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DOI: 10.25040/ntsh2023.02.15

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Надійшла до редакції: 26.05.2023

Прийнята до друку: 30.08.2023

Опублікована: 22.12.2023

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Конфлікт інтересів: автори заявляють про відсутність конфлікту інтересів.

Особистий внесок авторів: усі автори в однаковій мірі зробили внесок у підготовку рукопису, його редагування, а також прочитали та затвердили подану версію.

Дозвіл комісії з питань біоетики: 23 листопада 2020 р, протокол № 8 засідання комісії з питань біоетики експериментальних розробок і наукових творів Львівського національного медичного університету імені Данила Галицького

Фінансування: автори не отримали жодної фінансової підтримки свого дослідження.



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Клінічні, ультразвукові та цитогенетичні характеристики плодів першого триместру вагітності зі збільшеною товщиною комірцевого простору

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Вступ. На сучасному етапі розвитку медицини профілактика вроджених вад розвитку (ВВР) і спадкових захворювань набуває все більшого медичного та соціального значення. Поширеність ВВР в Україні стабільно висока і становить 23,7:1000 серед немовлят, народжених живими, і немає тенденції до суттєвого зниження. Маркером ВВР плода, анеуплоїдій та інших патологічних станів є вимірювання товщини комірцевого простору (КП). Відомо, що потовщення КП чи шийна кістозна гідрома в 50-80% випадків пов'язані з різними ВВР і анеуплоїдією плода.

Мета роботи – порівняти клінічні, ультразвукові та цитогенетичні дані в набряклих плодів зі збільшеною товщиною КП від вагітних першого триместру групи високого генетичного ризику для оптимізації алгоритму пренатальної діагностики.

Матеріали і методи. Проведено клінічні обстеження, ультразвукове дослідження (УЗД), генетичне консультування, застосовано інвазійні методи пренатальної діагностики (біопсія хоріона, амніоцентез), лабораторні генетичні методи, такі як каріотипування і FISH, статистичний аналіз отриманих результатів. В роботі було використано методи варіаційної статистики. Довірчий інтервал (ДІ) розраховували за методом Вальда або Фішера. Застосовували співвідношення шансів (ВШ) із відповідним тестом розподілу χ^2 та 95% довірчими інтервалами (95% ДІ). Для дослідження використовували 127 плодів зі збільшеною товщиною КП. Плоди було розподілено на дві групи, з яких 38 мали КП (2,5-3,5) мм (група 1) і 89 плодів мали КП більше 3,5 мм (група 2). Середній вік вагітних групи 1 становив 30.65 ± 0.92 (21-43) роки і був порівнювальним із віком жінок групи 2 – 31.44 ± 0.56 (21-44) роки ($p = 0.46$).

Результати. 65,4% випадків вагітностей з плодами зі збільшеною товщиною КП завершилися несприятливо. У жінок, які мали плоди групи 1, невдале завершення вагітності реєстрували в 63,2% випадків, а в тих, що мали плоди групи 2 – у 66,3% ($p = 0.73$). Завмирання вагітності спостерігали в 4,8% жінок. Хромосомну патологію було виявлено в 69,9% плодів, ВВР нехромосомної етіології – у 25,3%. У плодів групи 1 хромосомні аномалії було виявлено в 55,3% випадків, а в плодів групи 2 – у 41,6%. У спектрі хромосомних аномалій набряклих плодів переважали анеуплоїдії – додаткові копії автосом.

Висновки. Статистично не підтверджено істотної різниці за частотою хромосомних аномалій у плодів групи 1 порівняно з групою 2, що свідчить про високу інформативну цінність збільшеної товщини КП, враховуючи розміри (2,5-3,5) мм, у плодів першого триместру вагітності як маркера хромосомної патології. Статистично істотно вищу захворюваність на ВВР нехромосомної етіології було виявлено в плодів із патологією венотної протоки і КП більшим, ніж 3,5 мм, порівняно з плодами з цією ж патологією і КП (2,5-3,5) мм. Запропоновані зміни до алгоритму обстеження вагітних із набряклими плодами обов'язково мають охоплювати скринінг ВВР серця в першому триместрі. При виявленні ВВР серця у плодів із нормальним каріотипом варто виконувати молекулярно-генетичні дослідження для виключення синдрому Ді-Джоржі.

Ключові слова: потовщення комірцевого простору, вроджені вади розвитку, хромосомні аномалії, пренатальна діагностика, набряклість плодів, перший триместр вагітності.

Clinical, ultrasound and cytogenetic characteristics of fetuses with increased nuchal translucency thickness in the first trimester of pregnancy

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Background: Improving programs for early prenatal detection of congenital malformations remains a relevant scientific and practical problem. The prevalence of congenital anomalies in Ukraine is 23.7:1000 among live-born babies, and there is no significant decrease in it. The aim of this study was to compare clinical, ultrasound and cytogenetic data in swollen fetuses with increased nuchal translucency (NT) thicknesses from the group of pregnant women in the first trimester at high genetic risk to optimize the algorithm of prenatal diagnostics.

Materials and Methods: Clinical examinations, ultrasound diagnostics, invasive methods of prenatal diagnostics (chorion biopsy, amniocentesis), genetic testing techniques, such as karyotyping and FISH, genetic counseling and statistical analysis were carried out. The results of complex examinations of 127 fetuses with an increased NT thickness from the group of pregnant women were analyzed. Fetuses were divided into two groups with an NT thickness of 2.5–3.5 mm (group 1) (38 cases) and with an NT above 3.5 mm (group 2) (89 cases).

