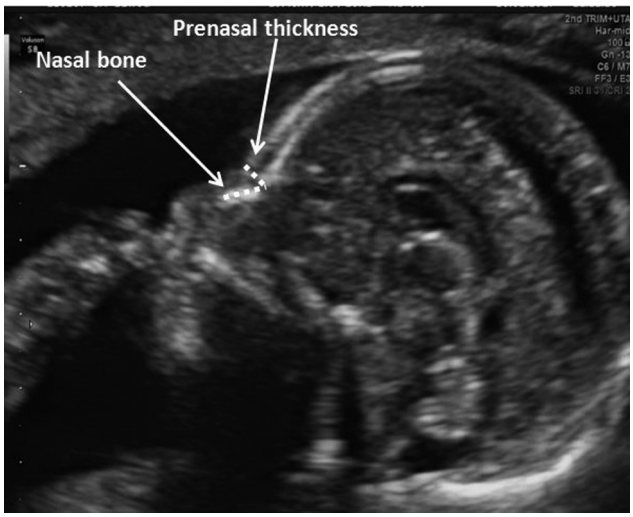

Fig. 1 Two-dimensional (2D) midsagittal plane used in measuring nasal bone length (NBL) and prenasal thickness (PNT).

and PNT. Maternal data and US findings were recorded in a database (Astraia software). US imaging data was stored in local digital imaging (Digital Imaging and Communications in Medicine) format.

The nomograms' percentiles weighted average method was used in the explore command. Scatter plots of NBL and PNT, with linear polynomial regression lines and percentile curves for each gestation, were created. The developed nomograms and scattered plots were validated by recruiting the DS fetuses (diagnosed by amniocentesis during the same study period) in a ratio of 10:1 (controls:cases). The detection rate of individual parameters was tested using receiver operating characteristic (ROC) analysis. Area under the curve (AUC), sensitivity, specificity, and diagnostic accuracy were compared between the markers to determine the most accurate marker.

Results

The study included 450 normal fetuses for the development of nomograms and 45 DS fetuses for its validation. The mean ± SD of maternal age of the former group was 28 ± 3 years, and 27 ± 1 years in the case of the latter. The mean ± SD of GA was found to be 28.3 ± 3 and 23.1 ± 2 weeks in normal and DS fetuses, respectively. Mean ± SD of NBL, PNT, and NBL/PNT ratio was found to be 5.11 ± 1.29, 2.66 ± 0.8, and 2 ± 0.53, respectively, in normal fetuses and 2.14 ± 1.76, 3.09 ± 1.18, and 0.77 ± 0.67, respectively, in case of DS fetuses with statistically significant difference between groups (p-value < 0.05) (►**Table 1**).

►**Supplementary Material S1–S4** (available in the online version) provides the nomograms of NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio of 3rd, 5th, 10th, 25th, 50th, 75th, 90th, and 95th percentile for GA 14 to 28 weeks.

The scattered plot of NBL and PNT for GA showed a steady linear increase in line with a correlation coefficient of r-value 0.56 and 0.59, respectively, which are statistically significant with a p-value of < 0.001, representing a moderate relationship between the variables. The other scatter plot of the NBL/PNT ratio and PNT/NBL ratio for GA showed a steady line with a correlation coefficient of r-value 0.03 and 0.06, which is statistically insignificant with a p-value of 0.45 and 019, representing a very weak relationship between the variables (►**Fig. 2**).

The validity of NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio as tested by the ROC curve reported AUC of 0.92, 0.595, 0.928, and 0.91, respectively (►**Fig. 3**).

With the predicted cutoff values of < 5th centile for NBL, > 95th centile for PNT, NBL/PNT ratio (< 5th centile), and PNT/NBL ratio (> 95th percentile), the diagnostic accuracy, sensitivity, specificity, PPV, NPV, PLR, NLR, FNR, and FPR were found to be 94, 51.11, 99, 83, 94, 47, 0.5, 38.89, and 1, respectively, for NBL; 99, 42.42, 96, 21, 92, 2.6, 0.9, 57.58, and 4, respectively, for PNT; 95, 48.89, 99.56, 92, 95, 110, 0.51, 51.11, and 0.44, respectively, for NBL/PNT ratio; and 94.8, 65.4, 96.7, 56.7, 97.7, 20.068, 0.358, 34.6, and 3.3 for PNT/NBL ratio (►**Table 2**).

We tried to test the efficacy of the variables in the 2.5th and 97.5th centiles. However, in the current study, none of the case groups had the measure of that centile and, therefore, were unable to be tested and left for future studies.

