Altered Doppler flow velocities in fetuses with increased nuchal translucency

ABSTRACT

Objective
We sought to assess blood flow in relation to jugular lymphatic distension in fetuses with increased and normal nuchal translucency (NT).

Study design
In all, 72 fetuses with normal NT and 71 fetuses with NT > 95th percentile were evaluated. NT-size, jugular lymphatic sacs (JLS), jugular vein and ductus venosus pulsatility index for veins (PIV), and intracardiac velocities were measured.

Results
JLS were visualized in 22/72 fetuses with normal and in 55/71 fetuses with increased NT. Jugular vein and ductus venosus PIV was higher in fetuses with increased NT compared to normal NT (P < .01). Visibility of JLS was associated with a higher ductus venous PIV (P < .05), but not with a higher jugular vein PIV. Larger NT and larger JLS volumes were associated with higher jugular vein and ductus venosus PIV (P < .05).

Conclusion
This study shows a relation between increased NT, jugular lymphatic distension and altered blood flow in jugular vein and ductus venosus.
BACKGROUND AND OBJECTIVE

The ultrasonographic measurement of the nuchal translucency (NT) in the first-trimester of pregnancy is a widely used screening method to identify chromosomal abnormalities in human fetuses.\textsuperscript{1,2} Increased NT is also associated with structural anomalies such as cardiac defects and several genetic syndromes.\textsuperscript{3} Recent studies implicate a disturbed lymphatic development as a likely explanation for the pathophysiology of increased NT.\textsuperscript{4-7} First-trimester fetuses with increased NT morphologically show nuchal edema, accompanied by distended jugular lymphatic sacs (JLS).\textsuperscript{7} Ultrasound studies of first-trimester fetuses show a similar association between increased NT and the presence of enlarged JLS.\textsuperscript{6,8}

Also, altered ductus venosus flow velocities have been described in fetuses with increased NT.\textsuperscript{9,10} These altered flow velocities and the frequently found cardiovascular malformations have led to cardiac failure as a possible explanation for increased NT.\textsuperscript{9,11} However, other signs of cardiac decompensation are rarely seen in fetuses with enlarged NT.

So far, no study has been performed to investigate if there is a relation between the jugular lymphatic development and haemodynamics in fetuses with increased NT.

In this study, we prospectively assessed first-trimester fetuses with normal and increased NT to evaluate presence and volumes of the JLS. The carotid artery, jugular vein, ductus venosus, intracardiac velocities and flow across the tricuspid valve were evaluated using Doppler flow to assess a possible relation between disturbed jugular lymphatic development and altered haemodynamics of fetuses with increased NT.

MATERIALS AND METHODS

Women referred to our hospital for tertiary care because of increased NT, and women attending our hospital for first-trimester screening, were asked to participate in the study. A total of 144 singleton pregnancies were examined. In all, 71 fetuses had increased NT and 73 fetuses had a normal NT. Increased NT was defined as a NT above the 95\textsuperscript{th} percentile. All patients received written information and gave informed consent. The medical ethical committee of the VU University Medical Center approved the study.

Gestational age was calculated based on the reported last menstrual period and adjusted according to crown-rump length if appropriate. Ultrasound examination was performed weekly from the initial scan between 11-13+6 gestational age until 17 weeks of gestation by 1 experienced ultrasonographer (Y.M.d.M.). The number of examinations differed because of different gestational ages at the initial scan and patients’ cooperation (Table1). During each ultrasound examination, the NT was measured using a transabdominal probe (4-8 MHz; Voluson 730 Expert series or Voluson E8, GE Medical Systems Kretz Ultrasound, Zipf, Austria) according to the guidelines of the Fetal Medical Foundation.\textsuperscript{1}
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The anterolateral region of the neck was examined both transvaginal and transabdominal for the presence of JLS, which appear as spheroid translucencies. If present, the volume of the JLS was calculated using the formula of a spheroid: length x height x width x π/6. Doppler measurements of the carotid artery, jugular vein and ductus venosus were performed as described previously. Care was taken to keep the interrogation angle as low as possible and always below 60 degrees. Flow velocity waveforms of the three vessels were used to analyze the peak systolic (S), diastolic, atrial contraction (a-V: for jugular vein and ductus venosus) and time-average velocity (TAV). The pulsatility index (PI) was calculated (carotid artery: PI= S-diastolic / TAV; jugular vein and ductus venosus: pulsatility index for veins (PIV)= S-aV / TAV ).