Results: Among pregnancies with fetuses with an increased NT thickness, there were 65.4% cases of adverse outcomes with chromosomal pathology (69.9%), congenital malformations of

OPEN ACCESS

DOI: 10.25040/ntsh2023.02.15

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Received: 26 May, 2023

Accepted: 30 Aug, 2023

Published: 22 Dec, 2023

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Disclosures: The authors declared no conflict of interest.

Author contributions: All authors contributed equally to the first draft of the manuscript, manuscript revision, and read and approved the submitted version.

Ethical approval: November 23, 2020, minutes No. 8 of the meeting of the commission on bioethics of experimental development and scientific works of the Danylo Halytsky Lviv National Medical University

Funding: The authors received no financial support for their study.



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non-chromosomal etiology (25.3%) and pregnancy loss (4.8%). The frequency of chromosomal abnormalities in fetuses of group 1 was 55.3% and 41.6% in group 2. Congenital malformations of various systems and organs in fetuses, the most frequent of which were cardiac defects, were diagnosed. The ratio of congenital heart defects in the fetuses of groups 1 and 2 was 23.7% and 43.8%, respectively ($p=0.03$; $OR=0.40$).

Conclusions: There is no significant difference between the frequency of chromosomal abnormalities in the fetuses of group 1 compared to group 2, which indicates a high informative value of an increased NT thickness, including the thickness of 2.5–3.5 mm in fetuses in the first trimester as a marker of chromosomal pathology. A significantly higher incidence of congenital malformations of non-chromosomal etiology was found in fetuses with venous duct pathology and NT thickness over 3.5 mm compared to fetuses with the same pathology and NT thickness of 2.5–3.5 mm. Proposed changes to the management algorithm for pregnant women with swollen fetuses include mandatory congenital heart defects screening in the first trimester.

Keywords: Increased nuchal translucency thicknesses, congenital malformations, chromosomal abnormalities, prenatal diagnostics, swollen fetuses, first trimester of pregnancy.

Introduction

Preventing congenital malformations (CM) and hereditary diseases is an increasing need to address medical and social problems. According to WHO, the frequency of births of children with CM is 4–6% of the total number of newborns. 2.7 million children with CM die annually across the globe. The prevalence of congenital anomalies in Ukraine is 23.7:1000 among live-born babies, and there is no dynamics towards its significant decrease. Infant mortality in 20–30% of cases is caused by genetic factors [1]. Therefore, improving programs for early prenatal detection of CM remains a relevant scientific and practical problem.

One of the most informative early prenatal markers of fetal CM, aneuploidies and other pathological conditions is the nuchal translucency (NT) thickness. In 50–80% of cases, NT thickening is known to be associated with different CM and fetal aneuploidy [2]. The use of NT in 1992 by Nicolaides et al. was a major breakthrough in screening for chromosomal aneuploidies pathology in fetuses at the end of the first trimester. Thus, during the 10-year study period, among 71 fetuses with an increased NT, chromosome aneuploidies were observed in 22% of cases [3]. Subsequently, a more extensive analysis of 374 fetuses with an increased NT was performed [4], where chromosomal abnormalities (CA) were detected in 29.1% of cases. Fetuses were divided into four groups depending on the NT

thickness: 1) 2.5–3.4 mm, 2) 3.5–4.4 mm, 3) 4.5–5.4 mm and 4) ≥ 5.5 mm, and within these groups, the frequency of CA varied and constituted 22.8%; 22.0%; 34.5% and 56.4%, respectively. There was a significant difference in the frequency of CA between groups with different NT thicknesses, with the degree of thickness positively correlated with CA ($r=0.208$, $P<0.05$). The authors confirmed that fetuses with an increased NT size also showed a higher incidence of CA, structural malformations, and adverse pregnancy outcomes. The incidence of CA in fetuses with a thickened NT combined with other CMs diagnosed with ultrasound was higher than in cases of isolated NT thickening.

After introducing the modern laboratory genetic method into clinical practice, such as chromosomal microarray analysis, which allows diagnosing microstructural CA, their detection in fetuses with an isolated NT thickening in the first trimester increased by an average of 5% compared to traditional karyotyping. However, even with a normal microarray result in the group of fetuses with isolated increased NT thickness, the risk of concurrent CM or intrauterine death was 28.8% higher [5]. Thus, the size of the fetal NT in the first trimester is a highly informative wide-spectrum echographic marker of fetal CM and is a mandatory parameter in early ultrasound protocols. NT thickness is measured at the first ultrasound screening

at fetal crown-to-rump length (CRL) of 45–84 mm according to the standards proposed by the Fetal Medicine Foundation (FMF) and requires an accurate sagittal cut [6]. In the first trimester, edema of the back of the head is diagnosed as thickened NT, or in the second trimester, as an increase of the nuchal fold thickness [7]. For the appropriate gestational age, fetuses with an NT thickness between the 95th percentile and a fixed value of 3.5 mm are classified as a separate group. This value is the 99th percentile for any gestational term, as recommended by the FMF (<https://courses.fetalmedicine>). A significant number of such fetuses may also be included in the high genetic risk group, where additional invasive or other examinations are required in the future. Increased NT thickness >99 percentile (≥ 3.5 mm) is found in approximately 1% of pregnancies. However, the visualization of this marker is not an unambiguous synonym for the presence of pathology in the fetus. Some such fetuses have normal karyotypes, and pregnancy success depends on the presence/absence of concomitant developmental abnormalities revealed at ultrasound examination [8, 9]. Thus, when an increase in NT is detected during an ultrasound performed to find combined systemic anomalies, the algorithm of prenatal diagnosis should be combined with a thorough anatomical ultrasound scan using echo markers.

