Research Article

The Impact of Nasal Bone Reporting on Down Syndrome Prognosis: A Prospective Study

Maryam Rahnama [1,2], Atefeh Manzoury [1], Neda Rashidmehr [1], Navid Ghasemzadeh[3], Majid Mojarrad [1, 4, *]

[1] *Genetic Foundation of Khorasan Razavi, Mashhad, Iran.*
[2] *Department of Applied Cell Sciences, School of Medicine, Urmia University of Medical Sciences, Urmia, Iran.*
[3] *Department of Clinical Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.*
[4] *Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.*

Article info:
Received: 3 July 2025
Revised: 30 August 2025
Accepted: 6 September 2025

[*] **Corresponding Author:**

Majid Mojarrad
Genetic Foundation of Khorasan Razavi, Mashhad, Iran
Email: majidmojarrad12@gmail.com

ABSTRACT

Objectives: Accurate prenatal screening for Down syndrome (trisomy 21) is essential for effective maternal-fetal management. The nasal bone (NB) is a critical marker in first-trimester ultrasound assessments, yet reporting inconsistencies can affect prognostic outcomes. This study aims to investigate the impact of reporting the presence or absence of the nasal bone on Down syndrome prognosis in a cohort of pregnant women.

Methods: A prospective study was conducted involving women undergoing contingent prenatal screening during the first trimester. Participants with documented present NB were classified as having unknown NB, while those with unknown reports were treated as having present NB. The risk of Down syndrome was assessed using Benetech-PRA software, and all flagged cases were monitored postpartum to evaluate outcomes.

Results: Of the fetuses assessed, only 5% were diagnosed with Down syndrome. Approximately 7.3% of sonographers reported unknown NB, which was associated with a false-positive screening rate of 16±1%. The findings indicate that NB reporting significantly influences prognostic outcomes, particularly in cases where the NB status is unclear.

Conclusion: Accurate examination and reporting of the nasal bone are critical for reliable Down syndrome prognosis. Inadequate or incorrect NB assessment can lead to misleading results and increased false-positive rates in prenatal screening, underscoring the need for standardized reporting practices in ultrasound examinations.

Keywords: Contingent prenatal screening test, Down syndrome, Nasal bone, Trisomy 21.

Use your device to scan and read the article online

Citation: Rahnama M, Manzoury A, Rashidmehr N, Ghasemzadeh N, Mojarrad M. The Impact of Nasal Bone Reporting on Down Syndrome Prognosis: A Prospective Study. Acta Biochimica Iranica. 2025;3(3):189-196.

https://doi.org/10.18502/abi.v3i3.20592

Introduction

Congenital anomalies are significant contributors to neonatal mortality, disability, childhood health issues, and long-term morbidity, as reported by the World Health Organization (WHO). Among these conditions, Down syndrome (trisomy 21) is one of the most prevalent congenital anomalies, occurring in approximately 1 in every 700 to 1,000 live births (1) . Unlike many congenital disorders, Down syndrome allows for survival beyond birth; however, it is associated with a range of serious medical complications. These challenges not only affect the health and quality of life of individuals with Down syndrome but also impose substantial economic and emotional burdens on families and society at large(2) .

Several laboratory tests and sonographic screenings are employed to detect and assess chromosomal abnormalities in the first trimester of gestation. Key markers include the measurement of the nasal bone (NB), nuchal translucency (NT), and serum biomarkers such as free β-human chorionic gonadotropin (free β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), typically assessed between 11 and 13 weeks plus 6 days of gestation. Studies indicate that these screening methods achieve detection rates exceeding 80%, accompanied by a false-positive rate ranging from 3% to 10% (3, 4).

Notably, hypoplasia of the nasal bone is correlated with trisomy 21, both prenatally and postnatally. Sonographic studies reveal that approximately 64% to 67% of fetuses diagnosed with trisomy 21 exhibit either a shortened or absent nasal bone in their ultrasound reports. This association underscores the importance of accurate sonographic evaluation in early prenatal screening for chromosomal abnormalities (5). At six weeks of gestation, the nasal bone (NB) begins to develop from aggregations of neural crest cells and undergoes ossification through intramembranous processes. Initially, the NB forms as two distinct structures separated by a gap that progressively narrows as pregnancy advances. Despite this gap's presence early in gestation, it does not impede the ability to differentiate between the absence and presence of the nasal bone during prenatal sonographic examinations (6, 7). By 11–14 weeks of gestation, the NB is not visible in sonographic assessments in less than 1% of chromosomally normal fetuses, while approximately 70% of fetuses with trisomy 21 exhibit absent nasal bones (8, 9) . Consequently, many sonographers report either absent or unknown NB findings in their evaluations. The implications of these reporting practices are significant, as various parameters including NB reports, serum markers, gestational age, and maternal age are integrated into prenatal prognosis software tools (10) .Inaccuracies in NB assessments can adversely affect congenital anomaly outcomes, leading to false-positive results that may induce unnecessary anxiety for expectant mothers and prompt invasive prenatal diagnostic procedures (9) .

