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Screening for Aneuploidies by Maternal Age, Fetal Nuchal Translucency and Maternal Serum Biochemistry at 11-13+6 Gestational Weeks

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SUMMARY

Introduction Aneuploidies are the major cause of perinatal death and early psychophysical disorders.

Objective In this study, we analyzed detection and false-positive rates of screening for aneuploidies in the first trimester by the combination of maternal age, fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotrophin (β-hCG), and pregnancy-associated plasma protein-A (PAPP-A) at 11-13+6 weeks of gestation, using the appropriate software developed by the Fetal Medicine Foundation.

Methods Our screening study for aneuploidies analyzed 4172 singleton pregnancies from January 2006 to December 2010. The sensitivities and false-positive rates using the combined aneuploidies determination for the risk cut-off of 1:275 were evaluated.

Results In the trisomy 21 pregnancies, the fetal NT was higher than 95th centile, in 72.8%, serum free b-hCG concentration it was above the 95th centile in 55% and serum PAPP-A was below the 5th centile in 47% of the cases. In the trisomy 18 and 13, the fetal NT was above 95th centile in 66.6% and 44.4% of the cases, respectively. The serum free b-hCG concentration was above the 95th centile in 0 and 10%, but serum PAPP-A was below 5th centile in 80.9% and 88.8% of pregnancies. In the trisomy 21 pregnancies the median free beta-hCG was 2.3 MoM and the median PAPP-A was 0.45 MoM. Chromosomal abnormalities were detected in 169 fetuses: trisomy 21 (97), Turner syndrome (19), trisomy 18 (28), trisomy 13 (11) and others (14). Detection rate of combined screening for aneuploides were 86.0% with false positive rate of 5.3% (mean age 33±4.9 years, >35 years in 35% of pregnancies).

Conclusion Our study suggests that the strategy of first-trimester combined screening of biochemical values and ultrasonographic parameters at 12 gestational weeks identifies higher percentage of aneuploidies with a lower false-positive rate than a single parameter strategy.

Keywords: aneuploidies; first-trimester screening; nuchal translucence

INTRODUCTION

The major causes of perinatal death and childhood handicap are aneuploidies. Approximately 10% of birth defects account for aneuploidies or chromosome abnormalities and are associated with high mortality and morbidity rates [1].

The nuchal translucency (NT) was visualized by ultrasonography as a transient subcutaneous collection of fluid behind the fetal neck at 11-13+6 weeks of gestation [2]. In the last two decades, many studies focused on the first trimester searching for the association of an increased fetal neck thickness with chromosomal abnormalities [2-5]. The first trimester combined screening for trisomy 21 has demonstrated that a combination of maternal parameters (maternal age and maternal serum free β-human chorionic gonadotrophin (β-hCG), pregnancy-associated plasma protein-A (PAPP-A) and fetal ultrasonographic parameters (fetal NT) can identify pregnancies with high risk for aneuploidies [2].

A beneficial effect of screening for trisomy 21 is the concomitant early diagnosis of trisomies 18 and 13, which are common chromosomal abnormalities. All three trisomies are associated with increased maternal age, increased fetal NT and decreased maternal serum PAPP-A. In trisomy 21, serum β-hCG increases whereas in trisomies 18 and 13 it decreases [2, 3, 4]. Trisomy 13 unlike trisomies 21 and 18 is associated with fetal tachycardia [5].

A screening strategy for trisomy 21 based on maternal age and two biochemical markers with fetal NT thickness had detection rate of 85-95% for a 5% false-positive rate [1, 2].

OBJECTIVE

In this study, we analyzed detection rate and false-positive rate of screening for aneuploidies in the period of five years by combination of maternal age, fetal NT and serum β-hCG and

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PAPP-A using the appropriate software developed by the Fetal Medicine Foundation (FMF).