One of the important minor echo markers is the fetal nasal bone (NB). Fetal profile examinations for absence or hypoplasia of the NB are performed at 11–14 weeks in ultrasound screening for aneuploidy, particularly trisomy 21 [10, 11]. Based on an examination of 15,822 fetuses, a group of FMF researchers found that the NB is absent in 60–70% of fetuses with trisomy 21 and 1.4% of normal fetuses at 11–14 weeks of development. Anthropometric studies have shown that the depth of the nose root is abnormally small in 50% of Down syndrome cases [12, 13]. The absence of the NB was found in 73% of fetuses with trisomy 21 and only 0.5% of fetuses with normal karyotype [14]. Thus, the NB absence or its hypoplasia is one of the sonographic markers helping in the recognition of chromosomal disorders and genetic syndromes associated with facial dysmorphism. Incorporating the assessment of the

fetal nose root into the ultrasound screening in the first trimester and considering this parameter in the estimation of the individual genetic risk of women may lead to a significant reduction in the need for unnecessary invasive interventions. A comprehensive cumulative assessment of NT thickness, maternal age, serum biochemical screening data of pregnant woman, and fetal profile examination for the presence or absence of the nose root may increase the effectiveness of Down syndrome detection up to 97% with a false positive rate of 5% [14, 15].

The pathology of the venous duct (VD) (ductus venosus blood flow) is considered to be another informative early echographic marker of aneuploidy and abnormalities of fetal development in the first trimester, especially in combination with other ultrasound indicators [16]. Determining VD pathology is particularly necessary in cases of suspected monosomy X, for which this feature is specific, and to differentiate this CA from others in cases of fetuses with a thickened NT. However, according to some publications, the VD pathology as an isolated defect is still insufficient to detect aneuploidy [17, 18].

There are known other pathological conditions which are also associated with an increased NT thickness. Thickened NT is often associated with abnormalities of different systems in the fetus, particularly cardiovascular, urinary, skeletal, articular and others, as well as with hypoproteinemia (<https://courses.fetalmedicine>). It is known that more than 2% of children are born with different CM, of which 25% are heart defects [19]. The fact that about 50% of heart defects are due to complex CM of the heart, where specialized medical care is necessary during the first days, months, and sometimes hours of the child's life, poses a difficulty. In general, congenital heart defects (CHDs) are the most common forms of birth defects and occur in 3–8/1000 fetuses. Currently, only 15–30% of cardiac malformations in newborns are detected prenatally. In this regard, determining NT thickness and VD pathology are informative methods used to find cardiac abnormalities in fetuses without chromosomal abnormalities. Evidence from publications suggests that the incidence of complex CHDs is clearly correlated with an increase in NT size [20]. At 11–13

weeks of gestation, fetal NT measurement and assessment of blood flow through the tricuspid valve and in the venous duct allows early diagnosis of cardiac malformations). Although the basic principles of prenatal diagnostics have already been developed, there is a constant improvement in specific examination algorithms, the search for optimal schemes and combinations of various methods (ultrasound, genetic, biochemical), the study of the epidemiology and etiology of CM, their manifestation in the prenatal period.

The aim of this study was to compare clinical, ultrasound and cytogenetic data in swollen fetuses with increased NT thicknesses from the group of pregnant women in the first trimester at high genetic risk to optimize the algorithm of prenatal diagnostics.

Materials and Methods

The results of complex examinations of 127 fetuses with increased NT thickness in the first trimester were analyzed (CRL 45-84 mm corresponds to the gestational age of 11 weeks and 2 days – 13 weeks and 6 days). Fetuses were divided into groups 1 with an NT thickness of 2.5–3.5 mm (38 cases, of which three cases were twins) and 2 with an NT of 3.5 mm or more (89 cases, including two cases of twins). The average age of pregnant women was 30.65 ± 0.92 (21–43) years in group 1 and was comparable with the age of women 31.44 ± 0.56 (21–44) years in group 2 ($p = 0.46$).

Clinical examinations, ultrasound diagnostics, invasive methods of prenatal diagnostics (chorion biopsy, amniocentesis), genetic testing techniques, such as karyotyping and FISH, genetic counseling and statistical analysis were carried out. Ultrasound, biometric, morphological and pathophysiological features in fetuses were evaluated. Echography was performed in real-time using a Voluson E8 device, an abdominal or transvaginal linear or convex 3-4 D transducer (frequency of 2–9 MHz). Ultrasound exposure was 15–30 min. A standard fetal assessment was used to obtain basic biometric indicators and describe changes in the condition of internal organs. Prenatal syndromological analysis was conducted when fetal congenital abnormalities were detected using ultrasound diagnostics. Invasive methods of prenatal di-

agnostics were recommended for pregnant women at high genetic risk (the cut-off value of first-trimester screening risk is 1:250 for trisomy 21 and 1:100 for trisomy 13 and 18, according to the National Protocol). Chorionic villus biopsy was performed by aspiration through abdominal access under constant ultrasound control, which was performed twice after the interventions: 10 min and 24 h after the procedure.