The primary objective of this study was to determine the diagnostic accuracy of incorporating NB assessment as a secondary ultrasound marker alongside NT, free β-hCG, and PAPP-A in first-trimester screening for trisomy 21, using data from a prospective cohort of pregnant women in Northeast of Iran. Specifically, we aim to evaluate how NB reporting influences overall screening efficacy, including detection rates and false-positive rates, in this regional population.

Materials and Methods

Study Design and Participants

A prospective study was conducted from March 2021 to March 2022 involving 5,449 women referred to the Genetic Foundation of Khorasan Razavi (GFKR) for contingent prenatal screening during the first and second trimesters of gestation. Comprehensive data were collected from all participants, including maternal age, gestational age, smoking status, blood grouping, medical history (including conditions such as hypertension, thyroid disorders, and diabetes), history of in vitro fertilization (IVF), previous abortions (spontaneous versus induced), and ultrasonography findings (NT and NB visibility). The inclusion criteria specified women with a gestational age between 11 weeks and 6 days to 13 weeks, while those with a gestational age exceeding 14 weeks were excluded from the study. This research adhered to the ethical standards set forth by the Iranian Ministry of Health and Medical Education regarding human experimentation and was conducted in accordance with the principles outlined in the Helsinki Declaration. All participants provided informed consent prior to their inclusion in the study.

Prenatal serum markers and NB examination

Blood samples were collected in first trimester of gestation for serum biomarkers (double marker tests) analysis. The first trimester serum biomarkers are maternal free β-hCG and PAPP-A serum levels. For this purpose, free β-hCG and PAPP-A were measured using Electro chemiluminescence assay (ECL) by Cubase E411, (Roche Germany), according to the manufacturer's instructions (Gene med Biotechnologies, South San Francisco, CA, USA). NB examination was performed in ultrasonography clinic by medical sonographers.

Down syndrome risk estimation

A risk of Down syndrome was calculated using Benetech-PRA software (version: 3.3, Benetech company, Canada) based on information obtained from PAPP-A and β-hCG serum levels and pregnant women information. The prognosis was conducted according to the software instructions. The threshold for determination of Down syndrome risk in the first-

trimester screening tests was accepted to be 1:270, as per the standard cutoff in Benetech-PRA for identifying high-risk cases equivalent to the age-related risk of trisomy 21 in a 35-year-old woman, and any women who had a risk greater than the established cutoff were considered high-risk for Down syndrome. Then, to evaluate the impact of potential misclassification in NB reporting on Down syndrome risk estimation, we conducted a sensitivity analysis by inverting the NB status in the Benetech-PRA software. Specifically, for women with ultrasound reports indicating a present NB, we reclassified it as "unknown"; conversely, for those with "unknown" NB reports, we reclassified it as "present." The Down syndrome risk was then recalculated for these simulated scenarios to compare outcomes against the original classifications and assess the effect on congenital anomaly screening results.

Follow up of patients with Down syndrome screening

For further examination, all high-risk cases underwent amniocentesis for confirmation of Down syndrome during pregnancy. Additionally, to ensure completeness, contact was made with mothers postpartum, and in cases of suspected or diagnosed affected infants, karyotype results were requested and reviewed.

Statistical analysis

Statistical analyses were performed using SPSS v.16 (SPSS Inc, Chicago, IL, USA), employing the Shapiro-Wilk test for normality assessment and Levene's test for variance homogeneity; categorical variables were evaluated via chi-square test, while continuous variables underwent independent t-tests or Mann-Whitney U tests for group comparisons and paired t-tests or Wilcoxon signed-rank tests, with statistical significance set at $P < 0.05$.

