The assessment of nuchal translucency and serum markers for down syndrome screening with ductus venosus Doppler measurements in the first trimester

Down sendromu taramasında birinci trimester nukal kalınlık ve serum belirteçlerinin duktus venozus doppler ölçümü ile değerlendirilmesi

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Abstract

Objective: The aim of the study was to improve nuchal translucency (NT) and serum marker Down syndrome (Tri21) screening methods by including fetal ductus venosus (DV) Doppler measurements.

Material and Methods: A total of 213 pregnant women were screened consecutively by combining maternal age, fetal NT and maternal serum pregnancy associated plasma protein A (PAPP-A) and free β-human chorionic gonadotropin (f β-HCG) values at 11-14 weeks of gestation. Also, a DV Doppler analysis was performed for the contribution to the screening for Tri21 and other fetal anomalies or adverse pregnancy outcomes.

Results: Twelve fetuses had DV PI measurements above the 95th percentile and two (17%) developed intrauterine growth retardation. DV PI values negatively correlated with birth weight (p=0.013, r=0.171). Two patients had T 21 among the study group (0.9%) with abnormal biochemical screening results. In these with Tri21, the combined test risk was above the suggested limit (>1/250). PAPP-A was <0.4 MoM in 23, and f β-HCG was >1.91 MoM in 49 patients. The rates of false positivity were 10% for PAPP-A and 22% for f β-HCG. The sensitivity, specificity, positive and negative predictive values of the combined test was 100%, 95%, 20% and 100%, respectively.

Conclusion: The combined test has high sensitivity and specificity for Tri21 detection. The addition of DV Doppler ultrasound in the first trimester might have the advantage of predicting some adverse pregnancy outcomes. However, in the Turkish population, further studies with larger numbers of patients will be needed to establish the usefulness of DV for the detection of Tri21 or the prediction of some major cardiac anomalies. (J Turkish-German Gynecol Assoc 2010; 11: 194-8)

Key words: Turkish population, Down syndrome, combined test, ductus venosus

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Özet

Amaç: Down sendromu (Tri21) taramasında kullanılan nukal kalınlık (NT) ve serum belirteçlerine fetal ductus venozus (DV) Doppler ölçümü eklenmesinin katkısını incelemek.

Gereç ve Yöntemler: Antenatal muayene için başvuran 11-14 haftalık 213 ardışık gebe anne yaşı, fetal NT, anne serum "pregnancy associated plasma protein A" (PAPP-A) ve serbest beta-human koryonik gonadotropin (s β-HCG) değerleri ile tarandı. Bu gebelere ayrıca Tri21, diğer fetal anomaliler ve olumsuz perinatal sonuçların taraması amacıyla DV Doppler analizi de gerçekleştirildi.

Bulgular: On iki fetusta DV PI ölçümü hesaplanan 95. persantilin üzerindeydi ve 2 tanesinde (%17) intrauterin gelişme geriliği gelişti. DV PI değerleri doğum ağırlığı ile negatif yönde ilişkili bulundu (p=0.013, r=0.171). Çalışmadaki 213 hastadan ikisinde Tri21 saptandı. Tri21 saptanan bu hastalarda kombine test sonucu riskli (>1/250) olarak saptandı. PAPP-A 23 hastada <0.4 MoM ve s β-HCG 49 hastada >1.91 MoM olarak bulundu. PAPP-A için yanlış pozitiflik oranı %10, s β-HCG için ise %22 bulundu. Kombine testin duyarlılık, seçicilik, olumlu ve olumsuz öngörü değerleri sırasıyla %100, %95, %20 ve %100 oranında saptandı.

Sonuç: Tri21 belirlenmesinde kombine test yüksek duyarlılık ve seçiciliğe sahiptir. Birinci trimesterde DV Doppler ultrasonografisi eklenmesi bazı olumsuz gebelik sonuçlarının öngörüsünde faydalı olabilir. Ancak Türk toplumunda DV incelemesinin Tri21 belirlenmesi ve bazı kalp anomalilerinin öngörüsünde yararını ortaya koymak için daha geniş serili çalışmalara ihtiyaç vardır.