Flow velocity waveforms were recorded across the mitral and tricuspid valves as described previously. Adjustment was made for the insonation angle which never exceeded 30 degrees. Peak flow velocities in early diastole (E) and late diastole with atrial contraction (A) were measured and the E/A ratio was calculated. The presence or absence of tricuspid regurgitation was determined by pulsed wave Doppler as described previously. Digital images of each examination were stored.

Karyotyping was performed by chorion villus sampling or amniocentesis. In case of termination of the pregnancy, suction aspiration was performed or labor was induced. Post mortem morphological examination was carried out if the patients approved. The fetus or aspiration tissue was fixed in formalin 4%. Subsequently, post-mortem evaluation of the whole fetus or the fetal heart (in case of suction aspiration) was carried out using a dissection microscope. A sequential segmental analysis of the heart was performed by an experienced cardiac morphologist. The neck region was analyzed by microscopic examination of paraffin-embedded serial sections, stained with the lymphatic marker LYVE-1. The size and morphology of the JLS were compared with those of euploid fetuses with normal nuchal skin (n=4).

In ongoing pregnancies, a second trimester ultrasound examination was performed in all cases. After delivery, the parents completed questionnaires concerning the newborns health.

<table>
<thead>
<tr>
<th>Examinations, n</th>
<th>Fetuses with normal NT (n= 72)</th>
<th>Fetuses with increased NT (n= 71)</th>
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<tr>
<td>1</td>
<td>10</td>
<td>28</td>
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<td>2</td>
<td>49</td>
<td>19</td>
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<td>3</td>
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Table 1 Number of examinations per fetus (n=143)

NT, nuchal translucency.
Statistical analysis

Doppler measurements of fetuses with and without visualized JLS were compared. Also, Doppler measurements of fetuses with normal and increased NT, with respect to the presence of a cardiac defect, were compared.

Data were studied using General Estimating Equations (GEE) analysis (SPSS version 15.0; SPSS Inc, Chicago, IL). This method takes into account that the same patients are repeatedly measured, which indicates that missing observations are allowed. Furthermore, GEE analysis is capable of dealing with irregularly spaced time intervals. Variables indicating JLS not visible and JLS visible, normal NT and increased NT, normal NT and increased NT without cardiac defect, increased NT without and increased NT with cardiac defect were used to create groups. It was analyzed whether Doppler flow measurements of the compared groups differed significantly in relation to advancing of gestational age. If necessary, a log-transformation was used to account for nonnormality of the data.

GEE analysis was also used to assess a possible relationship between Doppler flow measurements and JLS volume, and between Doppler flow measurements and NT-size (correlation coefficient). The statistical significance level was set on $P = .05$.

RESULTS

In the group of the 73 fetuses with normal NT, 1 patient was excluded from further analysis because of an intrauterine fetal death due to fetal growth restriction. Postmortem examination revealed no abnormalities. Follow-up was complete and in all 72 cases healthy infants were born.

The characteristics of the included 143 fetuses are listed in table 2. Invasive tests were offered in case of increased NT, but refused in 1 case. In this case, a healthy neonate without dysmorphic features was born and was considered to have a normal karyotype. A normal karyotype was