Results

Demographic data

Among 5,449 pregnant women referred to the GFKR, 72.6% (3,958/5,449) were in the first trimester of gestation, and 27.4% (1,491/5,449) were excluded due to gestational age exceeding 14 weeks. The demographic data were summarized in Table 1. Key findings include a mean maternal age of 28 ± 0.5 years, gestational age of 12 weeks ± 5 days, mean free β-hCG level of 44.5 ± 4 ng/mL, mean PAPP-A level of 9.69 ± 0.85 µg/mL, and low prevalence of risk factors such as positive Down syndrome history (0.1%), IVF (0.9%), and twin pregnancies (1.9%).

Double markers and NB examination results

Serum double markers were performed on 3958 pregnant women who were in first trimester of gestation. The mean free β-hCG and PAPP-A serum levels of patients were 44.62±4 and 9.69±0.85, respectively. In NB

Table 1. Demographic data of studied subjects

All participants in the study	Gestation age	Excluded participants	Included participants
5449	First trimester	None of them	3958 (72.6%)
	Second trimester	1491 (27.4%)	None of them
Variables		n*: 3958	
		Minimum	Maximum	Mean
Maternal age (year)		14	45	28±0.5
Gestational age (week+day)		11w+1d	13w+6d	12w±5d
Freeβ-hCG (ng/ml)		1.34	498	44.5±4
PAPP-A (µg/ml)		0.1	47.5	9.69±0.85
NT thickness (mm)		0.5	2.2	1.8±0.15
History		n: 3958		
		Results	Number	%
Dawn syndrome history		Negative	3955	99.9
		Positive	3	0.1%
Neural Tube Defects (NTD) history		Negative	3954	99.9%
		Positive	4	0.1%
Diabetes history		Negative	3940	99.5%
		Positive	18	0.5%
Smoker history		Negative	3955	99.9%
		Positive	3	0.1%
Rh blood grouping		Negative	315	7.9%
		Positive	2066	52.2%
		Unknown	1582	39.9%
IVF history		Negative	3924	99.1%
		Positive	34	0.9%
Singleton history		Singleton	3885	98.1%
		Twins	73	1.9%

*Pregnant women

terms, about 92.2%, 0.5% and 7.3% cases were reported as present, absent and unknown NB, respectively. All results were shown in table 2.

Effect of presence or unknown NB reports on Down syndrome screening

To investigate the reporting of present or unknown NB in Down syndrome screening, all women with present NB reports were considered as unknown NB. Conversely, women with unknown NB reports were considered as present NB. The chi-square analyses revealed that NB reports have a significant effect on Benetech-PRA software outcomes in women with unknown NB reports ($\chi^2 = 19.00$, df = 1, $P < 0.001$), but no similar effect in women with present NB reports ($\chi^2 = 5.00$, df = 1, $P = 0.025$), as shown in Fig. 1 and Table 3.

Final Down syndrome risk

Down syndrome diagnosis in pregnant women in first trimester of gestation using Benetech-PRA software revealed that 140 individuals had positive results. In NB examination terms, 75.7% (106/140), 18.5% (26/140) and 5.8% (8/140) of pregnant women were reported as present, unknown and absent NB, respectively (Fig. 2). All 140 individuals were followed up during the pregnancy period and postpartum, with no cases lost to follow-up. At the end of follow-up, it was found that only 5% (7/140) of fetuses had Down syndrome. Among fetuses with Down syndrome, 4, 2 and 1 pregnant women were reported as present,

Table 2. Double markers and NB examination results.

Double markers (n: 3958)		NB examination (n: 3958)		
Free β-hCG (ng/ml)	PAPP-A (µg/ml)	Present	absent	unknown
44.5±4	9.69±0.85	3648 (92.2%)	19 (0.5%)	291 (7.3%)

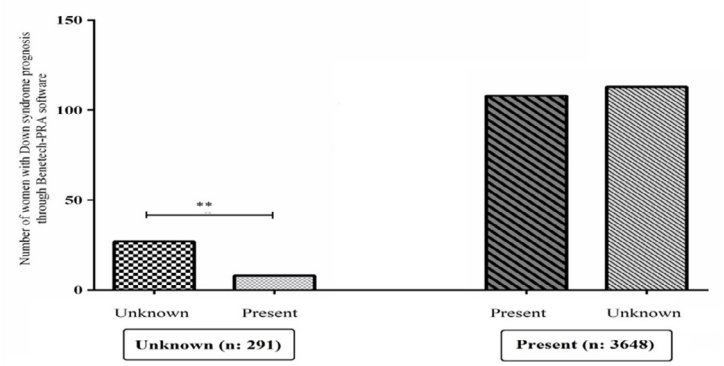

Figure 1. The chi-square analyses revealed that NB reports have significant effect on Benetech-PRA software outcomes in women with unknown NB reports, but no similar effect in women with present NB reports. (** P <0.001).

