Article published online: 2023-05-12

The Impact of Isolated Increased Nuchal Translucency ≥95th Centile on Perinatal Outcome: A Prospective Cohort Study from a North Indian Genetic Center

Sangeeta Khatter[1] Meena Lall[1] Shruti Agrawal[1] Sunita Bijarnia Mahay[1] Nandita Dimri[1] Nidhish Sharma[1] Kanwal Gujral[1] Ishwar C. Verma[1] Ratna D. Puri[1]

[1] Institute of Medical Genetics and Genomics, Sir Ganga Ram Hospital, New Delhi, India
[2] Fetal Medicine Unit, Sir Ganga Ram Hospital, New Delhi, India
[3] Institute of Obstetrics and Gynecology, Sir Ganga Ram Hospital, New Delhi, India

Address for correspondence Sangeeta Khatter, DNB, Institute of Medical Genetics and Genomics, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi 110060, India (e-mail: drsangeeta77@yahoo.com).

J Fetal Med 2023;10:9–15.

Abstract

Objectives The aim of this study was to determine the chromosomal abnormalities and other adverse outcomes like miscarriages, intrauterine deaths, structural defects, and genetic syndromes in fetuses with increased nuchal translucency (NT) more than or equal to 95th centile. This study also compared the outcomes in fetuses with NT between 95th and 99th centile and more than 99th centile.

Study Design A prospective cohort of 182 patients with isolated increased NT was evaluated by invasive testing. Fetal chromosomes were examined by fluorescent in situ hybridization and karyotype or chromosomal microarray. Euploid pregnancies were followed-up with level II ultrasound and fetal echocardiography. For pregnancies progressing to delivery, the neonates were followed-up till the age of 3 months. Final outcome was reported as normal or abnormal. Collated data for perinatal outcomes was analyzed and compared between fetuses with NT 95th and 99th centile (group I) and NT more than 99th centile (group II).

Results Of the 202 patients recruited, 182 patients consented for invasive testing and chromosomal analysis. Of the 182 patients, group I (NT 95–99th centile**)** included 92 patients and group II 90 patients. Chromosomal abnormalities were present in 50 (27.4%), 14 (7.6%) in group I, and 36 (19.4%) in group II. Of the 132 euploid pregnancies, adverse outcomes were present in 22 (16%) fetuses, 7 (5.3%) in group I, and 15(11.7%) in group II. A normal outcome was present in 110 (60.4%) pregnancies of the 182 fetuses with NT more than or equal to 95th centile. Normal outcome observed in group I was 77.1% and in group II, it was 43.4%.

Conclusion An increased NT is associated with poor perinatal outcomes in 39.6% patients. Chromosomal analysis and follow-up for adverse outcome in fetuses with NT more than or equal to 95th centile is important to enable a take home neonatal rate of 60.4%. No pregnancy with increased NT should be discontinued without detailed fetal evaluation for genetic disorders, structural malformation, and fetal growth.

Keywords
- nuchal translucency
- euploid
- perinatal
- chromosomes
- microarray
- CMA

article published online
May 12, 2023

DOI https://doi.org/10.1055/s-0043-57001.
ISSN 2348-1153.

Thieme Medical and Scientific Publishers Pvt. Ltd., A-12, 2nd Floor, Sector 2, Noida-201301 UP, India

Introduction

The incidence of congenital anomalies in live births is 2 to 4%.[1] First-trimester combined screen is the standard of care nowadays.[2–4] Fetal nuchal translucency (NT) measurement performed at 11 to $13^{6/7}$ is used solely or as a component of combined first-trimester screen for prenatal aneuploidies since the association between increased NT and chromosomal abnormalities was demonstrated.[2–5] It identifies 75% of fetuses with chromosomal aneuploidies, structural aberrations and serves as a marker for adverse pregnancy outcomes such as miscarriages, fetal deaths, genetic syndromes, and neurodevelopmental delays.[5–7] The incidence of poor perinatal outcomes increases with an increase in NT values from more than or equal to 95th centile to more than 99th centile. However, the cutoff for defining increased NT varies in different studies starting from more than or equal to 95th and in the majority NT more than 99th centile (e3.5 mm) in recent studies.[8–10] Hence in this study, we set out to evaluate systematically the incidence of chromosomal abnormalities, malformations, and adverse perinatal outcomes in patients presenting with NT more than or equal to 95th centile in a cohort of the population at the North Indian center. We compared this with the currently prevalent cutoff value of 3.5 mm (99th centile) to determine the appropriate increased NT value for invasive testing and detailed protocol-based follow-up.[8–11]