Discussion

Prenatal US-based detection aims at the identification of various distinctive characteristics of a DS child as described by John Langdon Down, and includes some of the unique facial features like a flat face, absent or small NB, and depressed nasal bridge. Since this evaluation is subjective, efforts should be made to objectify these parameters as US markers for DS screening.

Table 1 Comparison of demographic details and US markers between the groups

Parameter	Normal fetuses (N = 450)	DS fetuses (N = 45)	p-Value
Age	28.65 ± 4.17	31.49 ± 6.19	< 0.001
Gestational age (in weeks)	20.17 ± 2.59	19.76 ± 3.08	0.329
NBL	5.11 ± 1.29	2.14 ± 1.76	< 0.001
PNT	2.66 ± 0.8	3.09 ± 1.18	0.001
NBL/PNT ratio	2 ± 0.53	0.77 ± 0.67	< 0.001
PNT/NBL ratio	0.51 ± 0.13	0.95 ± 0.37	< 0.001

Abbreviations: DS, Down syndrome; NBL, nasal bone length; PNT, prenasal thickness; US, ultrasound.

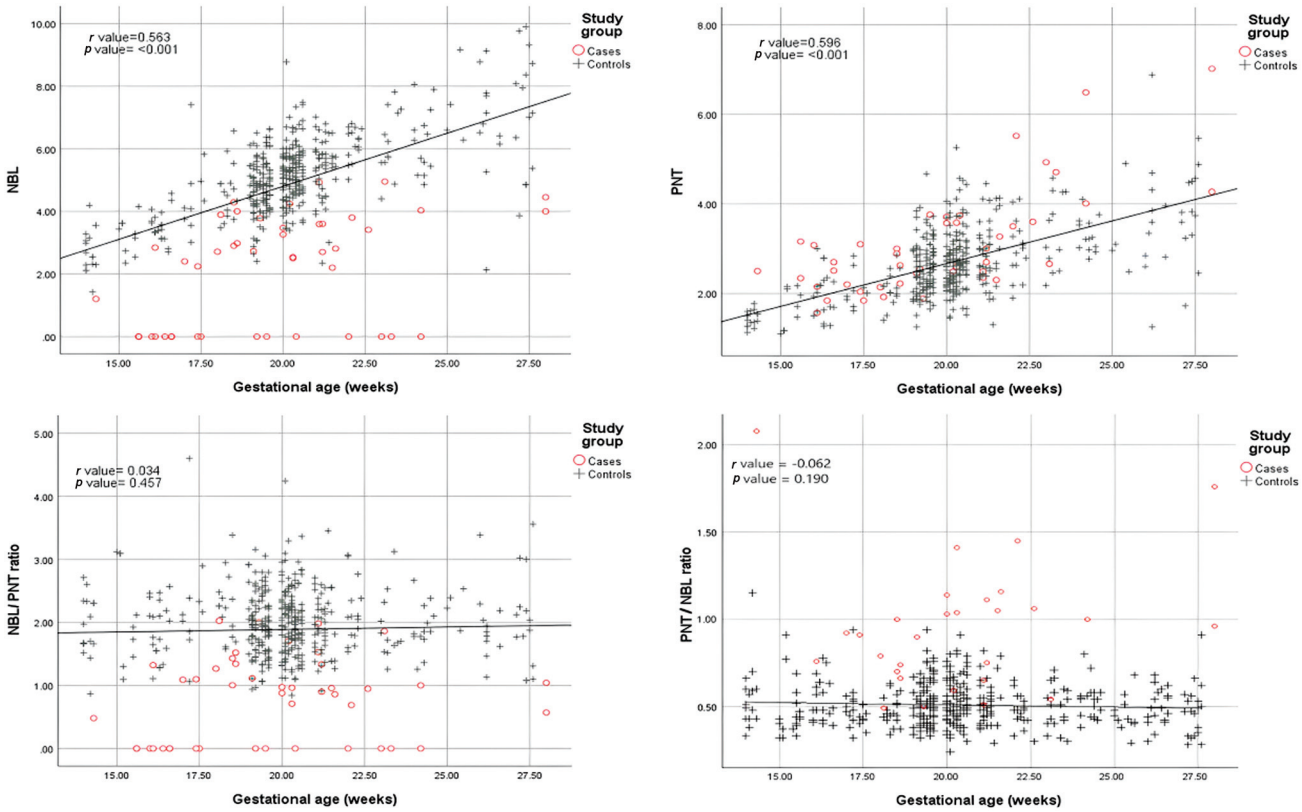

Fig. 2 Scatter plots of nasal bone length (NBL) against gestational age (GA), prenasal thickness (PNT) against GA, and NBL/PNT ratio against GA.