<table>
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<th>Table 2</th>
<th>Characteristics of fetuses with normal and increased nuchal translucency</th>
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<td>Normal NT (n=72)</td>
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<tr>
<td>Characteristics</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>34.4 (3.7)</td>
</tr>
<tr>
<td>Gestational age (weeks)*</td>
<td>$11^{+4} (0^{+3})$</td>
</tr>
<tr>
<td>Nuchal translucency (mm)</td>
<td>1.2 (0.4)</td>
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<tr>
<td>Crown-rump length (mm)*</td>
<td>54.0 (5.9)</td>
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*at first ultrasound examination (inclusion)
NT, nuchal translucency.
found in 46 of the 71 fetuses with increased NT (65%), 25 fetuses were aneuploid (35%). Follow-up was known in all cases. Figure 1 shows the disposition of the fetuses included in the study. The cardiac status was known by follow-up after birth or postmortem examination in 61 of the 71 (86%) fetuses with increased NT. The cardiac status was known only by prenatal ultrasound assessment in 5 of the 71 cases (7%), of which 3 were suspected to have a cardiac defect. The cardiovascular anomalies (n = 25) included septal defects (n = 9), hypertrophic cardiomyopathy (n = 5), polyvalvular disease (n = 4), hypoplastic left heart syndrome (n = 3), aberrant subclavian artery (n = 2), aortic stenosis (n = 1) and a persistent vena cava superior sinistra (n = 1).

Figure 1 Disposition of 71 fetuses with increased NT enrolled in study
*Cardiovascular anomalies (n=25) included septal defects (n=9), hypertrophic cardiomyopathy (n=5), polyvalvular disease (n=4), hypoplastic left heart syndrome (n=3), aberrant subclavian artery (n=2), aortic stenosis (n=1), persistent vena cava superior sinistra (n=1). ** Noonan syndrome confirmed with DNA analysis (n=2).
JLS, jugular lymphatic sacs; NT, nuchal translucency.

Neck region

Jugular lymphatic sacs
The fetal neck region was successfully evaluated for the presence of JLS in all fetuses. The JLS could be visualized in 22 (31%) of the 72 fetuses with normal NT and in 55 (77%) of the 71 fetuses with increased NT. In fetuses with normal NT, the mean volume of the left JLS was 10mm³
(range 2 - 30mm$^3$) and 8mm$^3$ (range 1 - 28mm$^3$) of the right JLS. The mean volumes of the JLS in fetuses with increased NT were significantly larger (left JLS: 75mm$^3$ (range 2 - 627mm$^3$); right JLS: 76mm$^3$ (range 2 - 849mm$^3$) as compared to normal NT ($P < .01$).

**Jugular vein**

Doppler measurement of the jugular vein could be recorded in 158 of the 221 examinations (71%) of the fetuses with normal NT and in 115 of the 146 examinations (79%) of fetuses with increased NT (Figure 2).

The jugular vein PIV of fetuses with increased NT was significantly higher than the PIV of fetuses with normal NT ($P < .01$). Within the group of fetuses with increased NT, the jugular vein PIV was significantly higher in case of a cardiac defect as compared to fetuses with a normal heart ($P < .01$; Figure 3). No significant differences in the jugular vein PIV were found between fetuses

![Figure 2](image-url)

**Figure 2** Midneck region of fetus showing Doppler flow waveforms of jugular vein in coronal view

Pulsed Doppler showing the triphasic pattern with reversed flow during atrial contraction from the jugular vein of a first trimester fetus with normal nuchal translucency (crown-rump length 82mm; nuchal translucency 1.2mm).

increased NT (Figure 2).

The jugular vein PIV of fetuses with increased NT was significantly higher than the PIV of fetuses with normal NT ($P < .01$). Within the group of fetuses with increased NT, the jugular vein PIV was significantly higher in case of a cardiac defect as compared to fetuses with a normal heart ($P < .01$; Figure 3). No significant differences in the jugular vein PIV were found between fetuses
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with and without visualized JLS. Larger NT-size and larger JLS volumes were associated with a higher jugular vein PIV \((P < .01)\) \((\text{NT-size}: r = 0.52, P < .01; \text{left} \ JLS \ r = 0.56, P < .01; \text{right} \ JLS \ r = 0.49, P < .01)\).

The jugular vein a-V of fetuses with increased NT was significantly lower than the a-V of fetuses with normal NT \((P < .01)\). Within the group of fetuses with increased NT, the jugular vein a-V was significantly lower in case of a cardiac defect as compared to fetuses with a normal heart \((P < .01)\);
Figure 4). No significant differences of the jugular vein a-V of fetuses with and without visualized JLS were found. Larger NT-size and larger JLS volumes were associated with a lower jugular vein a-V (P < .01) (NT-size: r = -0.50, P < .01; left JLS r = -0.44, P < .01; right JLS r = -0.42, P < .01).