Materials and Methods

This was a prospective cohort study conducted at the Institute of Medical Genetics and Genomics, Sir Ganga Ram Hospital in New Delhi. Patients referred to the genetic clinic for increased NT in the first trimester were evaluated for study inclusion. The NT percentiles were calculated for CRL by a validated and evidence-based NT percentile calculator.[11] Patients with nuchal translucency more than or equal to 95th centile at a crown-rump length of 45 to 84 mm were included. Although NT was measured by different operators, all images were reviewed and only those that adhered to the FMF criteria were included (►**Figs. 1** and **2).**

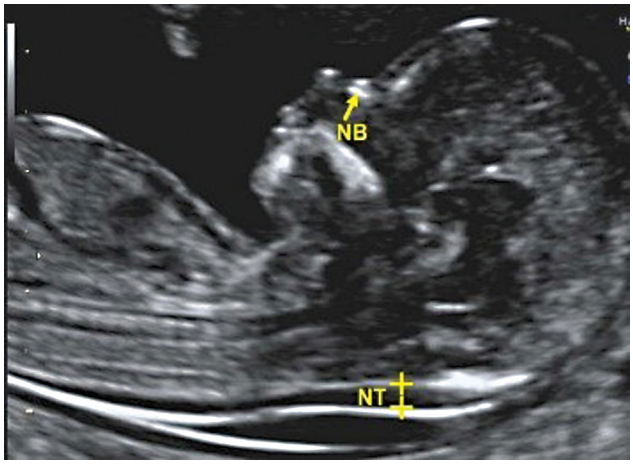

Fig. 1 Ultrasound image of normal nuchal translucency (NT). Image resource—Fetal medicine foundation.org.

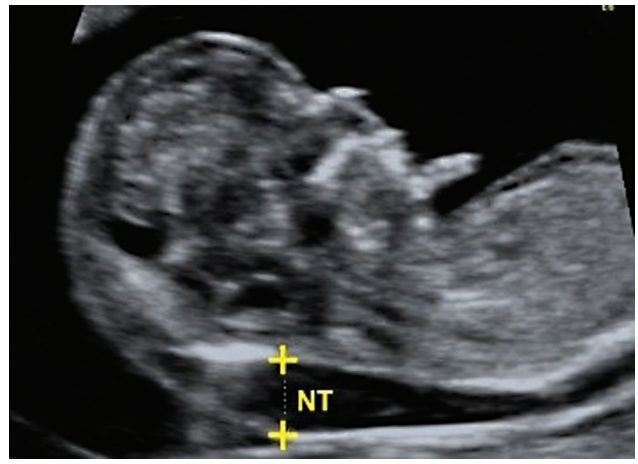

Fig. 2 Ultrasound image of increased nuchal translucency (NT). Image resource—Fetal medicine foundation.org

Pregnancies with other structural malformations like absent nasal bone, absent ductus venous flow, tricuspid regurgitation, and cystic hygroma were excluded from the study population. Patients were divided into two groups based on NT values—group I with NT 95th to 99th centile and group II with NT more than 99th percentile for gestation. Informed consent was obtained after appropriate genetic counselling for invasive testing and genetic tests including fluorescent in situ hybridization (FISH), karyotyping, or chromosomal microarray (CMA). The option of next-generation sequencing was discussed in cases with negative CMA. However, not all patients opted for CMA or exome sequencing in view of the high cost involved and lack of funding. Patients with normal chromosomes were followed-up with fetal structural anatomy screening including persistent nuchal edema (nuchal fold thickness > 6 mm) between the 18th and 22nd weeks of gestation and fetal echocardiography between the 16th and 22nd weeks of gestation.