Table 3. The effects of reporting NB on Down syndrome screening outcomes.

NB reports	Down syndrome prognosis through Benetech-PRA software outcomes	
	with present NB	with unknown NB
Present (n: 3648)	108 (2.9%)	113 (3%)
Unknown (n: 291)	8 (2.7%)	27 (9.2%)

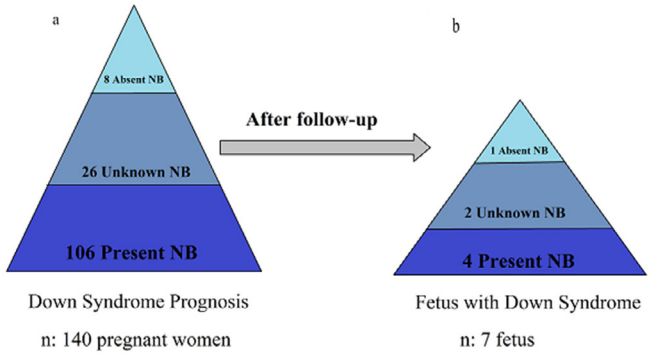

Figure 2. Down syndrome screening in 140 pregnant women in first trimester of gestation using Benetech-PRA software revealed that all of them had positive Down syndrome screening and 106 (75.7%), 26 (18.5%) and 8 (5.8%) pregnant women were present, unknown and absent NB reports, respectively (a). At the end of the follow-up, it was found that only 7 (5%) fetuses had Down syndrome which 4, 2 and 1 fetuses were present, unknown and absent NB reports, respectively (b).

Table 4. Serum marker and ultrasonic results from 7 women with Down syndrome pregnancy.

Patient No.	Maternal age	Gestational age	Rh blood grouping	NB reports	NT size (mm)	Freeβ-hCG (ng/ml)	PAPP-A (µg/ml)
1	26	12w+5d	Negative	Absent	2.98	39	1.7
2	27	13w+6d	Negative	Present	1.91	75.8	11.3
3	40	12w+1d	Positive	Unknown	2.93	248	5
4	40	13w+1d	Positive	Unknown	1.35	75.9	0.9
5	42	12w+1d	Positive	Present	1.8	42.8	11.3
6	40	13w+1d	Positive	Present	1.83	138	0.7
7	45	13w+5d	Positive	Present	1.48	67.6	2.8

Table 5. The effect of NB reporting on Down syndrome screening.

Patient No.	The effect of NB reporting on Down syndrome screening		
	NB reports	Benetech-PRA software outcomes	
		Risk with present NB report	Risk with unknown NB report
1	Absent	1:223	1:8
2	Present	1:38	1:20
3	Unknown	1:8	1:8
4	Unknown	1:14	1:8
5	Present	1:49	1:39
6	Present	1:41	1:8
7	Present	1:48	1:12

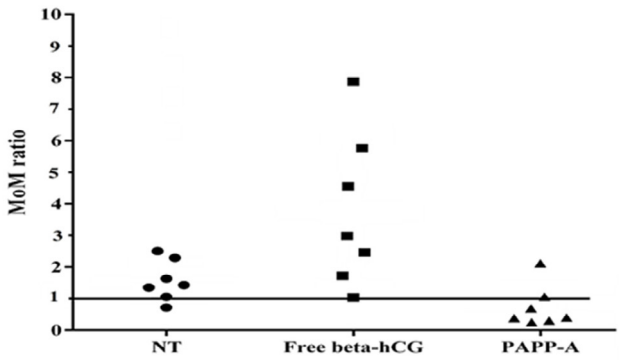

Figure 3. Distribution of NT, free β-hCG and PAPP-A MoM

Table 6. The MoM results from 7 women with Down syndrome pregnancy.