Fetal karyotype was determined using the biological material of the chorionic villi sampling (CVS) (11–13 weeks). A standard cytogenetic GTG method was used. Chromosome spread from CVS was prepared using the direct method [21]. A molecular cytogenetic fluorescence in situ hybridization (FISH) method [22], performed according to the protocol recommended by the manufacturer of DNA probes, was used for additional examination of fetuses with CM. The used laboratory equipment and reagents for biochemical screening fully complied with the requirements of the Resolution of the Cabinet of Ministers of Ukraine dated October 2, 2013, No. 754, "On Approving Technical Regulations for Medical Devices for In Vitro Diagnostics" (certificate of conformity No. PR. 117–18 of October 29, 2018). The studies were performed following user manuals for test kits manufactured by BRAHMS Kryptor. The estimation of the personal risk of pregnant women for common CA in the fetus was performed using the Astraia and Fast-Screen computer software (Germany).

The results are presented as an absolute value, and their relative number as a percentage and 95% confidence interval was calculated using the Fisher angular transformation method. The difference between groups of qualitative indicators was studied using a frequency table, and significance was established using Pearson's chi-squared test. If the expected value in one of the cells of the frequency table was less than 5, then Fisher's exact test was used. To determine the significance of the difference between the two groups with the Gaussian distribution, the independent t-test was used. If the distribution was non-Gaussian – the Mann-Whitney U test was applied. The difference in samples was considered to be significant at $p < 0.05$.

Results

Among pregnancies with fetuses with an increased NT thickness, 83/127 (65.4%) cases of adverse outcomes were reported. Chromosomal pathology was present in 58/83 (69.9%) cases, CM of non-chromosomal etiology in 21/83 (25.3%) cases, and pregnancy losses in 4/83 (4.8%) cases. In fetuses of group 1, adverse pregnancy outcomes were found in (24/38) 63.2% of cases and in group 2, in (59/89) 66.3% of cases ($p=0.73$). The CA was detected in (21/38) 55.3% of cases, and CM was diagnosed in (3/38) 7.9% of cases in fetuses of group 1. In group 2, the share of chromosomal pathology was lower than in group 1 – (37/89) 41.6%. However, CM cases significantly increased and constituted (18/89) 20.2%. In addition, pregnancy losses in (4/89) 4.5% of cases were recorded at a later gestational age ($p=0.16$; $p=0.09$ and $p=0.18$, respectively).

Chromosomal pathology in fetuses with increased NT thickness

The spectrum of chromosomal pathology diagnosed in fetuses of groups 1 and 2 from pregnant women at high genetic risk is shown in Table 1.

Aneuploidy with extra copies of autosomes is dominant in fetuses (Table 1). In fetuses from group 1, trisomy 21 was detected in 61.9% of all cases, including one mosaic karyotype: 47, XY,+21[25]/46, XY[27]*([]*means the quantity of the cell's clones). Trisomy 18 was the second most common abnormality, and monosomy X was diagnosed in only one case. The spectrum of CA in group 2 was more diverse. Although trisomy 21 and 18 dominated, 13.5% of cases showed monosomy X, characterized by specific and pronounced edema of cervical cystic hygroma type. However, in general, in both groups, a statistically significant increase in the incidence of abnormalities of autosome was observed compared to sex chromosomes: in group 1 – 20/21 (95.2%) and 1/21 (4.8%) ($p<0.001$; OR=400.00), and in group 2 – 31/37 (83.8%) and 6/37 (16.2%) ($p<0.001$; OR=26.69). At the same time, there was no statistically significant difference in the frequency of autosomal abnormalities between fetuses of groups 1 and 2 (95.2 and 83.8%, respectively, $p=0.20$; OR=3.87). The average age of women in group 1 who had fetuses with autosomal abnormalities was 33.5 ± 1.37 years and did not differ from that of women in group 2 with similar chromosomal pathology in fetuses (32.4 ± 1.11 years, $p=0.53$).

Table 1

Spectrum of chromosomal pathology in fetuses with increased nuchal translucency thickness from pregnant women with high genetic risk

Spectrum of chromosomal abnormalities	Number of fetuses with NT thickness (2.5–3.5 mm) (group 1) (absolute values, %) (n1=21)	Number of fetuses with NT thickness (> 3.5 mm) (group 2) (absolute values, %) (n2=37)	<i>p</i>	OR
Trisomy 21	13 (61.9%) (95% CI 40.7–81.0)	20 (54.1%) (95% CI 38.1–69.6)	0.56	1.38
Trisomy 18	5 (23.8%) (95% CI 8.5–43.8)	7 (18.9%) (95% CI 8.1–32.9)	0.66	1.34
Additional marker chromosomes	2 (9.5%) (95% CI 1.0–25.3)	0 (95% CI 0.0–2.6)	0.06	9.61
Deletion 22q11*	0 (95% CI 0.0–4.5)	2 (5.4%) (95% CI 0.5–14.9)	0.28	0.00
Triploidy (69,XXX)	0 (95% CI 0.0–4.5)	1 (2.7%) (95% CI 0.0–10.3)	0.45	0.00
Monosomy X (45,X)	1 (4.8%) (95% CI 0.0–17.7)	5 (13.5%) (95% CI 4.6–26.2)	0.29	0.32
Double aneuploidy (48, XY,+21,+mar)	0 (95% CI 0.0–4.5)	1 (2.7%) (95% CI 0.0–10.3)	0.45	0.00
Mosaic aneuploidy of sex chromosomes (47, XYY/48, XXYY)	0 (95% CI 0.0–4.5)	1 (2.7%) (95% CI 0.0–10.3)	0.45	0.00

Note: * abnormalities were detected using the FISH method in fetuses with congenital heart defects.