Carotid artery
Carotid artery Doppler measurements were obtained in 176 of 221 examinations (80%) of fetuses with normal NT and in 117 of the 146 examinations (80%) of fetuses with increased NT. No significant differences in carotid artery Doppler flow measurements between fetuses with and without visible JLS and fetuses with normal and increased NT, irrespective of the presence of a cardiac abnormality were found (data not shown). No relation of carotid artery Doppler measurements with NT-size and JLS volume were present.

Heart region
Intracardiac flow velocity waveforms were recorded in 187 (85%) of the 221 measurements of fetuses with normal NT and in 122 (84%) of the 146 measurements of fetuses with increased NT. No significant differences of the intracardiac velocities (E-wave, A-wave, E/A ratio) between fetuses with and without visible JLS and fetuses with normal and increased NT, irrespective of the presence of a cardiac abnormality were found (data not shown). No relation of intracardiac flow velocities with NT-size and JLS volume were present.

No tricuspid regurgitation was seen in the fetuses with normal NT. Tricuspid regurgitation was found in 6 of the 71 fetuses (8.5%) with increased NT. Karyotyping revealed trisomy 21 in 5 of these cases and trisomy 18 in one case.

Ductus venosus region
The ductus venosus of fetuses was successfully assessed in 181 of the 221 (82%) examinations with normal NT and in 117 of the 146 examinations (80%) with increased NT. A significantly higher ductus venosus PIV was found in fetuses with visible JLS compared to fetuses without visible JLS (P = .04; Figure 5). The ductus venosus PIV of fetuses with increased NT was significantly higher than the PIV of the fetuses with normal NT between 11 and 16 weeks of gestation (P < .01). Within the group of fetuses with increased NT, the ductus venosus PIV was significantly higher in case of a cardiac defect as compared to cases with a normal heart (11-16 weeks, P < .01, 16-17 weeks, no difference).

With advancing gestation, ductus venosus PIV in fetuses with visible JLS and fetuses with increased NT with or without a cardiac defect normalized. Larger NT-size and larger JLS volume were related with a higher ductus venosus PIV (NT-size: r = 0.59, P < .01; left JLS volume: r = 0.35, P < .01, right JLS volume: r = 0.27, P = .04).
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A significantly lower ductus venosus a-V was seen in fetuses with visible JLS compared to fetuses without visible JLS ($P = .04$; Figure 6). The ductus venosus a-V of fetuses with increased NT was significantly lower than the a-V of fetuses with normal NT. Within the group of fetuses with increased NT, the ductus venosus a-V was significantly lower in case of a cardiac defect as compared to cases with a normal heart ($P < .01$). A larger NT-size and larger JLS volume were
associated with a lower ductus venosus a-V (NT-size: \( r = -0.63, P < .01 \); left JLS: \( r = -0.36, P < .01 \); right JLS: \( r = -0.32, P = .02 \)).

**COMMENT**

This study shows a relation among increased NT, jugular lymphatic distension and altered blood flow in the jugular vein and ductus venosus. JLS were found in the majority of fetuses with increased NT (77%) and a small part of fetuses with normal NT (31%). Visibility of JLS was associated with a higher PIV and lower a-V of the ductus venosus. Larger JLS volumes were associated with a higher jugular vein and ductus venosus PIV and a lower jugular vein and ductus venosus a-V. No other studies have been performed before to assess a possible relation between increased NT, jugular lymphatic distension and altered Doppler blood flow velocities. Altered ductus venosus Doppler flow velocities, currently found in fetuses with visible and larger JLS volumes, have been described before in fetuses with increased NT. The altered ductus venosus flow and cardiac defects, frequently found in these fetuses, have led to the theory of cardiac failure playing a role in the development of increased NT. However, no significant differences in intracardiac velocities were found between fetuses with or without visible JLS and fetuses with normal and increased NT, irrespective of a cardiac defect. This is in agreement with earlier studies. Furthermore, tricuspid regurgitation, which is also suggested to be related with cardiac impairment, was found in only 6 of the 71 (8.5%) fetuses with increased NT. A large difference in incidence of tricuspid regurgitation, varying from 11 to 27%, in first-trimester fetuses with increased NT has been reported. These findings indicate that cardiac failure alone cannot explain the increased NT.