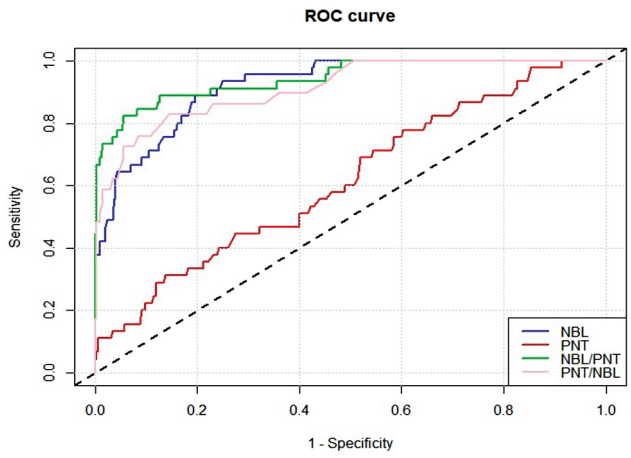

Fig. 3 Receiver operating characteristics curve of nasal bone length (NBL), prenasal thickness (PNT), NBL/PNT ratio, and PNT/NBL ratio.

These facial markers include PNT, prefrontal space ratio, NBL, PNT to NBL ratio, NBL to PNT ratio, and angles like frontomaxillary facial angle, frontonasal facial angle, maxilla-nasion-mandible angle, and mandibulomaxillary facial angle.[14] NBL as a second trimester marker of DS was first introduced in 1995 by Guis et al.[15] It is already an established marker for prenatal screening of DS, with a detection rate of 61.8% and an FPR of 1.2%.[11] PNT was proposed first by Maymon et al in 2005[12] and confirmed by several studies[16–19] as a marker for DS screening in the second trimester. Both measurements are taken in the same midsagittal profile view in 2D or three-dimensional US volumes.[20] The diagnostic value of each measurement alone appears to be moderate,[12] but their ratio of NBL/PNT can improve the detection of DS in the second trimester.[16–20]

The current prospective observational study performed to determine the diagnostic validity of second trimester fetal

Table 2 Comparison of US markers for diagnostic validity as reported by ROC analysis

US parameter	DA (%)	Sensitivity (%)	Specificity (%)	PPV	NPV	PLR	NLR	FNR (%)	FPR (%)
NBL (< 5th centile or absent)	94	11.11	99	83	94	47	0.5	88.89	1
PNT (> 95th centile)	99	42.42%	96	21	92	2.6	0.9	57.58	4
NBL/PNT ratio (< 5th centile)	95	48.89	99.56	92	95	110	0.51	51.11	0.44
PNT/NBL ratio (> 95th percentile)	94.8	65.4	96.7	56.7	97.7	20.068	0.358	34.6	3.3

Abbreviations: DA, diagnostic accuracy; FNR, false negative rate; FPR, false positive rate; NBL, nasal bone length; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PNT, prenasal thickness; PPV, positive predictive value; ROC, receiver operating characteristic; US, ultrasound.

facial US markers (NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio) in detecting DS fetuses included 450 normal fetuses in developing the nomograms, which were validated by matching with 45 DS fetuses, as confirmed with amniocentesis and fetal karyotype. The mean ± SD of the maternal age was 28.65 ± 4.17 and 31.49 ± 6.19 years, and that of gestation age was 20.17 ± 2.59 and 19.76 ± 3.08 weeks among normal fetuses and DS fetuses, respectively. The correlation between NBL and PNT with GA was found to be moderate, with a positive linear increase and *r*-values of 0.56 and 0.59, respectively, which were statistically significant with *p*-values < 0.001. Correlation with that of NBL/PNT ratio and PNT/NBL ratio with GA was found to be weak with a steady line and *r*-value of 0.03 and 0.06, which was statistically insignificant with a *p*-value of 0.45 and 019. The diagnostic accuracy of NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio was found to be 94, 99, 95, and 94.8, respectively. Sensitivity and specificity were found to be 51.11, 42.42, 48.89, and 65.4 and 99, 96, 99.56, and 96.7 for NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio, respectively. FNR and FPR were 38.89, 57.58, 51.11, and 34.6 and 1, 4, 0.44, and 3.3 for NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio, respectively.