There was no statistically significant difference in the frequency of sex chromosome abnormalities (4.8% vs. 16.2%, $p=0.20$; $OR=0.26$). In group 2, microdeletion of the long arm of chromosome 22 (DiGeorge syndrome) was detected in two fetuses, double aneuploidy (karyotypes 48, XY,+21,+mar and 47, XYY/48, XXYY) in the other two cases and triploidy (karyotype 69, XXX) in one case. Thus, the frequency of CA in fetuses from group 1 was 55.3% (21/38), and in fetuses from group 2 – 41.6% (37/89), indicating a high informational value of the increase in the fetal NT thickness in the first trimester as a marker of chromosomal pathology.

Congenital malformations of non-chromosomal etiology in fetuses with an increased NT thickness

Fetuses with NT greater than 3.5 mm (group 2) were diagnosed with CM of various systems and organs, the most frequent of which were heart defects. The spectrum of CHDs in swollen fetuses with different NT thicknesses detected by ultrasound is shown in Table 2.

Fetuses from group 1 had predominant AV communication among the CHDs, and group 2 fetuses had a broader spectrum of CM with predominating conotruncal anomalies. In the fetuses of group 1, only one case of a heart defect was identified – VSD. In the fetuses of group 2, a broader spectrum of cardiac CM was observed, but conotruncal malformation prevailed (Table 2). Among other CHDs, group 2 fetuses were diagnosed with tricuspid valve failure in 6/37 (15.7%) cas-

es, aortic coarctation in 3/38 (7.91%) cases, open oval window in 2/38 (5.3%) cases, and one case per each of such defects as aortic stenosis, double aortic arch, heterotaxia syndrome, and open arterial duct. The ratio of cardiac CM in fetuses of groups 1 and 2, respectively, was 9/38 (23.7%): 39/89 (43.8%) (95% CI 11.7–38.3: 33.7–54.2) ($p=0.03$; $OR=0.40$).

When detecting cardiac CM in fetuses with thickened NT during invasive diagnostics, molecular cytogenetic testing shall be performed together with standard karyotyping to exclude DiGeorge microdeletion syndrome accompanied by congenital abnormalities of large vessels (Petersen, 2020). Due to this approach, two cases of 22q11 microdeletion were diagnosed in the sample of fetuses with increased NT thickness. An example of the FISH analysis result is presented in Figure 1.

We compared the prevalence of the entire CM spectrum in both groups' fetuses with a normal karyotype. A normal karyotype was found in 17/38 (44.7%) fetuses from group 1 and 52/89 (58.4%) from group 2. In fetuses from group 1 with normal karyotype, CM was found in 3/17 (17.6%) cases and in group 2 – in 18/52 (34.6%) cases. The study results of the clinical characteristics of swollen fetuses with different NT thicknesses and a normal karyotype are shown in Figure 2 and Figure 3.

CHDs dominated the pathological NB fetuses of group 2 in comparison with other malforma-

Table 2

Spectrum of congenital heart defects in swollen fetuses with different nuchal translucency thickness

Spectrum of CHDs	Number of fetuses with NT thickness (2.5–3.5 mm) (group 1) (absolute value, %) (n1=9)	Number of fetuses with NT thickness (> 3.5 mm) (group 2) (absolute value, %) (n2=39)	<i>p</i>	<i>OR</i>
Conotruncal anomalies in the abnormal V-sign	2 (22.2%) (95% CI 2.7–53.2)	15 (38.5%) (95% CI 24.0–54.0)	0.36	0.46
AV-communication	4 (44.4%) (95% CI 15.4–75.8)	5 (12.8%) (95% CI 4.3–25.0)	0.03	5.44
VSD	2 (22.2%) (95% CI 2.7–53.2)	4 (10.3%) (95% CI 2.8–21.6)	0.33	2.50
Other defects	1 (11.1%) (95% CI 0.0–38.2)	15 (38.5%) (95% CI 24.0–54.0)	0.12	0.20

Note: AV-communication – antioventricular communication; VSD – ventricular septal defect.