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Anahtar kelimeler: Türk toplumu, Down sendromu, kombine test, ductus venozus

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Introduction

Prenatal screening for Down syndrome (Tri21) was developed by the introduction of nuchal translucency (NT) and ultrasound to the first trimester of pregnancy. In pregnancies with fetal Tri21, low maternal serum pregnancy associated plasma protein A (PAPP-A) and elevated free β-human chorionic gonadotropin (f β-HCG) values were observed by the 1990s (1, 2). Screening for Tri21 by combining maternal age, fetal NT thickness and maternal serum f β-HCG and PAPP-A at 11-13 weeks was associated with a detection rate of about 90% for a false-positive rate of 5% (3, 4). However, since measurements of NT varied considerably between centers and clinicians, the sensitivity can be as low as 31%, thus it could hardly be reliably incorporated into the test (5). Doppler ultrasound of the ductus venosus (DV) has also been added to expert antenatal screening programs for chromo-

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somal abnormalities. An association between abnormal flow in the DV and fetal aneuploidy has been introduced. The use of DV velocimetry in combination with NT has been asserted as better than either test alone, since it increased the sensitivity in the detection of Tri21 (6-11). In fetuses with cardiac defects or fetal hypoxia some abnormal patterns of the "a" wave on DV, which represents atrial contraction, can be observed (12). Matias et al. analyzed fetuses at 10-14 weeks of gestation with increased NT and found that 57 of 63 had chromosomal defects, whereas only 13 out of 423 with normal chromosome had abnormal DV flow patterns (13). Likewise, in fetuses with Tri21, absence of flow or reverse flow of the "a" wave can be observed (14).
In this study, our aim was to improve Tri21 screening methods based on NT and serum markers by including fetal DV Doppler measurements.

Materials and Methods

The study was performed in Trakya University Faculty of Medicine, Department of Obstetrics&Gynecology, on 213 consecutive pregnant women aged between 18 and 43 years admitted for antenatal care at 11-14 weeks of gestation. Twins or higher order pregnancies, pregnancies ending in spontaneous abortion or with congenital anomalies detected at the first trimester and patients that did not deliver in our clinic or were lost during follow-up were excluded from the study. All patients were delivered in our department and the newborns were examined after birth for possible anomalies. in the Neonatology Department by a pediatrician The study was approved by the Ethics Committee for Human research at Trakya University, Turkey, and informed consent was obtained from the patients. The study population consisted of Turkish women living in the Trakya Region of Turkey. Gestational age was based on the last menstrual period and according to a reliable menstrual history confirmed by ultrasonography.
Age, maternal smoking habit, previous fetuses with anomalies, presence of diabetes were noted, height and weight were obtained and body mass index calculated from all women. A detailed structural survey by ultrasound (Shimadzu SDU-2200, Japan) was performed on each fetus with a 3.5 MHz transabdominal transducer. Crown rump length (CRL), NT and DV flow patterns were measured by the same clinician (OY) during periods without uterine contractions and in the absence of fetal body movements. Three measurements for NT were obtained and the highest was accepted for calculation of risk for the combined test.
The pulsatility (PI) of DV was estimated from the Doppler waveforms. The mean value assessed from five consecutive waveforms was analyzed. Color Doppler imaging was used to optimize placement of the pulsed wave Doppler gate by adjusting the velocity scale to identify area and direction of maximum blood flow. The size of the sample gate was enlarged to encompass the entire vessel, and transducer position was adjusted to eliminate aliasing, in order to minimize the Doppler angle. All measurements were obtained from the sagittal plane of the fetus. DV was identified from where it appeared from the umbilical vein and all measurements were taken from the beginning of the vessel since the flow pattern changes from the beginning to the end of DV.
Blood samples were obtained from the subjects through venipuncture to perform the PAPP-A and f β-HCG assays. Samples were assayed immediately. Serum concentrations of PAPP-A and f β-HCG levels were all analyzed by chemiluminescent immunometric assays (Immulite 2000, Diagnostic Products Co., LA, USA), following the instructions of the manufacturers. All values were calculated by multiples of median (MoM) according to gestational age. Risk analysis for trisomies was made by the computer program PRISCA version 3.4. In this first trimester biochemical tests, values <0.4 MoM for PAPP-A, and >1.91 MoM for f β-HCG were accepted as high risk for Tri21 as suggested (15). 'Screen-positive' risk for Tri21, based on combined PAPP-A, f β-HCG and NT was accepted with a cut-off ≥ 1 : 250. A second level genetic ultrasound examination was performed on all patients for anomaly screening at 18 - 23 weeks. Amniocentesis for chromosomal anomalies was carried out in women who had a high risk according to the first trimester screening or had anomalies in genetic ultrasound, as suggested (16, 17).
Data were stored and analyzed by SPSS program (Statistical Package for Social Science, release11.0; SPSS, Chicago, IL) for Windows. Kruskal-Wallis test was used for inter-group comparisons of non-normally distributed variables. Continuous variables were analyzed with student *t*-test if distributional assumptions were consistent with normality. Otherwise, we performed Mann-Whitney U tests for the parameters that were not normally distributed. Spearman and Pearson correlation analysis was used for linear correlations. A *P* value less than 0.05 was considered statistically significant.