Patient No.	Mean NT size (mm)	NT MoM	Mean Freeβ-hCG (ng/ml)	Freeβ-hCG MoM	Mean PAPP-A (µg/ml)	PAPP-A MoM
1		2.30		1		0.4
2		1.35		4.55		0.38
3		2.5		7.87		2.12
4	2	1	98.1	2.47	4.8	0.25
5		1.65		1.74		1
6		1.43		5.78		0.3
7		0.72		2.99		0.72
Mean ± SD	-	1.56 ±0.65	-	3.77 ± 2.43	-	0.74 ± 0.66

unknown and absent NB, respectively (Fig. 2 and Table 4). Recoding the NB status in Benetech-PRA software (comparing risks assuming "present" vs. "unknown" NB) for these 7 confirmed cases demonstrated variable impacts on calculated Down syndrome risk; for instance, switching to "unknown" NB often increased risk (e.g., from 1:223 to 1:8 in one case), highlighting potential overestimation in screening outcomes (Table 5). The sonographers reported that about 7.3% (291/3958) of unknown NB reports resulted in 16±1% false-positive Down syndrome prognosis. In addition, the Multiple of the Median (MoM) values resulting from 7 pregnant women with a Down syndrome newborn were shown in Fig. 3 Table 6.

Discussion

Present studies performed on first trimester maternal serum biomarkers has shown that the double marker test helps to identify 90% of women with high risk for trisomy 21(11). The first trimester screening is performed at the 11-13w+6d of gestation. The serum double markers are used in the first screen test for Down syndrome diagnosis. Maternal serum levels of free β-hCG and PAPP-A are increased and decreased in Down syndrome, respectively (4) .(12) Our results showed that the serum level markers may be normal in some pregnant women with Down syndrome pregnancy (Table 4 and 6); however, total mean free β-hCG (98.1 ng/ml) and PAPP-A (4.8 µg/ml) were higher and lower than normal mean range (Normal mean range: 44.62 ng/ml and 9.11µg/ml, respectively).

The second screening test is sonographic examination including measurement of NT thickness and NB examination. The NT measurement needs to be conducted by experienced sonographers and should be obtained at the 11-13w+6d of gestation. Most fetuses with Down syndrome exhibit increased NT thickness in comparison with normal fetuses at the same gestational age(13). Nevertheless, the role of ethnicity and region on the value of NT is highly controversial. Several studies reported that ethnicity has significant effects on the value of NT(14) and suggested that region and ethnic reference value of NT could have impact on prenatal screening efficacy(15). In our study, the NT examination results revealed that NT thickness could be normal in some fetuses with Down syndrome (Table 4 and 6); however, the average of NT (2 mm) was higher than normal mean range (Normal mean range: 1.7 mm). In NB examination terms, most children with Down syndrome have a low nasal bridge and according to the sonographic studies(16), approximately 64-67% of fetuses with Trisomy 21 have short or absent NB in sonographic reports(17). Different studies showed that at the 11–14 weeks of gestation, the NB is not visible in the sonographic screening in about 60-70% and 0.1-1% of fetuses with trisomy 21 and normal chromosome, respectively. However, NB might be present in fetuses with trisomy 21(18-20). The measurement of NT and NB examination serves as an objective technique for prenatal Down syndrome diagnosis (21){Sasaki, 2021 #2394}. The sensitivity of NB sonographic findings in the second trimester of gestation is 77.7% at a 0.9% false-positive rate in screening for Down syndrome(22, 23). In study by Cicero. S. *et al.*, it was demonstrated that the minimum number of scans required to become competent in examining the NB is about 80 with a range of 40–120 scans for expert sonographers(24) .Therefore, many medical sonographers report an absent or unknown NB in their sonographic reports.

Since many parameters such as NB reports, serum markers, pregnancy age and maternal age are used in prenatal screening software tools, the failure in sonographic examination of NB could impact on Down syndrome outcomes and lead to false-positive results (9, 10, 25). This will expose the pregnant women to excessive stress and invasive prenatal diagnostic tests, both of which may cause irreparable harm to fetus. Another study by Cicero. S. *et al.* stated that a present or absent fetal NB report has a major impact on the estimated risk for Down syndrome in prenatal screening, which results in the patient's decision for or against invasive testing(18). Moreover, the NB examination is more difficult compared to the measurement of NT, and consequently, it is obligatory that clinical sonographers, who examine the fetal profile, receive appropriate training in performing tests such as scans(26, 27). In the current study, about 14.2% (1/7) and 28.5% (2/7) of Down syndrome fetuses were reported with absent and unknown NB. Overall, the NB was not visible in about 41.7% (3/7) of fetuses with Down syndrome. The study by Larose. C. *et al.* reported that the NB was absent in 47.6% (10/21) of the fetuses with trisomy 21 in the X-ray examination(28). In our study, the sonographers reported about 7.3% (291/3958) of unknown NB reports resulted in 16±1% false-positive Down syndrome diagnoses; in addition, the correct NB examination had reportedly a significant effect on Down syndrome diagnosis in fetuses with unknown NB reports.