METHODS

Our prospective screening study for trisomy 21 analyzed 4,172 singleton pregnancies by using the criteria of the FMF in the period of five years, from January 2006 to December 2010, "in High-Risk Pregnancies Department, Obstetrics and Gynecology Clinic "Narodni front". The FMF has established a widely accepted method of training, certification and ongoing audit of results to ensure high quality in the performance of the 11-13+6 week scan [1]. This screening included maternal age, fetal NT thickness and maternal serum β-hCG and PAPP-A for a first-trimester assessment of risk at 11-13+6 weeks of gestation. Transabdominal ultrasound examination was performed to diagnose any major fetal defect and to measure crown-rump length (CRL) and fetal NT thickness (Figure 1). The pregnancy was dated according to the last menstrual period, but if the date was uncertain or the estimated gestation by CRL was discordant by more than 7 days from estimated gestation dates, the CRL was used to date the pregnancy. Maternal demographic characteristics, ultrasonographic measurements and biochemical results were recorded in a computer database.

All the sonographers involved had completed training and received the FMF Certificate of Competence in the 11-13+6 weeks scan [1].

In biochemical testing it is necessary to make adjustments in the measured maternal serum concentration of β-hCG and PAPP-A to correct for certain maternal and pregnancy characteristics. Each measured level is first converted to a multiple of expected normal median (MoM) specific to a pregnancy of the same gestational age, maternal weight, ethnicity, smoking status, method of conception and parity. The MoM distribution of each metabolite in unaffected and trisomy 21 pregnancies was implicit to be Caucasian [1, 2].

The distribution for fetal NT for CRL using the 95th and 99th centiles (FMF range), for maternal serum β-hCG using the 95th centile and for PAPP-A using 5th centile were determined. Sensitivities and false-positive rates were calculated by multiplying the maternal age-related risk with the likelihood ratio (LR) derived from the fetal NT and the maternal serum biochemistry using the FMF software [1]. The sensitivities and false-positive rates for the risk cut-off 1:275 were calculated.

Measurements of maternal serum β-hCG and PAPP-A were carried out on the Kryptor analyzer (Brahams, Henningsdorf, Germany) and were corrected for maternal weight. After informed consent was obtained from each woman, amniocentesis was done transabdominally under direct ultrasound visualization with a 20 G needle (Cook Medical). All amnion samples were sent for karyotype analysis to the genetics laboratory of our hospital or Genetics Laboratory, Institute of Mental Health.

Screening accuracy was assessed by performing receiver-operating characteristics (ROC) curves analysis for maternal age, NT, β-hCG and PAPP-A in the prediction of aneuploidy. All calculations were performed using the SPSS version 17 software package [6].

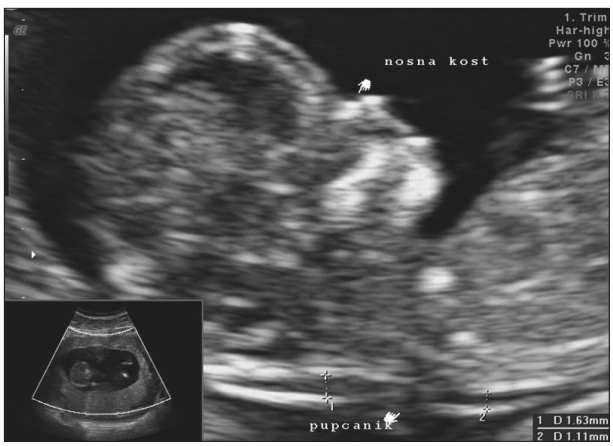


Figure 1. Longitudinal view of a 12-week fetus with thickened nuchal translucency (NT) and nasal bone

RESULTS

During a five-year study period, a total of 4,172 singleton pregnancies with live fetuses underwent screening for aneuploidy by a combination of fetal NT and maternal serum β-hCG and PAPP-A. The mean maternal age was 33 years, and 35% of the mothers were 35 years or older (Table 1). Mean body weight was 78 kg (range 53-98), and 42.3% of women were smokers. In 91.9% of women conception was spontaneous and in 8.1% it was the result of assisted reproduction techniques. The mean gestation at screening was 12.2 weeks (range 11.0-13.6 wk) and the mean CRL was 65 mm (range 45-84 mm) (Table 1). Chromosomal abnormalities were detected in 169 fetuses; 97 had trisomy 21 and 72 had other, including Turner syndrome (19), Edwards syndrome (28), Patau (11) syndrome and other (14) (Table 2).