Results

Mean±SD age, gestational age at admittance, and gestational age at delivery were 27.8±4.9 years, 12.4±0.72 and 38.1±1.5 weeks, respectively. Only 20 (9.4%) women were older than 35 years. Twenty-four women (11%) were smokers. Mean weight at delivery was 3278±445 (min. 1090, max. 4260) gr., of which 117 (56%) were boys (3345±443 gr.) and 94 (44%) were girls (3199±435 gr.). Birth weight was significantly higher in boys (p=0.017). Ten amniocenteses were performed in women ≥ 35 years (n=20) and no Tri21 syndrome was detected.
Mean CRL, NT, DV PI values were 58.5 9.1 mm, 1.16±0.3 mm and 1.05±0.13, respectively. Fetal heart rate measurements significantly changed (162±8 to 164±7.4 beats/min., p<0.01, r=0.869) after Doppler analysis. NT and DV PI measurements according to gestational age were shown in Table 1. The 95th percentile was high in 13-13.4 weeks, because there was a fetus with Tri21 with a NT value of 3.6 mm in that group. NT measurement increased significantly with the CRL value (p<0.001, r=0.457). DV PI values showed a plateau during 11th and 14th weeks of gestation (p>0.05, r=0.009). Twelve fetuses had DV PI measurements above the 95th percentile and two (2/12, 17%) developed intrauterine growth retardation (IUGR) in the third trimester. Also, DV PI values negatively correlated with birth weight (p=0.013, r=0.171). Of 213 women detected, 2 had Tri21 (Table 2). In these pregnancies with Tri21, the combined test risk was above the suggested limit (>1/250). In the first , no absence or reversal of flow during atrial contraction was observed, but DV PI measurement could not be obtained in the second case.
In all patients, mean±SD values for PAPP-A and f β-HCG were 0.82±0.41 (min.:0.18, max.: 2.4) MoM and 1.61±1.31 (min.:0.11, max.: 8.74) MoM, respectively. PAPP-A values decreased (p>0.05, r=0.032) and f β-HCG increased (p>0.05, r=0.003)

nonsignificantly with the development of pregnancy (Table 3). Ten women had PAPP-A values under the 5th percentile, whereas 9 had f β-HCG above the 95th percentile. Smoking habits and sex did not correlate with PAPP-A and f β-HCG values (p>0.05). PAPP-A was <0.4 MoM in 23, and f β-HCG was >1.91 MoM in 49 patients. The rates of false positivity were 10% for PAPP-A and 22% for f β-HCG. In patients who had PAPP-A <0.4 MoM, 2 had Tri21 syndrome detected by amniocentesis and were terminated, whereas 17 (74%) had uneventful pregnancy outcomes. One patient had pericentric translocation on the 9th chromosome which had no effect on phenotype, 2 had IUGR and one developed gestational hypertension. However, in another 5 patients who developed IUGR, PAPP-A values were >0.4 MoM. In patients who had f β-HCG >1.91 MoM, one had had Tri21 and was terminated, 37 (75%) had uneventful pregnancy outcomes.

Table 1. Percentiles for fetal nuchal translucency and for ductus venosus pulsatility index

Gestational weeks	NT				DV		
	N	5th p	50th p	95th p	5th p	50th p	95th p
11-11.4	22	0.72	0.83	1.56	0.66	1.02	1.27
11.5-11.9	37	0.73	1.04	1.50	0.78	1.08	1.23
12-12.4	53	0.76	1.10	1.48	0.83	1.03	1.26
12.5-12.9	61	0.91	1.25	1.64	0.79	1.06	1.26
13-13.4	27	0.85	1.29	2.80	0.88	1.13	1.28
13.5-14	13	0.86	1.42	1.64	0.77	1.08	1.26

DV: Ductus venosus; NT: Nuchal translucency; p: Percentile

Table 2. Calculated parameters in pregnancies with Down syndrome

Case	Age	CRL (mm)	NT (mm)	PAPP-A (MoM)	f β-HCG (MoM)	DV PI	Combined test risk
1	27	66.9	3.6	0.39	2.31	0.98	1/50
2	26	66.7	1.6	0.2	1.7	-	1/114