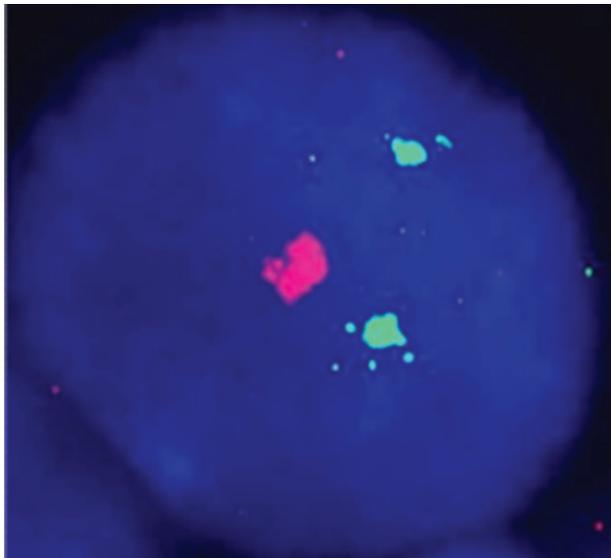
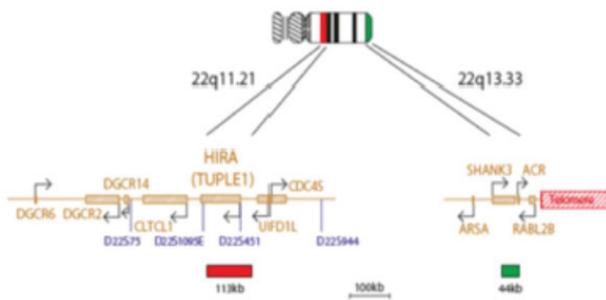


Figure 1. 22q11 microdeletion in a swollen fetus with an NT thickness over 3.5 mm

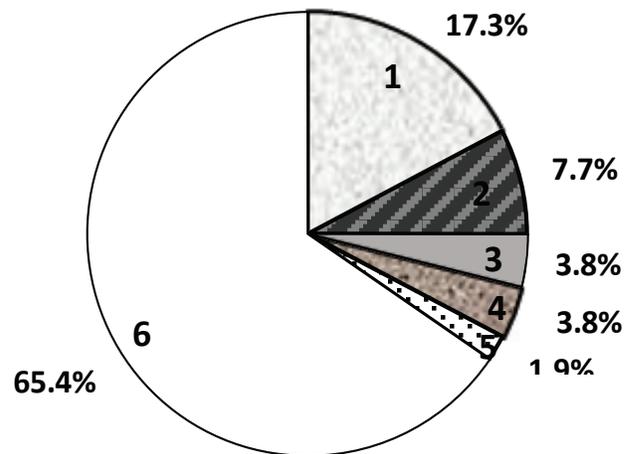


Figure 3. The spectrum of congenital malformations in swollen fetuses with normal karyotype (group 2) (NT thickness of > 3.5 mm):

1. cardiac abnormalities – 9 (17.3%);
2. multiple malformations – 4 (7.7%);
3. central nervous system – 2 (3.8%);
4. osseous and articular system – 2 (3.8%);
5. urinary system – 1 (1.9%);
6. without congenital malformations – 34 (65.4%).

tions. In the fetuses of group 1, three various CMs were diagnosed: 1) cardiac (VSD), which closed during the pregnancy and a healthy child was born; 2) central nervous system (dysgenesis of the corpus callosum) was diagnosed in the third trimester and confirmed after birth; 3) osseous and articular system (skeletal dysplasia, short rib polydactyly syndrome).

Increased NT thicknesses in combination with other echo markers

A prevalence analysis of other minor echo markers detected by ultrasound was performed in swollen fetuses with different NT thicknesses. In fetuses of group 1, pathological NB (aplasia/hypoplasia) was detected in 20/38 (52.6%) cases and in fetuses of group 2 – in 38/89 (42.7%) cases. Thus, the ratio of pathological NB between the groups was 52.6%:42.7% (95% CI: 36.9–68.1: 32.7–53.1) ($p=0.30$; $OR=1.49$). The pathology of VD was found in 14/38 (36.8%) fetuses of group 1 and in 26/89 (29.2%) fetuses of group 2. The VD pathology ratio between groups of fetuses is as follows: 36.8%:29.2% (95% CI: 22.4–52.6: 20.3–39.0) ($p=0.40$; $OR=1.41$).

Among group 1 of fetuses with an increased NT thickness and pathological NB, in 12/20 (60.0%) cases, CA was diagnosed, and in the

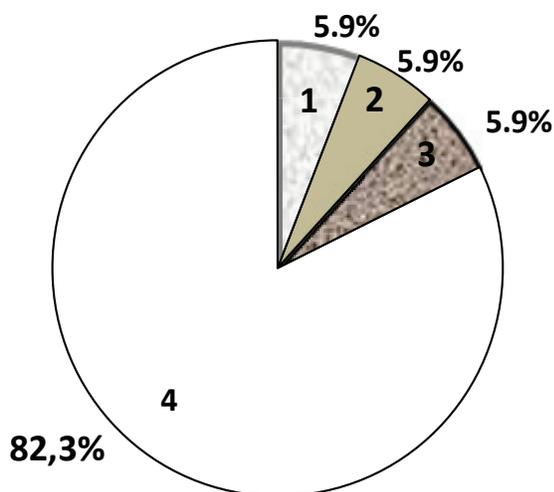


Figure 2. The spectrum of congenital malformations in swollen fetuses with normal karyotype (group 1) (NT thickness of 2.5–3.5 mm):

- cardiac abnormalities – 1 (5.9%);
- central nervous system – 1 (5.9%);
- osseous and articular system – 1 (5.9%);
- without congenital malformations – 14 (82.3%).

group 2 fetuses – in 24/38 (63.2%) cases, the difference was not significant ($p=0.81$). These results are consistent with publications on the absence of NB or its hypoplasia in 58.8% of Down syndrome cases and 1.7% of unaffected pregnancies, respectively [23]. CM of non-chromosomal etiology was found in 7/20 (35.0%) fetuses of group 1 and in 23/38 (60.5%) fetuses of group 2 with pathological NB (Table 3).