The concentration of double markers in first-trimester screening tests is expressed as MoM for unaffected pregnancies at the same gestational age(29). These MoM values are calculated by dividing individual marker levels by the median levels for the entire population at corresponding gestational ages within a specific laboratory. In normal pregnancies, the mean MoM values for pregnancy-associated plasma protein A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) are standardized to one(30, 31). A study by Spencer et al. reported that the MoM values for PAPP-A and free β-hCG in Down syndrome pregnancies were 0.15 and 2.15, respectively. Furthermore, they observed a decrease in PAPP-A mean MoM from 0.65 at 11 weeks to 0.38 at 13 weeks in Down syndrome cases, while the expected values for free β-hCG increased from 1.8 at 11 weeks to 2.09 at 13 weeks(32). Notably, our findings indicate that some women with Down syndrome pregnancies exhibit normal MoM values in certain parameters, suggesting variability in marker expression that may complicate risk assessment for trisomy 21 (Fig. 3 and Table 6).

Research has indicated that there are no significant differences in fetal NT thickness or maternal serum levels of free β-hCG and PAPP-A between fetuses diagnosed with Down syndrome and those without a visible NB(26, 33). In the present study, it was demonstrated that maternal biochemical markers could still fall within the normal range (Tables 4 and 6). This suggests that a combined approach utilizing both biochemical markers and sonographic assessments may

enhance early screening efficacy for Down syndrome. Given that any fetus may be at risk for trisomy 21, it is essential to first evaluate the woman's prior risk based on gestational and maternal age. This prior risk is calculated using likelihood ratios derived from various parameters, including serum biochemistry results, ultrasound findings, and both gestational and maternal age. The correlation between advancing maternal age and an increased risk for trisomy 21 is well established; thus, pregnant women over the age of 35 are routinely advised to consider invasive prenatal diagnostic testing(34). In our study, among the seven pregnancies diagnosed with Down syndrome, five participants were over 40 years of age, with a risk cutoff of less than 1:50. This highlights the importance of integrating maternal age into risk assessment protocols for more accurate Down syndrome screening outcomes.

In summary, the inadequacy of NB examinations conducted by sonographers can significantly affect the outcomes of congenital anomaly screenings through prenatal software, leading to false-positive results. These inaccuracies not only impose unnecessary economic burdens on pregnant women but also contribute to emotional distress. Conversely, accurate assessment and reporting of the nasal bone are crucial, as they markedly enhance the prognostic accuracy for Down syndrome in fetuses classified with unknown nasal bone status. Ensuring thorough and precise NB evaluations can mitigate the risks of misdiagnosis and improve the overall management of prenatal care, ultimately benefiting both maternal and fetal health.

Ethical consideration

This trial procedure was conducted under the ethical standards of the Iranian Ministry of Health and Medical Education on human experimentation (Ethical code: IR.RUMS.REC.1400.024) and in concordance with the Helsinki Declaration. Finally, all participants signed a consent form.

Acknowledgement and Funding

This study was supported and financially funded by the Genetic Foundation of Khorasan Razavi, a Charity Foundation, Khorasan Razavi, Iran. The authors would like to thank Seyed Majid Sezavar Kamali and Seyed Ali Taghavian for their support and assistance.

Authors' contributions

MR: methodology, writing – original draft. AM: Investigation, Visualization. NR: methodology, Validation. NG: Writing – review & editing, Validation and MM: Conceptualization, Project administration