CRL: Cranium rump length; DV PI: Ductus venosus pulsatility index; f β-HCG: free β human chorionic gonadotropin; NT: Nuchal translucency; PAPP-A: Pregnancy associated protein A

Table 3. Calculated multiples of median (MoM) values for pregnancy associated protein A (PAPP-A) and free β human chorionic gonadotropin (f β-HCG) in the whole group

Gestational weeks	PAPP-A				f β-HCG		
	N	5th p	50th p	95th p	5th p	50th p	95th p
11-11.4	22	0.42	0.87	2.16	0.26	1.22	5.04
11.5-11.9	37	0.31	0.88	1.79	0.41	1.14	6.88
12-12.4	53	0.34	0.75	1.55	0.48	1.33	3.15
12.5-12.9	61	0.22	0.70	1.91	0.52	1.35	5.61
13-13.4	27	0.24	0.66	1.25	0.55	1.28	5.09
13.5-14	13	0.29	0.60	1.33	0.67	1.22	2.81

f β-HCG: free β human chorionic gonadotropin; PAPP-A: Pregnancy associated protein A; p: Percentile

Five (10%) had IUGR (one of which had also PAPP-A <0.4 MoM), one had coarctation of the aorta, 2 developed gestational diabetes, 1 preeclampsia, 2 threatened preterm labor.

Ten patients (4.7%) had ahigh risk for Tri21 according to the combined test (risk ≥1/250) and 2 fetuses had Tri21 detected by amniocentesis. Two women had a high risk on combined test, but did not accept amniocentesis. However, neither had adverse pregnancy outcomes nor any fetal anomaly detected at birth. The sensitivity, specificity, positive and negative predictive values of the combined test was 100%, 95%, 20% and 100%, respectively.

Amniocentesis was performed in 21 patients (9.8%). Indications were maternal age (n=10), high risk in screening test (n=8), findings on genetic ultrasound (n=2) and history of recurrent abortion (n=1).

Two cardiac anomalies were observed in the study group. One had coarctation of the aorta and the other had secundum type atrial septal defect with duodenal atresia. Both fetuses had normal phenotype and normal NT, PAPP-A and DV PI values, whereas one had a f β-HCG level above the 95th percentile. However, these two abnormalities could not be detected by the II. level ultrasound.

Discussion

The most sensitive method for Tri21 screening was introduced as the combination of maternal age, serum screening for PAPP-A, f β-HCG with fetal NT with 90% detection and 5% false positive rate (18). Nearly 70% of fetuses with Tri21 are born of mothers <35 years-old (19). Similarly, our two patients with Tri21 fetuses were below age 35. Also, not only did the amniocentesis reveal no Tri21, no case was found in patients who did not accept amniocentesis in women >35 years in our study. In a study evaluating NT in a low risk population of 1473 women, only 67% of fetuses with Tri21 would have been detected with a 24% invasive testing rate, if the only screening criteria was maternal age. If NT measurement had been added to the screening policy, the sensitivity would have been 100% with a 19.1% invasive testing rate (20). In the study by Snijders et al. (3) the estimated Tri21 risk, from maternal age and fetal NT, was 1 in ≥ 300 in 7907 (8.3%) of 95476 normal pregnancies, but in 268 (82.2%) of 326 with Tri21. The number of invasive procedures performed to detect one Tri21 was calculated as 30. In line with that study, others observed that the main benefit of the addition of first trimester NT measurements to the risk screening protocol was a very high detection rate with a moderate false-positive rate (21). Different studies have used the combined test for the screening of chromosomal anomalies in low and high risk populations, or used pooled data with patients with Tri21 and reported a detection rate of about 80% (18, 22-24). The detection rate of Tri 21 with only NT measurement was reported as 77%, with a 5% false positive rate (3). However, biochemistry tests alone, consisting of PAPP-A and f β-HCG, detected about 60% of the cases with 5% false positive rate (5, 24). For screening purposes, a cut-off threshold value for NT of ≥3 mm gave a sensitivity ≥50%, a false positive rate <5% and a positive predictive value >1% for chromosomal anomalies (25). In our study, only one case had NT> 3 mm. If only NT was considered for screening of Tri21, the sensitivity would have been 50%, specificity 100%, positive and negative predic-

tive values 100% and 99%. Likewise, according to our results, the sensitivity, specificity and positive and negative predictive values of the combined test were 100%, 95%, 20% and 100%, respectively However, these high rates seem to result from the limited number of patients in our study.