FISH studies on fetal tissue were performed using Vysis aneuvysion DNA probes on uncultured amniocytes or chorionic villi cells as a targeted approach for rapid aneuploidy detection of the five commonly involved chromosomes 13, 18, 21, X & Y.[10]

For karyotyping, chorionic villi or amniocytes were cultured and processed for GTG banding. Both numerical and structural abnormalities were classified using the International System of Chromosome Nomenclature.[10]

CMA studies were performed on AGILENT catalogue 4×180k array slides. This array contains approximately 120,000 CGH (comparative genomic hybridization) probes and 60,000 SNP (single-nucleotide polymorphism) probes. Abnormalities were identified using aberrant detection method-2 algorithm with a sensitivity of six CGH region calling. The log threshold values for aberrations are ≥ -04 for copy number losses and $\geq +0.4$ for copy number gains.[12] The software utilized for analysis is also based on UCSC build 37 (hg19). The resolution cutoff was taken as less than 200 kb for pathogenic deletions and 400 kb for pathogenic duplications across the genome, or smaller (≥50 kb) for clinically

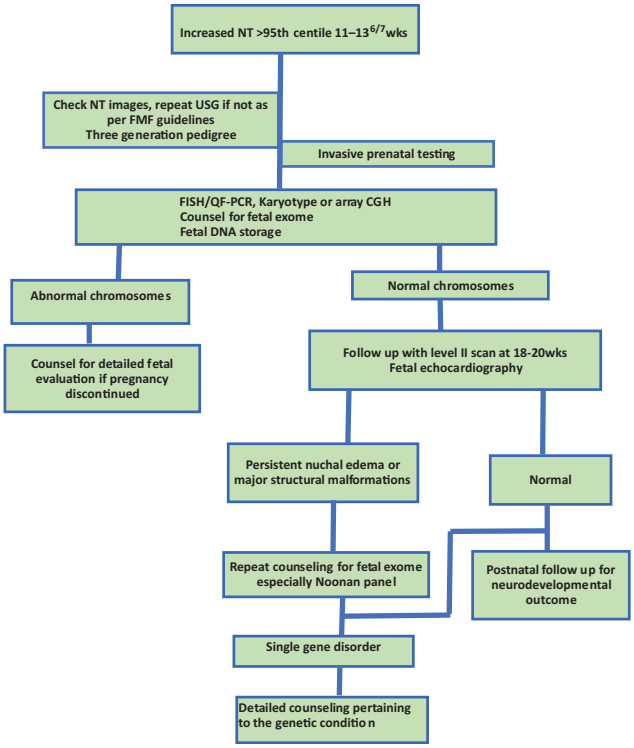

Fig. 3 Algorithm for patients with increased nuchal translucency more than 95th centile on first trimester ultrasound.

relevant deletions/duplications syndromes, subtelomeric, and pericentric regions, or targeted genes.

For clinical exome, DNA extracted from chorionic villi and amniocytes was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean more than 80 to 100X coverage on the Illumina sequencing platform. Clinically relevant mutations were annotated using published variants in literature and a set of diseases databases - ClinVar, OMIM (updated on May 11, 2020), GWAS, HGMD (v2020.2), and Swiss Var.[13–16] Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v2.1), EVS, dbSNP (v151), 1000 Japanese Genome, and laboratory's internal Indian population database.[16–19] The nonsynonymous variant's effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2, and LRT.

Outcomes were measured as adverse or normal. Adverse outcomes were observed as miscarriage, intrauterine fetal death, structural malformations, and a genetic syndrome. Normal outcomes were documented with normal karyotype or CMA and delivery of a child without structural defects. A one-point postnatal follow-up after birth till 3 months was done through telephonic interviews or by follow-up meetings with the families and babies. The flowchart of the protocol is shown in ►**Fig. 3**.

The SPSS package and chi-squared test were used for statistical analysis. For statistical significance, a two-tailed *p*-value less than 0.05 was taken as significant

Institutional ethics committee approval was obtained for the study.