In chromosomally normal pregnancies, the fetal NT was above the 95th centile of the reference range in 4.2 % (168/4003) of cases, maternal serum β-hCG concentration

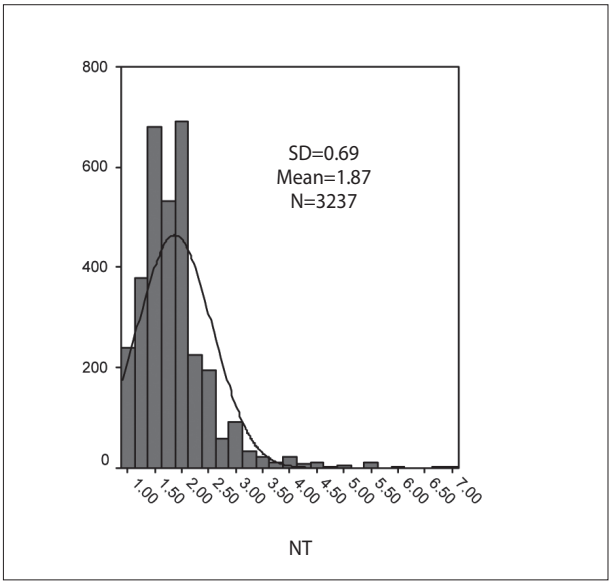
Table 1. Clinical characteristics of study population

Parameter	Value
Number of pregnancies	4172
Maternal age (years)	33±4.89
Weight, median (kg)	78±2.33
Spontaneous conception (%)	91.9
Smoker (%)	42.3
Gestational age (weeks)	12.2±0.4
Crown-rump length (mm)	65.0±2.3
Karyotype, normal	4003
Trisomy 21	97
Other aneuploidies	72

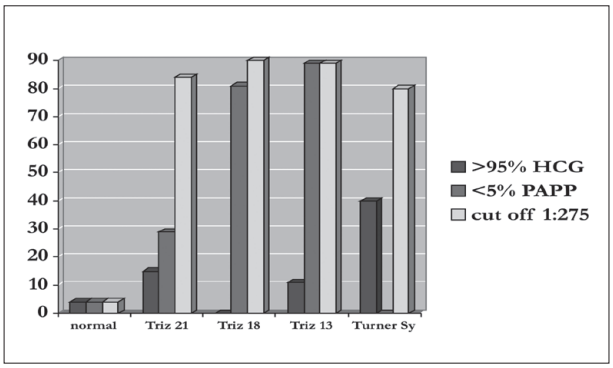
Table 2. Cases of each chromosomal group with values above or below certain cut-off levels in maternal age, fetal nuchal translucency thickness (NT) and maternal free beta-human chorionic gonadotropin (HCG) and pregnancy-associated plasma protein-A (PAPP-A)

Group	Number	Maternal age ≥35 years		NT ≥95%		Free β HCG ≥95%		PAPP-A ≤5%		Estimated risk for Sy Down cut-off ≥275	
		N	%	N	%	N	%	N	%	N	%
Normal	4003	1401	35	168	4.2	164	4.2	156	3.9	212	5.3
Trisomy 21	97	49	51	70	72.8	53	55	45	47	83	86
Trisomy 18	28	13	47	18	66.6	0	0	22	80.9	25	90
Trisomy13	11	9	88.8	5	44.4	1	10	10	88.8	10	88.8
Turner Sy	19	0	0	17	93.3	8	40	0	0	15	80
47 XXX, XXY or XYY	9	0	0	5	66.6	0	0	3	33.3	6	66.6
Other	5	1	33.3	3	66.6	0	0	3	66.6	3	66.6
Total	4172	1473	35.3	286	6.8	226	5.4	239	5.7	354	8.4

N – number of pregnancies



Graph 1. The distribution of nuchal translucency for crown-rump length in the studied fetuses



Graph 2. Distribution of multiple median (MoM) values of free beta -human chorionic gonadotropin (HCG) and regnancy-associated plasma protein-A (PAPP-A) in normal population and aneuploidy

was above the 95th centile in 4.2% (164/4003) and serum PAPP-A concentration was below the 5th centile of our reference ranges in 3.9% (156/4003) of the cases (Table 2; Graphs 1 and 2).

The NT in unaffected population above 95th centile was found in 4.2% and above 99th centile in only 1.5% of the chromosomally normal fetuses. In the trisomy 21 pregnancies, the fetal NT was above the 95th centile in 72.8% and in trisomies 18 and 13 above the 95th centile in 66.6% and 44.4%, respectively (Graph 1).