A significantly higher incidence of CM of non-chromosomal etiology was found in fetuses with VD pathology and NT thickness over 3.5 mm compared to fetuses with the same pathology and NT thickness of 2.5–3.5 mm.

Discussion

This study is devoted to analyzing the effectiveness of an early ultrasound marker, such as measuring NT thicknesses, to identify CM and common chromosomal pathology of fetuses to optimize the algorithm of prenatal diagnostics in the first trimester.

Obtained data indicate that 14/38 (36.8%) fetuses with an NT thickness of 2.5–3.5 mm had normal karyotype without CM, of which three fetuses from dichorial twins had normal NT thickness; 30/89 (33.7%) fetuses with an NT thickness of more than 3.5 mm also had normal karyotype without CM, of which two from dichorial twins had an NT thickness within the normal range (95% CI: 22.4–52.6: 24.3–43.8 $p=0.73$; OR=1.15). These data are coherent with the findings of the world's leading experts in fetal medicine, according to which NT thickening can be detected in normal preg-

nancies, and the likelihood of chromosomal pathology in the fetus depends on the presence or absence of concomitant abnormalities diagnosed by ultrasound [8]. In swollen fetuses with a normal karyotype, the ratio of healthy fetuses compared to those with CM is 82.4%:17.6% in group 1 and 62.5%:37.5% in group 2, $p=0.13$. Fetuses with an NT thickness of over 3.5 mm (group 2) with a normal karyotype showed a wider spectrum of pathology than fetuses of group 1. The CA frequency and spectrum vary depending on the degree of NT thickening. They are slightly higher in the study sample than the results obtained by other authors, especially in fetuses with an NT thickness of 2.5–3.5 mm. Thus, according to the literature data [24, 25], 38% of fetuses with NT >3.5 mm had CA, of which 28% were trisomy 21, 18, 13, 7% of fetuses had abnormalities of sex chromosomes, 0.8% had triploidy karyotype, and 1.6% had unbalanced chromosomal aberrations.

CHDs dominated pathological NB fetuses of group 2 in comparison with other malformations. Multiple CMs were typical for this group, accounting for 8.3% of all cases. The ratio of CHDs in fetuses of groups 1 and 2, respectively, was 23.7%: 43.8% ($p=0.03$; OR=0.40). Thus, the probability of detecting a cardiac abnormality was higher with an increase in the NT thickness over 3.5 mm, which is consistent with other researchers' data [26] and necessitates in-depth fetal echocardiography when detecting this echo marker at routine screening ultrasound check. This is confirmed by the world's leading experts in fetal medicine. In

Table 3

Chromosomal abnormalities and congenital malformations in fetuses with increased NT thicknesses and with combined echo markers (hypoplasia of the nasal bone, pathology of the venous duct)

Detected abnormalities	Number of fetuses with NT thickness (2.5–3.5 mm) (group 1) with NB hypoplasia	Number of fetuses with NT thickness (> 3.5 mm) (group 2) with NB hypoplasia	<i>p</i>	OR
1) CA	12/20 (60.0%) (95% CI 38.3–79.8)	24/38 (63.2%) (95% CI 47.4–77.6)	0.81	0.88
2) CM	7/20 (35.0%) (95% CI 16.2–56.7)	23/38 (60.5%) (95% CI 44.7–75.3)	0.06	0.35
Detected abnormalities	with VD pathology	with VD pathology	<i>p</i>	OR
3) CA	9/14 (64.3%) (95% CI 38.4–86.3)	13/26 (50.0%) (95% CI 31.3–68.7)	0.39	1.80
4) CM	4/14 (28.6%) (95% CI 8.8–54.0)	20/26 (76.9%) (95% CI 59.2–90.8)	0.003	0.12

particular, it was shown that in fetuses with a normal karyotype and without non-cardiac abnormalities, adding a cardiac scan to the combined parameters of the first-trimester screening significantly improved the detection of the main cardiac CM [27].

The algorithm for the management of pregnant women with swollen fetuses and manda-

tory CHDs is based on the performed comprehensive study of pregnancy outcomes in the cases of increased NT thicknesses (Fig. 4).

This will allow for creating a group of high genetic risk more accurately, as well as planning the necessary range of verification diagnostics and laboratory genetic methods. Based on the obtained results, we consider it necessary