Results

Of a cohort of 9,659 patients attending the genetic clinic from July 2015 through August 2017, 202 women having singleton pregnancies and an increased NT were evaluated and counselled. Of these, 20 patients did not conform to evaluation as per the study protocol and were excluded from the analysis. The final study group comprised 182 patients. The mean maternal age was 32 years ± 3SD (range: 22–45), mean gestation at the first screening ultrasound was 12^{+5} weeks ± 2SD (range: 11–14), mean CRL-66.3 mm (range: 45–84 mm), mean NT thickness 3.9 mm ± 4SD (range: 2.2–8.0). Of the 182 study group cohort, 87 opted for chorionic villus sampling at 11 to 14 weeks of gestation and 95 for amniocentesis at 16 to 24 weeks. No procedure-related miscarriage was reported after CVS or amniocentesis.

Fetal Chromosomes Analysis

FISH was performed in all patients and karyotype in 128 patients who had a normal report on FISH testing. Fifty-four patients with normal FISH results consented for CMA. FISH/Karyotype/CMA results were normal in 132 (72.6%) cases and abnormal in 50 (27.6%) cases. Of these, 7.6% (14 of 182) cases were from group I versus 19.4% (36 of 182) in group II ($p = 0.0001$; ►**Table 1**). Trisomy 21 was the commonest aneuploidy as compared with other aneuploidies. A fourfold increased trisomy 21 was seen in group II compared with group I. All the patients with fetal chromosomal anomalies underwent termination without further evaluation by ultrasound or fetal autopsy. CMA was performed in 54 of 132 cases after excluding common numerical and structural chromosomal anomalies by FISH and karyotype. Among these, 28 and 26 patients were in group I and group II, respectively. Four pathogenic structural abnormalities were identified through CMA (►**Table 2**) of which three were from group I. Three variations in uncertain significance were identified on

Table 1 Increased nuchal translucency and chromosomal outcomes

NT values	Total	Normal chromosomes, *n* (%)	Abnormal chromosomes on karyotype and CMA, *n* (%)
Total cases with NT≥ 95th centile	182	132 (72.6)	50 (27.6)
NT:95th–99th centile (group I)	92	78 (84.8)	14 (15.2)
NT > 99th centile			
Group II	90	54 (60)	36 (40)

Abbreviations: CMA, chromosomal microarray; NT, nuchal translucency.

Table 2 Details of chromosomal abnormalities in fetuses with increased nuchal translucency, $n = 50/182$

Categories, *n* (%)	Numerical chromosomal anomalies, *n* (%); *n* = 46 (24.7)				Structural chromosomal anomalies, *n* = 4 (7.4)			
	Tr 21	Tr 18	Tr 13	SCA	15q11.3 duplication (26Mb gain)	2q32 deletion (10.2Mb loss)	22q11.2 deletion (2.6Mb loss)	4q32.3 deletion (8Mb loss)
Total *n* = 50 (27.4)	34 (66)	6 (12)	1 (2)	5 (10)	1 (2)	1 (2)	1 (2)	1 (2)
Group I, *n* = 14 (15.2)	9 (64.2)	0 (0)	0 (0)	2 (14.2)	1 (7.1)	1 (7.1)	1 (7.1)	0 (0)
Group II, *n* = 36 (40)	25 (69.4)	6 (16.6)	1 (2.7)	3 (8.3)	0 (0)	0 (0)	0 (0)	1 (2.7)

Abbreviation: SCA, sex chromosome anomalies.

CMA. These three variants were inherited from asymptomatic parents and the infants are having normal development on postnatal follow-up.

Overall, microarray analysis was found more valuable in NT values between 95th and 99th centile for the detection of submicroscopic copy number variations. However, further data would be required to confirm the results with a larger sample size.

Structural Malformations and Adverse Outcomes

Persistent nuchal edema was identified in the second-trimester scan in 6 (4.5%) of 132 euploid fetuses. No pathogenic copy number variation was identified by CMA analysis in these cases. This nuchal edema resolved and neonates were normal on follow-up. Of the 132 euploid fetuses, 16 (10.6%) had structural malformations on ultrasound of which 11 were in group II and five in group I. Of these16 fetuses, 13 patients opted to discontinue the pregnancy and the rest three patients were detected to have intrauterine fetal death on follow-up. No further evaluation could be performed in fetuses with structural malformations due to lack of consent by parents. One neonate was detected with a complex cardiac defect (transposition of great vessels) after delivery that was not identified on fetal echocardiography. The adverse outcomes in euploid fetuses in the two groups were statistically significant ($p = 0.000009$). Details of the structural malformations and outcomes are shown in ►**Table 3** and overall perinatal outcome in patients with increased NT more than 95th centile is depicted in ►**Table 4**.