In Turner syndrome NT above 95th centile was detected in 93.3% of cases.

Pregnant women were older than 35 years in 51% of trisomy 21. In the trisomy 21 pregnancies, the fetal NT was higher than 95th centile in 72.8% (70/97), serum free b-hCG concentration was above the 95th centile in 55% (53/97) and serum PAPP-A was below the 5th centile in 47% (45/97) of the cases (Table 2 and Graph 3). Screening by a combination of maternal age, fetal NT and maternal serum biochemistry using a cut-off of 1:275 identified 86% (83/97) of the fetuses with trisomy 21 with a false-positive rate of 5.3% (212/4003). In the remaining 14 (14%) pregnancy with trisomy 21, NT was bellow 95th centile and maternal age of each of these 14 pregnancies was above 35 years (41±2.1 years).

The rate of the maternal age greater than 35 years was 47.0% and 88.8% in all pregnancies with diagnosed trisomy 18 and 13. In the trisomy 18 and 13, the fetal NT was above 95th centile in 66.6% and 44.4% of the cases, respectively. The serum free b-hCG concentration was above the 95th centile in 0 and 10%, but serum PAPP-A was below 5th centile in 80.9% and 88.8% cases (Table 2 and Graph 2).

Distribution of centiles for free b-HCG and PAPP-A in trisomy 21, 18 or 13, Turner syndrome and unaffected pregnancies is shown in Graph 3. In the unaffected pregnancies the median free β-hCG was 1.0 (range 0.4-2.98) MoM and the median PAPP-A was 0.97 (range 0.1-2.0)

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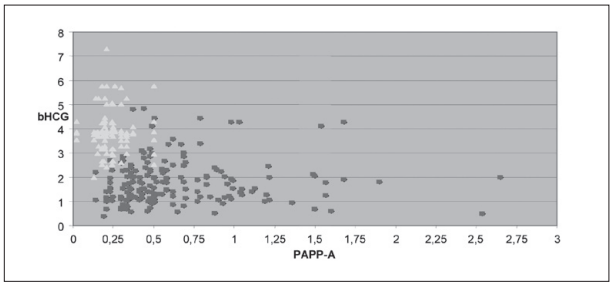
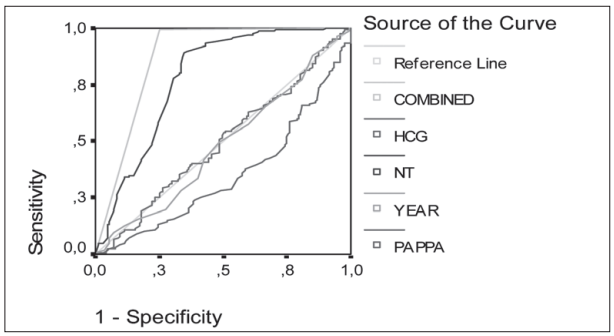
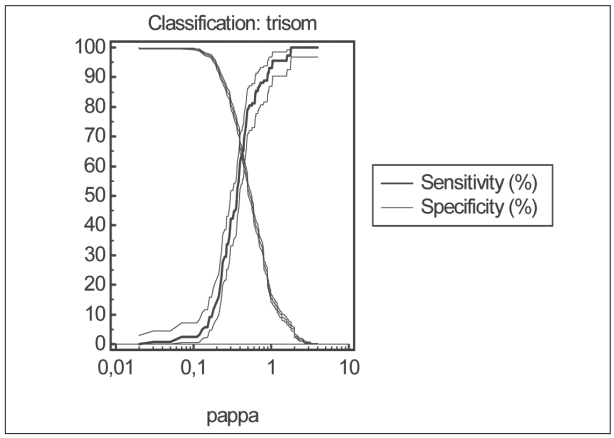


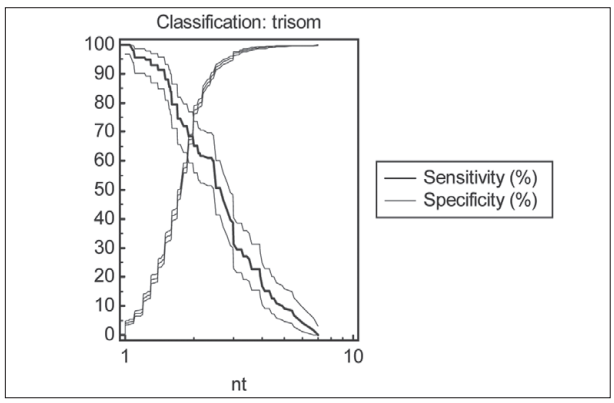
Graph 3. Distribution of MoM values of free beta-HCG and PAPP-A in chromosomally normal pregnancy (dark gray) and trisomy 21 (light gray)