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Jensen KM BP, Santoro S. Down syndrome. In: Care of Adults with Chronic Childhood Conditions: A Practical Guide: Springer; 2024.https://doi.org/10.1007/978-3-031-54281-7_30.
2. Bull MJ, Trotter T, Santoro SL, Christensen C, Grout RW, GENETICS TCO. Health Supervision for Children and Adolescents With Down Syndrome. Pediatrics. 2022;149(5). https://doi.org/10.1542/peds.2022-057010.
3. LeFevre NM, Sundermeyer RL. Fetal Aneuploidy: Screening and Diagnostic Testing. Am Fam Physician. 2020;101(8):481-8.
4. Ozdemir S, Sahin O, Acar Z, Demir GZ, Ermin E, Aydin A. Prediction of Pregnancy Complications With Maternal Biochemical Markers Used in Down Syndrome Screening. Cureus. 2022;14(3):e23115.https://doi.org/10.7759/cureus.23115.
5. Wagner P, Sonek J, Klein J, Hoopmann M, Abele H, Kagan KO. First-trimester ultrasound screening for trisomy 21 based on maternal age, fetal nuchal translucency, and different methods of ductus venosus assessment. Prenat Diagn. 2017;37(7):680-5.https://doi.org/10.1002/pd.5065.
6. Wojda KM, Moczulska H, Sieroszewski PJ. The absence of fetal nasal bones in ultrasound examination between 11 + 0 and 13 + 6 weeks of gestation versus the occurrence of trisomies 21, 18, and 13. Ginekol Pol. 2019;90(10):604-6. https://doi.org/10.5603/gp.2019.0104.
7. Sonek JD, Cicero S, Neiger R, Nicolaides KH. Nasal bone assessment in prenatal screening for trisomy 21. Am J Obstet Gynecol. 2006;195(5):1219-30.https://doi.org/10.1016/j.ajog.2005.11.042.
8. Spencer R HH, McCarthy L, Wimalasundera R, Pandya P. Non-invasive prenatal testing for aneuploidy screening. BMJ. 2020:371. .https://doi.org/10.1136/bmj.m3930.
9. Cicero S, Longo D, Rembouskos G, Sacchini C, Nicolaides KH. Absent nasal bone at 11-14 weeks of gestation and chromosomal defects. Ultrasound Obstet Gynecol. 2003;22(1):31-5.https://doi.org/10.1002/uog.170.
10. Aghaz F, Ojagh SZ, Khanjari S, Vaisi-Raygani A, Khazaei M, Bakhtiari M. The Contingent Prenatal Screening Test for Down's Syndrome and Neural Tube Defects in West of Iran. J Reprod Infertil. 2019;20(4):244-51
11. Shiefa S, Amargandhi M, Bhupendra J, Moulali S, Kristine T. First Trimester Maternal Serum Screening Using Biochemical Markers PAPP-A and Free β-hCG for Down Syndrome, Patau Syndrome and Edward Syndrome. Indian J Clin Biochem. 2013;28(1):3-12.https://doi.org/10.1007/s12291-012-0269-9.
12. A K. Maternal Serum Screening and Counselling. In: Down Syndrome Screening: A Practical Guide2024.https://doi.org//10.1007/978-981-99-7758-1_4.
13. Sagi-Dain L, Singer A, Ben Shachar S, Josefsberg Ben Yehoshua S, Feingold-Zadok M, Greenbaum L, et al. Risk of Clinically Significant Chromosomal Microarray Analysis Findings in Fetuses With Nuchal Translucency From 3.0 mm Through 3.4 mm. Obstet Gynecol. 2021;137(1):126-31. https://doi.org/10.1097/aog.0000000000004195.
14. Huang T, Wang FL, Boucher K, O'Donnell A, Rashid S, Summers AM. Racial differences in first trimester nuchal translucency. Prenat Diagn. 2007;27(12):1174-6.https://doi.org/10.1002/pd.1866.
15. Sevón E NH. False negatives in screening for chromosomal abnormalities2021.
16. Moreno-Cid M, Rubio-Lorente A, Rodríguez MJ, Bueno-Pacheco G, Tenías JM, Román-Ortiz C, et al. Systematic review and meta-analysis of performance of second-trimester nasal bone assessment in detection of fetuses with Down syndrome. Ultrasound Obstet Gynecol. 2014;43(3):247-53.