Recent studies suggest a disturbed lymphatic development as a likely explanation for the pathophysiology of increased NT. It was demonstrated that the majority of fetuses with increased NT show enlarged JLS on ultrasound. The lymphatic development starts in the neck by the formation of the JLS. Small buds of lymphatic endothelial cells arise from the internal jugular veins, which fuse and form the JLS. At 14 weeks gestation the formation of the lymphatic system is completed by the ingrowth of the right thoracic duct into the left JLS, thereby forming the main drainage-site of lymphatic fluid into the systemic circulation. A delayed reorganization of the JLS into lymph nodes could explain both transient and regional character of the increased NT. This is supported by the fact that the development of the NT and the JLS volume is related, with NT expansion preceding the JLS enlargement. The jugular vein has a strong anatomic relation with the developing JLS. Drainage of the lymphatic fluid takes place through the jugular vein. An increased amount of lymphatic fluid in enlarged JLS of fetuses with increased NT could cause a higher drainage through the jugular vein. This might result in a higher venous pressure and higher PIV of the jugular vein as found in the present longitudinal study in fetuses with distended JLS.
A wide range of chromosomal abnormalities, structural defects and genetic syndromes is associated with increased NT.\cite{3,26} One single explanation for the origin of increased NT is therefore not likely. A disturbance in endothelial development and differentiation could be the common process related to the lymphatic abnormalities, cardiac defects and altered jugular vein and ductus venosus flow found in fetuses with enlarged NT. Morphological studies of both aneuploid human and mouse embryos with nuchal edema showed a distended and abnormal endothelial differentiation of the jugular lymphatic system.\cite{4,7,25} Also, abnormal endothelial processes have been described to play a role in relation to development of cardiovascular defects.\cite{27,28} Previous research in trisomy 16 mouse embryos, an animal model for trisomy 21, showed a disturbed interaction between neuronal and endothelial pathways in the JLS, aortic arch and ductus venosus region.\cite{29} Beside the higher venous pressure due to the enlarged JLS, abnormal innervation of the jugular vein and ductus venosus could attribute to the abnormal flow velocities in fetuses with increased NT which previously has been suggested for the ductus venosus.\cite{29}

Our finding of a significantly higher PIV of the jugular vein in fetuses with increased NT compared to fetuses with normal NT are in contrast to a previous study of Martínez et al. who found no significant differences of the jugular vein PIV.\cite{12} However, this was a cross-sectional study with a relatively small group of fetuses with increased NT (n = 22).

In our study, JLS were visualized in 31% of the fetuses with normal NT. Previous cross-sectional research reported visible JLS in about 2% of fetuses with normal NT.\cite{6,30} However, a recent longitudinal ultrasound study with weekly follow-up demonstrated visible JLS in 33% of the fetuses with normal NT.\cite{31} Though, the JLS volumes of fetuses with increased NT are about 8 times larger compared to fetuses with normal NT. We hypothesise that there is a spectrum in lymphatic development, varying from physiological with visibility of small JLS on ultrasound for normal NT, to a delayed or disturbed lymphatic development with visibility of large JLS for increased NT. A similar spectrum is acknowledged for the development of the NT.\cite{32}

The finding of the small JLS in 22 fetuses with normal NT, most probably reflect a physiological variation in jugular lymphatic development, and therefore no significant differences in the jugular vein PIV and a-V were found between fetuses with and without visualized JLS. Also, the study group of examined fetuses was relatively small.

In conclusion, this study shows a relation between increased NT, distension of the jugular lymphatic system and altered Doppler flow in jugular vein and ductus venosus. Larger JLS were associated with a higher jugular vein and ductus venosus PIV and a lower jugular vein and ductus venosus a-V. The developmental background of these findings, in relation to endothelial differentiation and innervation, should be further investigated.
REFERENCES


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