Table 3 Details of follow-up for structural malformations and other adverse outcomes in fetuses with normal chromosomal complement

Categories of euploid fetuses *n* (%)	[a]Prenatal SM *n* (%); *n* = 16 (12.1)			Postnatal SM	Total SM	GS	Spontaneous IUD	TAO
	CNS[b] *n* (%)	Cardiac[c], *n* (%)	Others[d], *n* (%)	Cardiac				
Total *n* = 132	2 (1.5)	7 (5.3)	7 (5.3)	1 (0.75)	17 (12.8)	2 (1.5)	3 (2.2)	22 (16.6)
Group I, *n* = 78	1 (1.2)	3 (3.8)	1 (1.2)	1 (1.2)	6 (7.6)	0 (0)	1 (1.2)	7 (8.9)
Group II, *n* = 54	1 (1.8)	4 (7.4)	6 (11.1)	0 (0)	11 (20.3)	2 (3.7)	2 (3.7)	15 (27.7%)

Abbreviations: CMA, chromosomal microarray; GS, genetic syndrome; IUD, intrauterine demise; LB, live birth; SM, structural malformation; TAO, total adverse outcome; TM, trimester; TOP, termination of pregnancy; WM, with malformation, WOM, without malformation.
[a]Details of Structural malformations on level II ultrasound.
[b]CNS—Holoprosencephaly-1, agenesis of corpus callosum-2.
[c]Cardiac—Ventricular septal defect-4; hypoplastic left heart-2; double outlet right ventricle-1.
[d]Others—Diaphragmatic hernia-1; hydrops-3; severe oligohydramnios with hydronephrosis and hydroureter-1; skeletal dysplasia and oligohydramnios.

Table 4 Total perinatal outcome of increased NT ≥95th centile

Categories	Number of patients in each category, *n* (%)	Chromosomal anomalies [A], *n* (%)	Adverse outcome in euploid fetuses [B], *n* (%)	Normal live births, *n* (%)
Total patients with increased NT	182	50 (27.4)	22 (16.6)	110 (60.4)
Group I	92	14 (15.2)	7 (8.9)	71 (77.1)
Group II	90	36 (40)	15 (16.6)	39 (43.3)

Abbreviation: NT, nuchal translucency.

Single-Gene Disorders

In our cohort, only five of 132 patients with negative CMA consented for clinical exome analysis. The clinical exome is a next-generation sequencing technique that tests for approximately 4,000 disease-related genes including rasopathy genes. Out of five, two fetuses were identified with a monogenic disorder due to pathogenic gene variants. One fetus with a 5.6 mm NT had a novel, heterozygous, splice site variant in *KMT2D* (c.4131 + 3A > C) gene, consistent with Kabuki syndrome. The variation was pathogenic by ACMG criteria.[13] The second fetus with 6.2 mm NT was detected with a pathogenic variant in *PACS1* gene (c.607C > T), previously reported to cause an autosomal dominant Schuurs-Hoeijmakers syndrome. Both variants were de novo on segregation analysis.

Discussion

In this study, 182 fetuses with nuchal translucency more than or equal to 95th centile, ranging from 2.2 to 8 mm, were prospectively followed-up for perinatal outcomes. Additionally, we wanted to examine if the adverse outcome measures were significant in the cohort with NT between 95th and 99th centiles. This is in variation to the majority of the current studies where NT more than 3 mm or 3.5 mm (which is >99th centile) are commonly evaluated.[20–26] In this study, 27.4% of fetuses with NT more than or equal to 95th centile had abnormal chromosomes. This is consistent with previous studies on NT enlargement and chromosomal outcomes.[21–26] A meta-analysis of 17 studies showed that CMA identifies additional 4% pathogenic CNVs in isolated increased fetal NT of more than 99th centile.[27] In this study, we observed the majority of abnormal CMA results in fetuses with NT between 95th and 99th centiles suggesting that evaluation in mild increased nuchal translucency may also be relevant and missed if the NT cutoff of 3 /3.5 mm is used.[9] Since all patients did not opt for CMA following a normal karyotype report and the postnatal follow-up was limited to 3 months, it is likely that not all abnormalities have been picked up.