Graph 4. Receiver-operating characteristic curves of screening for aneuploidy by maternal age, serum free beta-hCG, pregnancy-associated plasma protein-A, fetal nuchal translucency thickness and combined screening



Graph 5. Receiver-operating characteristics curves for the performance of screening for trisomy 21 pregnancy-associated plasma protein A, cut off = 0.44 MoM, y=60.10 %



Graph 6. Receiver-operating characteristics curves of the performance of screening for trisomy 21 NT, cut-off = 1.97 MoM; y=67.00.

MoM. In the trisomies 13 and 18, the median free β-hCG was 0.37 (range 0.1-0.55) MoM and the median PAPP-A was 0.17 (range 0.02-0.48) MoM.

MoM values for free β-hCG and PAPP-A in trisomy 21 and unaffected pregnancy are shown in Graph 2. In the trisomy 21 pregnancies the median free beta-hCG was 2.3 (range 0.1-4.6) MoM and the median PAPP-A was 0.45 (range 0.02-2.0) MoM.

Comparison of screening for aneuploidies between a single parameter, either maternal age, free β-hCG, PAPP-A or nuchal translucence thickness alone, and combined screening is shown in Graph 4. For a 5.3% false-positive rate, the respective detection rate were 30.0%, 45.0%, 63.9%, 73.0% and 86.0%.

The values of ROC curves for PAPP-A (AUC 0.66; standard error 0.025; 95% 0.64-0.67; Z- statistic 6.33; $p<0.0001$) and for NT (AUC 0.78; standard error 0.03; 95% 0.73-0.76; z statistic 8.04; $p<0.0001$) are shown in Graphs 5 and 6.

DISCUSSION

The aim of the first trimester scan is not only to screen for aneuploidies but also to detect other fetal malformations and in this respect the ability for better visualization of fetal anatomy at 12-13 gestational weeks.

The major finding of our study is that the first-trimester screening consisting of the combination of maternal age, fetal NT, free β-hCG and PAPP-A, identified 86.0% of all pregnancies with trisomy 21 with a false-positive rate of 5.3%. This detection rate is considerably higher than that obtained with the traditional methods of screening by maternal age (30%), PAPP-A in a first trimester serum biochemistry (63.9%) or fetal NT alone (73.0%). It has been reported that screening for trisomy 21at 11-14 weeks by a combination of the sonographic markers of nasal bone and NT and the biochemical markers free β-hCG and PAPP-A could result in the detection rate of about 97% for a false-positive rate of 5% [7, 8]. The observed sensitivity of 86% by the combined first-trimester method is likely to be underestimated because during the study period women with a high fetal NT undergo invasive testing before biochemical screening. When the fetal NT is above 99th percentile, the estimated risk for trisomy 21 is invariably higher than 1:275 even if the maternal serum-free β-hCG or PAPP-A are within the normal range.

The findings of this study demonstrate that fetal NT follows two distributions, one of which is dependent on CRL while the other is independent of CRL. The distribution in which NT increases with CRL is the same for chromosomally abnormal or unaffected pregnancies. The proportion of cases in which NT does not change with gestation is small for unaffected pregnancies and large for the abnormal group. NT thickness greater than 95th centile has been associated with an increased risk for a chromosome abnormality, congenital heart malformation and other structural defects. If the NT thickness ranges between 3.5-4.4 mm the risk of a fetal chromosome abnormality is

21%, increasing to 33% if 5 mm, 50% if 6 mm and 65% if greater than 6.5mm [7].

