Increased nuchal translucency is associated with jugular lymphatic distension

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BACKGROUND: Measurement of nuchal translucency (NT) is a widely used method of screening for chromosomal abnormalities. Increased NT is seen in a diversity of fetal malformations. The mechanism explaining the abnormal fluid accumulation and the transient nature of NT remains unexplained. METHODS: The nuchal regions of normal and trisomy 16 mouse embryos were examined for (lympho)vascular abnormalities using immunohistochemical markers against lymphatic vessels (LYVE-1) and smooth muscle (1A4) and endothelial (CD34) cells. Additionally, an ultrasonographic study was carried out on 17 human fetuses with an increased NT. Two of these fetuses were examined morphologically. RESULTS: In both abnormal human and mouse specimens, we found a mesenchyme lined cavity within the posterior nuchal region as well as bilaterally enlarged jugular LYVE-1 positive lymphatic sacs. The persistence of jugular lymphatic sacs was also confirmed by ultrasound in 14 human fetuses with increased NT. CONCLUSION: Our findings identify the cause of increased NT as mesenchymal oedema in the presence of distended jugular lymphatic sacs, detected by the hyaluronan receptor LYVE-1. The delayed organization and connection of these lymphatic sacs to the venous circulation might explain the transient nature of NT. Disturbance in timing of endothelial differentiation might be a common denominator in the origin of NT, linking cardiovascular and haemodynamic abnormalities.

Key words: lymphangiogenesis/lymphatic pathogenesis/mouse trisomy 16/nuchal translucency/trisomy 21

Introduction

Ultrasonographic measurement of nuchal translucency (NT) in the human fetus at a gestational age of between 10 and 14 weeks is a widely used and sensitive screening method for chromosomal abnormalities (Pandya et al., 1995a,b; Sherer et al., 1997; Economides et al., 1998; Hafner et al., 1998; Snijders et al., 1998). An increased NT is associated with trisomy 21 and other chromosomal abnormalities. It may also be present in fetuses with a normal karyogram. These latter fetuses may have a normal outcome as well as a variety of structural abnormalities such as cardiac malformations (Van Vugt et al., 1996,1998; Hyett et al., 1997a; Souka et al., 1998; Matias et al., 1999). The ultrasonographic entity of NT is explained as subcutaneous fluid accumulation in the neck region of unknown origin.

A common morphological denominator explaining the complete spectrum of malformations in which an enlarged NT is found is still lacking. Proposed pathophysiological explanations are alterations in the extracellular matrix (Brand-Saberi et al., 1994a,b; von Kaisenberg et al., 1998a) or cardiac failure (Montenegro et al., 1997) because of the association with congenital heart malformations and abnormal Doppler flow velocity waveforms in the fetuses with an enlargement of the NT.

The aim of this study was to investigate the morphology of the nuchal region in wild type and trisomy 16 mouse embryos, the latter have been reported as the animal model for Down’s syndrome (Miyabara et al., 1982; Holtzman et al., 1992; Reeves et al., 1995). In this model, the concept of jugular lymphatic distension was developed and subsequently applied to study 17 human fetuses with increased NT by ultrasound. In two of these human fetuses with enlarged NT, which had an abnormal karyotype, morphological examination was carried out.

Materials and methods

Pathomorphology of the nuchal region in normal and trisomy 16 mouse embryos

The nuchal region was studied in four wild-type (WT) Swiss strain and four trisomy 16 mouse embryos [one case each at embryonic
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Figure 1. (a) Wild type mouse embryo, day 14 of development. (b) Trisomy 16 embryo, day 14 of development, with increased NT.

day (ED) 12 and 13 and two cases at day 14. The trisomy 16 mouse is widely accepted as the animal model for human Down’s syndrome (Miyabara et al., 1982; Holtzman et al., 1992), presenting with heart malformations and macroscopic nuchal oedema (Reeves et al., 1995) (Figure 1). The mouse embryos were embedded in paraffin after fixation in paraformaldehyde. Transverse serial sections of 5 µm were haematoxylin/eosin stained and examined by light microscopy. To explore the origin and nature of structures in the nuchal region, sections were stained for several immunohistochemical markers. Antibodies against anti-α-smooth muscle actin (1A4; Sigma Aldrich), against the pan-endothelial markers CD34 (Monsoon) and against lymphatic vessel endothelial hyaluronic acid receptor-1 (LYVE-1) (provided by D.G.Jackson). LYVE-1 is a recently described hyaluronan receptor that has proved to be specifically expressed on lymphatic endothelium (Banerji et al., 1999; Prevo et al., 2001).