Structural malformations were identified in 12.8% fetuses and cardiac malformations being predominant ones in our cohort consistent with other studies.[8,25,28] Structural malformations on follow-up scans were significantly increased ($p = 0.033$) in group II as compared with group I. There are limited studies that report increased structural defects in NT values 95th to 99th. Bilardo et al and other authors reported 15% abnormal karyotypes, 2% miscarriage and intrauterine demises, and 6.2% structural and genetic anomalies at this mild increase in NT.[24,25,27,29]

Persistent nuchal edema was seen in around 4.5% of fetuses on follow-up ultrasound and was not associated with any anomalies or apparent genetic syndromes till 3 months postnatal follow-up. Saldanha et al reported similar results.[28] In contrast, another study reported a 10% chance of an abnormal outcome as perinatal death or hydrops fetalis in all cases of persistent nuchal edema.[9] Large-scale multicentric studies are required to establish this outcome.

Increased NT is reported to be associated with various single-gene syndromes in different studies.[30–32] Till recently, studies on DNA sequencing were limited to Noonan syndrome panel testing.[32] With availability of next-generation sequencing in the clinics, multiple studies report its significant role in increased NT to identify monogenic disorders with an additional diagnostic yield ranging from 3.2 to 32% in CMA-negative samples.[33–37] In this study, two fetuses had single-gene variations identified on next-generation sequencing. Neither of these two syndromes are previously reported to be associated with increased NT.[6,8,24,25,27,28]

Noninvasive prenatal screening with cell-free fetal DNA evaluation for common aneuploidies is recommended in NT between the 95th and 99th centile.[38–40] However, other studies proposed that NIPT should not be recommended as an alternative to invasive diagnostic testing in fetuses with ultrasound anomalies including increased NT.[40] We would have missed 2.7% cases of copy number variation by doing only NIPT in NT 95th to 99th centile, thereby asserting the importance of evaluation of the entire chromosomal complement in fetuses with increased nuchal translucency.[41]

In low-resource countries, genetic testing is predominantly an out-of-pocket expenditure. A detailed family history to evaluate a baseline disease risk, an appropriately measured NT, and evidence-based practice for fetal evaluation is important to define the best approach to prevent fetal morbidity and mortality. The present data with the outcome's measures could assist geneticists and fetal medicine specialists in counseling in such situations.

The strength of this study is that it is a prospective study where all the families were followed-up at one center with a uniform protocol. A postnatal follow-up till 3 months allowed for appropriate infant evaluation. The limitations include the small sample size that gave a limited statistical power to the results and lack of long-term follow-up for neurodevelopmental outcomes. Further larger cohort studies to substantiate the results of this study would impact counseling strategies for patients with increased nuchal translucency in the first trimester.

Conclusion and Implication for Clinical Practice

In this prospective study of 182 fetuses with increased nuchal translucency more than 95th centile, there was a normal live birth rate of 60.4%, chromosomal abnormality in 24.7% and an adverse outcome was present in 16.6% cases. Overall, outcomes are more favorable at 91.1% in NT between 95th and 99th centile. CMA is the preferred test for chromosomal testing. Data on monogenic disorders in isolated increased NT is limited and extreme caution is called upon for its incorporation in the diagnostic algorithm. It must only be offered in research setting at present as stringent guidelines for genomic data evaluation must be adhered to for reporting in the prenatal setting.[42] Termination of pregnancies based on an ultrasound finding of increased NT is strongly discouraged without detailed genetic, anomaly, and growth evaluation of fetuses.

Funding
None.

Conflict of Interest
None declared.

Acknowledgment
All patients and their families participating in the study, whole team of clinical genetics, cytogenetics, and molecular genetics are highly acknowledged. I extend my acknowledgment to Dr. Umesh Jindal, Head of Jindal IVF Hospital, for her moral support and guidance.