A retrospective study performed by Bernardeco et al reported that the NBL in the first and second trimesters was associated with aneuploidy.[21] A study conducted in the Thai population reported statistically significant differences in the NBL, PNT, and NBL/PNT ratio between normal and DS fetuses, which is in consonance with the current study reports.[22] A retrospective study by Vos et al in the Netherlands reported the correlation coefficient of NBL and PNT against GA as 0.69 and 0.74, which is closely aligned with the findings of the current study. The same study reported the diagnostic accuracy of NBL and PNT as 61.9 and 63.4, respectively, which are far less than that of the current study, interpreted as due to the difference in the population characteristics.[23]

A retrospective study in the Afro-Caribbean population reported a weak correlation of NBL to GA as 0.354. In contrast, the current study found a moderate correlation of 0.56, which is not in line.[24] This correlation differences can also be plotted to the difference in the population characteristics. A prospective study conducted in the Caucasian population by Szabó et al reported sensitivity of 75.75, 75.75, and 96.97 for NBL, PNT, and NBL/PNT ratio, which are not in great understanding with the current study results. However, the specificity reported in the same study was found to be in line.[13]

Several other US parameters have been found to be efficacious in the early identification of DS. Since it is very crucial for risk stratification of DS fetuses and better fetal assessment, a meta-analysis performed by Agathokleous et al to identify the US markers associated with DS has reported that intracardiac echogenic focus, ventriculomegaly, increased nuchal fold, hyperechogenic bowel, short femur and humerus, ARSA, and absent or hypoplastic NB were associated. The study concluded that the absence of these markers would decrease the risk of DS.[10] However, in contrast to these findings, highlighting isolated absent NB in the second trimester and its association with the DS was found to be unlikely, as per the study conducted by Singh et al.[25] Similar confrontations exist for all the variables in the evidence. Hence, to draw an endpoint, the study performed by Rumi Kataguiri et al reported that the risk of DS would increase by 10.5 times in the presence of a single parameter and by 13.5 times in more than one parameter.[26] Adding to this further, in identifying the potential second trimester US markers, we evaluated the facial parameters NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio, which were found to have better diagnostic accuracy and were also strengthened by a few other similar study findings, encouraging the clinicians to include this parameter as well to predict the risk.

The studies in disagreement with the current findings were found to be performed in a population with different sociodemographic characteristics, emphasizing the necessity of evaluating the efficacy of these parameters in different population characteristics before implementation in clinical care protocols. In this instance, the current study performed in India has proved that second trimester US markers NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio can be deployed in the Indian clinical settings for the early identification of DS fetuses, aiding in the counseling of parents and allowing them to take a decision on whether to prepare for a child with a chromosomal abnormality or terminate the pregnancy.[27]

Furthermore, the NBL/PNT ratio performs slightly better than its inverse counterpart (PNT/NBL ratio) for detecting DS fetuses, primarily because it produced less false positive cases.[13] In our study, we also found that PNT/NBL has a FPR of 3.3%, which was higher when compared with the NBL/PNT ratio of 0.4%. It indicates that the NBL/PNT ratio is a better US predictor for T21 detection in the second trimester. The added advantage of the NBL/PNT ratio is that it can be calculated in all cases of both hypoplastic and absent NBs. PNT/NBL (reverse of NBL/PNT ratio) cannot be calculated in cases with absent NB, as the denominator is zero, making the ratio infinity.

The current study has a limitation of a smaller sample size for the validation of nomograms. We recommend future studies to validate the nomograms in different ethnic groups and use a larger sample size to empower these nomographs further.

Conclusion

This 2D US study demonstrates that second trimester fetal facial US markers (NBL and PNT) measurements should be incorporated into routine second trimester anatomy scans, and their ratios appear to be highly sensitive and specific markers for euploid and DS fetuses. The diagnostic accuracy, sensitivity, specificity, PPV, NPV, PLR, NLR, FNR, and FPR of NBL, PNT, NBL/PNT ratio, and PNT/NBL ratio were evaluated, and showed a high diagnostic accuracy for PNT followed by NBL/PNT ratio, PNT/NBL ratio, and NBL. However, considering the sensitivity and specificity of markers together, we found the NBL/PNT ratio to be a good diagnostic marker in predicting DS.

Furthermore, the NBL/PNT ratio performs slightly better than its inverse counterpart (PNT/NBL ratio) for detecting DS fetuses. It produced fewer false positive cases and can be used in cases where the NB is absent. If the NBL/PNT ratio is

less than the fifth centile, a search for other aneuploidy soft markers and invasive fetal testing should be considered.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, Dr. Arati Singh, upon reasonable request.

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None.

Conflict of Interest

None declared.

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