https://doi.org/10.1002/uog.13228.
17. Ozcan T, Özlü T, Allen J, Peterson J, Pressman EK. Predictive role of prenasal thickness and nasal bone for Down syndrome in the second trimester. Eur J Obstet Gynecol Reprod Biol. 2013;171(2):220-4.https://doi.org/10.1016/j.ejogrb.2013.08.039.
18. Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: an observational study. Lancet. 2001;358(9294):1665-7.https://doi.org/10.1016/s0140-6736(01)06709-5.
19. Orlandi F, Bilardo CM, Campogrande M, Krantz D, Hallahan T, Rossi C, et al. Measurement of nasal bone length at 11-14 weeks of pregnancy and its potential role in Down syndrome risk assessment. Ultrasound Obstet Gynecol. 2003;22(1):36-9.https://doi.org/10.1002/uog.167.
20. Das S, Sharma C, Yadav T, Dubey K, Shekhar S, Singh P, et al. Absent or hypoplastic nasal bone: What to tell the prospective parents? Birth Defects Res. 2024;116(5):e2348. https://doi.org/10.1002/bdr2.2348.
21. Kumar S, Selvakumar K. Various Methods for Computing Risk Factors of Down Syndrome in Fetus. Arch Computat Methods Eng. 2025;32(1):485-98.https://doi.org/10.1007/s11831-024-10158-8.
22. Dal Y, Akkuş F, Karagün Ş, Coşkun A. Comparison of the ratio of second trimester fetal biometric measurements to fetal nasal bone length in fetuses with normal karyotype and trisomy 21. J Clin Ultrasound. 2024;52(4):368-76.https://doi.org/10.1002/jcu.23638.
23. Viora E, Errante G, Sciarrone A, Bastonero S, Masturzo B, Martiny G, et al. Fetal nasal bone and trisomy 21 in the second trimester. Prenat Diagn. 2005;25(6):511-5.https://doi.org/10.1002/pd.848.
24. Cicero S, Dezerega V, Andrade E, Scheier M, Nicolaides KH. Learning curve for sonographic examination of the fetal nasal bone at 11-14 weeks. Ultrasound Obstet Gynecol. 2003;22(2):135-7.https://doi.org/10.1002/uog.176.
25. Alldred SK, Takwoingi Y, Guo B, Pennant M, Deeks JJ, Neilson JP, et al. First trimester ultrasound tests alone or in combination with first trimester serum tests for Down's syndrome screening. Cochrane Database Syst Rev. 2017;3(3):Cd012600.https://doi.org/10.1002/14651858.Cd012600.
26. Karim JN, Bradburn E, Roberts N, Papageorghiou AT. First-trimester ultrasound detection of fetal heart anomalies: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2022;59(1):11-25.https://doi.org/10.1002/uog.23740.
27. Liao Y, Wen H, Ouyang S, Yuan Y, Bi J, Guan Y, et al. Routine first-trimester ultrasound screening using a standardized anatomical protocol. Am J Obstet Gynecol. 2021;224(4):396.e1-.e15.https://doi.org/10.1016/j.ajog.2020.10.037.
28. Larose C, Massoc P, Hillion Y, Bernard JP, Ville Y. Comparison of fetal nasal bone assessment by ultrasound at 11-14 weeks and by postmortem X-ray in trisomy 21: a prospective observational study. Ultrasound Obstet Gynecol. 2003;22(1):27-30.https://doi.org/10.1002/uog.169.
29. Tørring N. First trimester combined screening - focus on early biochemistry. Scand J Clin Lab Invest. 2016;76(6):435-47.https://doi.org/10.1080/00365513.2016.1200131.
30. Korevaar TI, Steegers EA, de Rijke YB, Schalekamp-Timmermans S, Visser WE, Hofman A, et al. Reference ranges and determinants of total hCG levels during pregnancy: the Generation R Study. Eur J Epidemiol. 2015;30(9):1057-66. https://doi.org/10.1007/s10654-015-0039-0.
31. Honarjoo M, Kohan S, Zarean E, Tarrahi MJ. Assessment of β-human-derived chorionic gonadotrophic hormone (βhCG) and pregnancy-associated plasma protein A (PAPP-A) levels as predictive factors of preeclampsia in the first trimester among Iranian women: a cohort study. BMC Pregnancy Childbirth. 2019;19(1):464.https://doi.org/10.1186/s12884-019-2526-x.
32. Spencer K, Heath V, El-Sheikhah A, Ong CYT, Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first trimester: a study of Oriental, Asian and Afro-Caribbean populations. Prenat Diagn. 2005;25(5):365-9.https://doi.org/10.1002/pd.1153.
33. Cicero S, Bindra R, Rembouskos G, Spencer K, Nicolaides KH. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta-hCG and PAPP-A at 11 to 14 weeks. Prenat Diagn. 2003;23(4):306-10.https://doi.org/10.1002/pd.588.
34. Abele H, Lüthgens K, Hoopmann M, Kagan KO. Impact of the maternal age-related risk in first-trimester combined screening for trisomy 21. Fetal Diagn Ther. 2011;30(2):135-40.https://doi.org/10.1159/000327157.