References

1 International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR) Annual Report 2007 with data for 2005. Rome, Italy: The International Centre on Birth Defects; 2007
2 Roozbeh N, Azizi M, Darvish L. Pregnancy outcome of abnormal nuchal translucency: a systematic review. J Clin Diagn Res 2017; 11(03):QC12–QC16
3 Bardi F, Bosschieter P, Verheij J, et al. Is there still a role for nuchal translucency measurement in the changing paradigm of first trimester screening? Prenat Diagn 2020;40(02):197–205
4 Nicolaides KH. The 11–13 +6 Weeks Scan. London: Fetal Medicine Foundation; 2004[cited2015May10]. Accessed March 3, 2023 at: http://www.fetalmedicine.com/synced/fmf/FMF-English.pdf
5 Michailidis GD, Economides DL. Nuchal translucency measurement and pregnancy outcome in karyotypically normal fetuses. Ultrasound Obstet Gynecol 2001;17(02):102–105
6 Sotiriadis A, Papatheodorou S, Makrydimas G. Neurodevelopmental outcome of fetuses with increased nuchal translucency and apparently normal prenatal and/or postnatal assessment: a systematic review. Ultrasound Obstet Gynecol 2012;39(01): 10–19
7 Bilardo CM, Timmerman E, Pajkrt E, van Maarle M. Increased nuchal translucency in euploid fetuses–what should we be telling the parents? Prenat Diagn 2010;30(02):93–102
8 Maya I, Yacobson S, Kahana S, et al. Cut-off value of nuchal translucency as indication for chromosomal microarray analysis. Ultrasound Obstet Gynecol 2017;50(03):332–335
9 Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. Am J Obstet Gynecol 2005;192(04):1005–1021. Doi: 10.1016/j.ajog.2004.12.093 Erratum in: Am J Obstet Gynecol. 2005 Jun;192(6):2096
10 Shaffer LG, McGowan-Jordon J, Schmid M, , Eds. An International System for Human Cytogenetic Nomenclature. Karger Medical and Scientific Publisher; 2013
11 Crown Rump Length and Nuchal Translucency Calculator 1135 S Sunset, Suite 402, CA 91790,. West Covina: Focus Information Technology; 2019. Accessed March 3, 2023 at: perinatology.com/calculators/Crown%20Rump%20and%20Nuchal%20Translucency.html
12 Accessed March 3, 2023 at: http://www.chem.agilent.com/library/applications/5988_9498 en_scanner_paper 72.pdf
13 Richards S, Aziz N, Bale S, et al;ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(05): 405–424
14 Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res 2016;44(D1):D862–D868
15 Stenson PD, Matthew M, Edward V Ball, et al. The Human Gene Mutation Database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. Hum Genet 2017;136 (06):665–677
16 Mottaz A, David FP, Veuthey AL, Yip YL. Easy retrieval of single amino-acid polymorphisms and phenotype information using SwissVar. Bioinformatics 2010;26(06):851–852
17 1000 Genomes Project Consortium. et al., A global reference for human genetic variation. Nature 526(7571):68–742015
18 Exome Variant Server. NHLBI GO Exome Sequencing Project (ESP). Accessed May 1, 2023 at: http://evs.gs.washington.edu/EVS/
19 Nagasaki M, Yasuda J, Katsuoka F, et al;ToMMo Japanese Reference Panel Project. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. Nat Commun 2015; 6:8018
20 American Institute of Ultrasound in Medicine. AIUM practice guideline for the performance of obstetric ultrasound examinations. J Ultrasound Med 2013;32(06):1083–1101
21 Dane B, Dane C, Cetin A, Kiray M, Sivri D, Yayla M. Pregnancy outcome in fetuses with increased nuchal translucency. J Perinatol 2008;28(06):400–404
22 Lithner CU, Kublickas M, Sverker Ek. Pregnancy outcome for fetuses with increased nuchal translucency but normal karyotype. J Med Screen 2016;23:1–6
23 Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004;191(01):45–67
24 Bilardo CM, Müller MA, Pajkrt E, Clur SA, van Zalen MM, Bijlsma EK. Increased nuchal translucency thickness and normal karyotype: time for parental reassurance. Ultrasound Obstet Gynecol 2007;30(01):11–18
25 Tahmasebpour A, Rafiee NB, Ghaffari S, Jamal A. Increased nuchal translucency and pregnancy outcome. Iran J Public Health 2012; 41(11):92–97
26 Ayräs O, Tikkanen M, Eronen M, Paavonen J, Stefanovic V. Increased nuchal translucency and pregnancy outcome: a retrospective study of 1063 consecutive singleton pregnancies in a single referral institution. Prenat Diagn 2013;33(09):856–862 Erratum in: Prenat Diagn. 2016 Feb;36(2):199
27 Grande M, Jansen FA, Blumenfeld YJ, et al. Genomic microarray in fetuses with increased nuchal translucency and normal karyotype: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015;46(06):650–658
28 Saldanha FA, Brizot Mde L, Moraes EA, Lopes LM, Zugaib M. Increased fetal nuchal translucency thickness and normal karyotype: prenatal and postnatal follow-up [in Portuguese]. Rev Assoc Med Bras (1992) 2009;55(05):575–580
29 Bakker M, Pajkrt E, Bilardo CM. Increased nuchal translucency with normal karyotype and anomaly scan: what next? Best Pract Res Clin Obstet Gynaecol 2014;28(03):355–366
30 Pergament E, Alamillo C, Sak K, Fiddler M. Genetic assessment following increased nuchal translucency and normal karyotype. Prenat Diagn 2011;31(03):307–310
31 Coletta Lucas J. Increased nuchal translucency and normal karyotype: Value of additional testing. Am J Obstet Gynecol 2014; 208:85–87
32 Mula R, Goncé A, Bennásar M, et al. Increased nuchal translucency and normal karyotype: perinatal and pediatric outcomes at 2 years of age. Ultrasound Obstet Gynecol 2012;39(01):34–41
33 Carss KJ, Hillman SC, Parthiban V, et al. Exome sequencing improves genetic diagnosis of structural fetal abnormalities revealed by ultrasound. Hum Mol Genet 2014;23(12):3269–3277
34 Hillman SC, Willams D, Carss KJ, McMullan DJ, Hurles ME, Kilby MD. Prenatal exome sequencing for fetuses with structural abnormalities: the next step. Ultrasound Obstet Gynecol 2015;45 (01):4–9
35 Xue S, Yan H, Chen J, et al. Genetic examination for fetuses with increased fetal nuchal translucency by genomic technology. Cytogenet Genome Res 2020;160(02):57–62