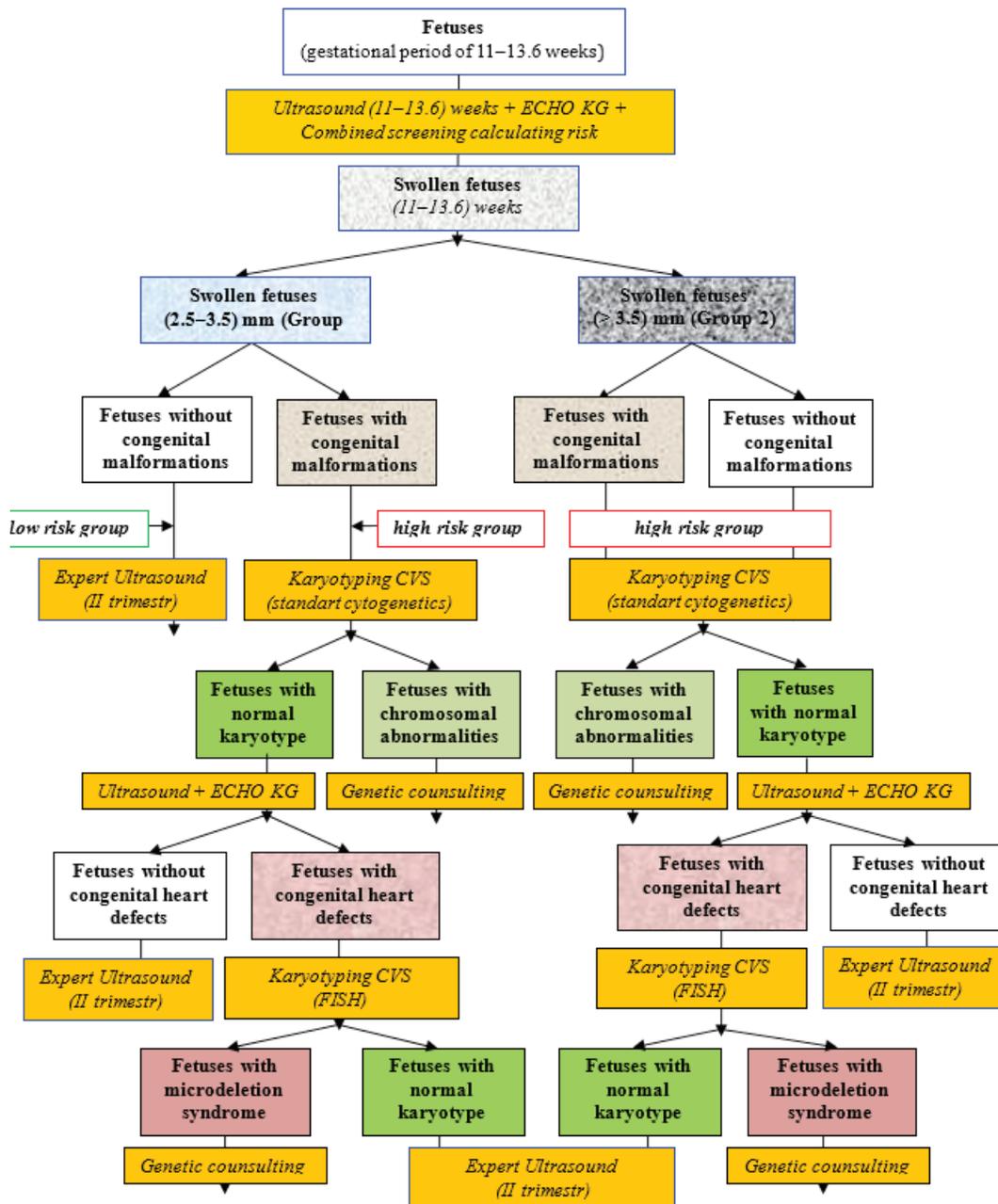


Figure. 4. Algorithm for prenatal diagnostics of swollen fetuses with increased NT thickness (2.5-3.5 up to > 3.5) mm in the gestational period of 11-13.6 weeks

to conduct genetic studies of swollen fetuses precisely at early gestational age (11–13.6 weeks), as evidenced by the high frequency of CA in these fetuses with an NT thickness of 2.5–3.5 mm, which did not significantly differ from CA with increased NT thickness > 3.5 mm. Molecular cytogenetic studies should be performed if CHDs are detected in fetuses with a normal karyotype for exclusion of Di-George syndrome. The algorithm for prenatal diagnostics of swollen fetuses with increased NT thickness > 3.5 mm in a gestational period of 11–13.6 weeks is shown in Figure. 4.

Thus, methods of comprehensive prenatal diagnostics of swollen fetuses in the first trimester of gestational age of 11–13.6 weeks from women at high genetic risk allow for the early detection of fetal CA and CM, which significantly increases the effectiveness of genetic counseling that will narrow down to the prevention of this pathology both in a separate family and in the population as a whole. The analysis results are significant for a deeper clinical study and for improving the effectiveness of prenatal fetal assessment in families at risk of congenital and hereditary pathology, which will improve genetic assistance to the population [28, 29]. The similarity of the prevalence of additional echo markers at different NT thicknesses indicates the need for the combined use of all screening echographic and biochemical mark-

ers to assess the individual's combined genetic risk (in the case of an increase in NT thickness in the fetus, more than 3.5 mm).

In conclusions: Among pregnancies with fetuses with an increased NT thickness, 65.4% of cases of adverse outcomes were reported. There is no significant difference between the frequency of chromosome abnormalities in fetuses of group 1 as compared to group 2, which indicates a high informative value of an increased NT thickness, including of 2.5–3.5 mm in fetuses in the first trimester as a marker of chromosomal pathology.

A significantly higher incidence of CM of non-chromosomal etiology was found in fetuses with venous duct pathology and NT thickness over 3.5 mm compared to fetuses with the same pathology and NT thickness of 2.5–3.5 mm. Fetuses with thickened NT were diagnosed with CM of various systems and organs, the most frequent of which were cardiac abnormalities. The ratio of CHDs in fetuses of groups 1 and 2, respectively, was 23.7%: 43.8% ($p=0.03$; $OR=0.40$).

Proposed changes to the management algorithm for pregnant women with swollen fetuses include mandatory CHD screening in the first trimester.

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