The effect of smoking on PAPP-A and f β-HCG values has been defined (26). We did not find any effect of smoking on biochemical markers of the combined test. Smoking reduced serum PAPP-A and f β-HCG levels in women who smoked ≥5 cigarettes a day in a Turkish population (27). However, it was demonstrated that the effect of adjusting for smoking on the combined test is small, with an estimate of less than half percentage point increase in the detection rate (28). Smoking decreases trophoblast invasion and proliferation (29, 30). So the clinical effect on placental function may be obvious by IUGR. However, in our study smoking neither decreased PAPP-A nor had a relationship with the development of IUGR. Besides, out of 213 patients, 23 had PAPP-A<0.4 MoM; 2 had Tri21 and 2 (9.5%) developed IUGR. In the other 5 fetuses that developed IUGR, PAPP-A values were within normal limits. Thus, PAPP-A, the marker for placental function, has not been shown to predict IUGR.

The reference range for DV PI has been shown to have a biphasic pattern; with an initial non-significant increase up to a CRL of 63 mm and a fall thereafter, as in our study (31). Doppler studies of the DV have been applied as an adjunct to NT measurements. In 1998, increased DV PI values above the 95th percentile have been observed in 73% of the fetuses with Tri 21 between the 10th and 18th weeks (14). In a further study, the same investigators found that the median DV PI in Tri21 was 1.70 times higher than in unaffected pregnancies in women between 10 and 14 weeks. Also, the addition of PI to NT alone will increase the detection rate from 76 to 85%, and, combined with serum markers, from 88 to 92% (8). Murta et al. analyzed absent or reversed flow during atrial contraction in 93.1% of chromosomally abnormal fetuses (32). However, abnormal ductal blood flow was observed in 5.2% of euploid fetuses and 70.8% of fetuses with Tri21 (6). Inclusion of DV flow in first-trimester screening by maternal age, fetal NT and maternal serum free β-HCG and PAPP-A would detect about 96% of trisomy 21 fetuses at a false-positive rate of about 2.5% (6). Assessment of DV flow is time consuming and requires appropriately trained sonographers, and sonographers with extensive experience in the first trimester scan require an average of 80 examinations to achieve this level of competence (33). The alternative strategy is to reserve this examination for the subgroup of pregnancies with an intermediate risk (between one in 51 and one in 1000) after combined fetal NT, FHR, free β-HCG and PAPPA screening. Even when NT is normal, reverse flow during atrial contraction in DV has a strong association which predicts adverse outcome such as IUGR, cardiovascular abnormalities and renal abnormalities (34). Although we could not measure the flow pattern in a case with Tri21 in our study, the addition of DV Doppler to the combined test did not improve the detection of Tri21 or the development of IUGR and cardiovascular abnormalities. In line with our results, some authors observed a lack of correlation of DV PI values with NT or with serum markers (8, 10), but the association between reversed a-wave on DV and increased NT may be explained by the coincidence of cardiac defects or transient cardiac dysfunction (6). Abnormal DV flow may result from abnormal cardiac preload, cardiac compliance or afterload. When there is an overlap in the pathophysiology leading to an increased NT and abnormal DV blood flow, their combination improved the sensitivity and specificity of aneuploidy prediction (13).

Matias et al. (13) observed significantly higher DV PI values in Tri21,18,13,Turner syndrome and triploidy, but multivariate regression analysis demonstrated that only the height of the a wave provided a significant independent contribution in distinguishing between the chromosomally normal and abnormal groups. We did not find any absent or reverse flow during atrial contraction on DV Doppler, but 12 fetuses had DV PI >95th percentile and 2 (17%) developed IUGR in the third trimester, while DV PI negatively correlated with birth weight in our study. The first case with Tri21 and the other two cases with cardiac defects without chromosomal anomaly also revealed normal flow patterns. Favre et al. (35) observed abnormal flow and increased NT in 36% of fetuses with a normal chromosome but a major cardiac defect, and the authors have concluded that in chromosomally normal fetuses with increased NT, assessment of DV blood flow velocimetry could improve the predictive capacity for an underlying major cardiac defect. However, we could not find any pathologic pattern of flow in DV in our two cases.

In conclusion, the combined test has a distinctive effect on Down syndrome detection with high sensitivity and specificity. The addition , DV Doppler ultrasound might have the advantage of predicting some adverse pregnancy outcomes. However, further studies in the Turkish population will be needed to rectify these screening tests; in the current study we could not establish the usefulness of DV Doppler analysis for the detection of Tri21 or the prediction of some major cardiac anomalies.

Conflict of interest
None declared.

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