36 Choy KW, Wang H, Shi M, et al. Prenatal diagnosis of fetuses with increased nuchal translucency by genome sequencing analysis. Front Genet 2019;10:761
37 Petrovski S, Aggarwal V, Giordano JL, et al. Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study. Lancet 2019;393(10173):758–767
38 Khalil A, Mahmoodian N, Kulkarni A, et al. Estimation of detection rates of aneuploidy in high-risk pregnancy using an approach based on nuchal translucency and non-invasive prenatal testing: a cohort study. Fetal Diagn Ther 2015;38(04):254–261
39 Huang L-Y, Pan M, Han J, Zhen L, Yang X, Li DZ. What would be missed in the first trimester if nuchal translucency measurement is replaced by cell free DNA foetal aneuploidy screening? J Obstet Gynaecol 2018;38(04):498–501
40 Beulen L, Faas BHW, Feenstra I, van Vugt JMG, Bekker MN. Clinical utility of non-invasive prenatal testing in pregnancies with ultrasound anomalies. Ultrasound Obstet Gynecol 2017;49(06):721–728
41 Petersen OB, Smith E, Van Opstal D, et al. Nuchal translucency of 3.0-3.4 mm an indication for NIPT or microarray? Cohort analysis and literature review. Acta Obstet Gynecol Scand 2020;99(06): 765–774
42 Mellis R, Eberhardt RY, Hamilton SJ, et al;PAGE Consortium. Fetal exome sequencing for isolated increased nuchal translucency: should we be doing it? BJOG 2022;129(01):52–61