The median NT was 1.87 for the whole examined population and NT more the 99th centile was found in trisomy 21, 18, 13 and Turner syndrome. Fetal NT above the 99th centile was only 1.5% of the chromosomally normal fetuses. In a fetus with increased NT thickness and a normal chromosome complement, the prevalence of major cardiac defects increased exponentially with the thickness of the NT. The prevalence of major cardiac defects was 1-2% in fetuses with an NT>3.5 mm, increasing to 3% if 3.5-4.4 mm, 7% if 4.5-5.4 mm, 20% if 5.5-6.4 mm and 30% if 6.5 mm or greater [7, 9]. Ultrasound scan from first-trimester onward can effectively lead to the correct diagnosis or at least raise suspicions so that follow-up scans and echocardiography in the second trimester can detect or exclude major cardiac and structural malformations that could not be identified in the first trimester [10].

The ideal gestation for combined testing in the same visit would be 12 weeks when estimated detections rates of trisomy 21 are 85-90%, whereas the false positive rates are 5% [11]. The alternative strategy for the first-trimester combined screening is that it can be carried out in two separate visits; biochemical testing at 9 weeks and ultrasound scanning at 12 weeks. The estimated detection rates in that strategy would be 90-93%, respectively, with a false-positive rate of 3 and 5% [12]. It has been reported that the performance of combined screening for trisomy 21 decreased with gestation at 3% of a false-positive rate [11, 13, 14, 15]. The estimated detection rate was 92%, 85% and 79% at 11, 12 and 13 weeks respectively. In our study the mean gestation age of the first trimester in combined screening arm was 12.2 weeks. This age is in line with our strategy to detect not only aneuploidies but also other fetal malformations which could be better visualized at 12-13 gestational weeks than at earlier age. Finally, the cost and patients acceptability of the two alternative policies of first trimester screening would also depend on the existing infrastructure of antenatal care.

CONCLUSION

Our study suggests that the strategy of first-trimester combined screening of biochemical values and ultrasonographic parameters at 12 gestational weeks identifies higher percentage of all pregnancies with aneuploidies with a lower false-positive rate than a single parameter strategy.

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Испитивање анеуплоидија на основу животне доби труднице, дебљине набора врата фетуса и биохемијских параметара у серуму труднице током једног прегледа између 11 и 13+6 недеља гестације

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КРАТАК САДРЖАЈ

Увод Анеуплоидије су главни узрок смрти фетуса и сметњи у раном психофизичком развоју детета.

Циљ рада Циљ нашег истраживања био је да се одреди успешност скрининга првог триместра на анеуплоидије коришћењем следећих параметара: животне доби труднице, дебљине набора врата фетуса (енгл. *nuchal translucency – NT*) и хормона слободног бета хуманог хорионског гонадотропина (*β-hCG*) и плазма-протеина А удруженог с трудноћом (*PAPP-A*) у серуму труднице између 11 и 13+6 недеља гестације.

Методе рада Студијом су испитане 4.172 једноплодне трудноће од јануара 2006. до децембра 2010. године. Расподела и процена ризика при комбинованом испитивању на анеуплоидије одређена је у односу на граничну вредност од 1:275.

Резултати У групи физиолошких трудноћа учесталост *NT* изнад 95. центила била је 4,2%. Код трудница с тризомијом 21 *NT* изнад 95. центила дијагностикован је у 72,8% случајева, а код трудница с тризомијом 13 и 18 у 44,4%, односно 66,6% трудноћа. Код физиолошких трудноћа средња вредност *β-hCG* била је 1,0 *MoM*, а средња вредност *PAPP-A* 0,97 *MoM*. У групи жена с тризомијом 13 и 18 средња вредност *β-hCG* била је 0,37 *MoM*, а *PAPP-A* 0,17 *MoM*. Код трудница с тризомијом 21 средња вредност *β-hCG* била је 2,3 *MoM*, а *PAPP-A* 0,45 *MoM*. Анеуплоидије су утврђене код 169 фетуса: 97 с тризомијом 21, 19 с Тарнеровим (*Turner*) синдромом, 28 с тризомијом 18, 11 с тризомијом 13, и 14 фетуса са другим тризомијама. Сензитивност комбинованог скрининга код анеуплоидија била је 86% с учесталошћу лажно позитивних вредности од 5,3% (средња старост трудница била је 33±4,9 година, с тим што је 35% трудница било старије од 35 година).

Закључак Студија је показала да се комбинованим скринингом првог триместра препознаје висок проценат анеуплоидија уз малу учесталост лажно позитивних налаза.

Кључне речи: анеуплоидије; скрининг првог триместра; дебљина вратног набора

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