Ultrasonographic study of the nuchal region in normal and abnormal human fetuses

A clinical study was undertaken to explore whether the pathomorphological microscopic findings in mouse embryos could be visualized by ultrasound in the human fetus. The medical ethical committee of the VU Medical Center approved the study. During the period from August 1, 1999 to March 15, 2001, 1050 nuchal translucency measurements were performed in our centre on women undergoing risk assessment for Down’s syndrome. The measurements were performed according to the guidelines of the Fetal Medicine Foundation (Pandya et al., 1995a). In 50 cases (4.76%) the NT was above the 95th percentile (range 2.4–16 mm). Videotapes and digital images of these 50 cases were studied. In 17 of these 50 cases, the electronic images or videotapes contained transverse planes of the posterior and anterior neck region. These 17 were included in the study. In the remaining 33 cases the videotapes and digital images only contained longitudinal planes, to measure the NT. No pre-selection was made on NT thickness. Furthermore, in a control group of 15 fetuses with normal NT, the neck was examined extensively. In addition to the standard longitudinal plane to measure the NT, transverse planes of the fetal neck were also made for examination. All women had given informed consent for participation in this study. Gestational age varied between 11+0 and 14+0 weeks (crown–rump length between 41 and 82 mm). Transvaginal ultrasound was performed with a 4–8 MHz probe.

Pathomorphology of the nuchal region in human fetuses with increased NT

Two of these 17 fetuses with increased NT on ultrasound, were available for post mortem examination. In one fetus, karyotyping with chorionic villus sampling revealed a trisomy 18 (NT 10.0 mm, CRL 70 mm), and in the other a trisomy 21 (NT 4.2 mm, CRL 69 mm). In the first fetus the posterior nuchal skin was removed. In the second fetus a transverse section was made of the whole fetal neck. The specimens were fixed in paraformaldehyde, embedded in paraffin and transverse serial sections of 5 µm were made. To explore the origin and nature of structures in the nuchal skin of these fetuses, immunohistochemical markers for α-smooth muscle actin and lymph vessel endothelium (LYVE-1 antibody) were used.

Results

Pathomorphology of the nuchal region in normal and trisomy 16 mouse embryos

Nuchal region

No posterior nuchal cavity was found in the WT mouse embryos (ED 12–14, Figure 2a,c,e). In the trisomy 16 mouse embryo of 12 days gestation, the cells in the nuchal region formed a loosely organized mesenchyme without a specific subepithelial nuchal cavity formation (Figure 2b). In the trisomy 16 embryo of ED 13, spongy mesenchyme with intercellular spaces was seen (Figure 2d). In the oldest trisomy 16 embryos (ED 14) the spaces had merged to form a substantial subepithelial mesenchymal cavity (Figure 2f). This cavity covered the posterior nuchal area and extended laterally. The posterior cavity was negative for LYVE-1 (Figure 2f), 1A4 and CD34.

Lymphatic changes

Both in the WT as in the trisomy 16 mouse embryos, spaces were seen at the antero-lateral side of the neck, accompanying
Figure 2. Sections from mouse embryos of gestational age ED 12, 13, 14 showing the increasing size of the lymphatic sacs (positive for LYVE-1) in trisomy 16 embryos compared with wild type mouse embryos. The loose mesenchyme in the posterior region (d) and a large posterior cavity (f), which is non-staining for LYVE-1, can be seen in trisomy 16 embryos. (a–d) Haematoxylin/eosin stained with insets of LYVE-1 stained details. (e) and (f) LYVE-1 stained. WT = wild type, ED = embryonic day, L = jugular lymphatic sacs, A = artery, V = veins. Bars = 25 µm.

The jugular vein and the carotid artery (Figures 2 and 3). In the trisomy 16 mouse embryos these spaces were substantially larger than in WT mouse embryos. The spaces were present at day 12 both in WT as is trisomy 16 embryos, but in the trisomy 16 embryos their size was increased substantially on embryonic days 13 and 14. To identify the nature of the cells lining the antero-lateral spaces, the immunohistochemical markers 1A4, CD 34 and LYVE-1 were used. The antero-lateral spaces were positive for LYVE-1 on days 12, 13 and 14 of gestation and thus considered to be lymphatic vessels. The jugular vein showed patches of positivity. At day 14, the jugular lymphatics, veins and arteries could clearly be differentiated (Figure 3a,b).

These spaces are considered to be the jugular lymphatic sacs as described early last century by Sabin (Sabin, 1909). The trisomy 16 embryos showed extremely distended bilateral jugular sacs next to a normally sized vein and a slightly increased artery (Figures 2f and 3c, d). The distension of these bilateral jugular lymphatic sacs in the trisomy 16 embryos was always seen prior to the development of the nuchal oedema (Figure 2d).

Ultrasonographic study of the nuchal region in normal and abnormal human fetuses

In 17 fetuses with an increased NT between a gestational age of 11 and 14 weeks, transverse planes through the fetal neck were available for retrospective examination of the fetal neck. The median NT was 6.1 mm (mean 6.9 mm; SD 3.1; range 3.1–10.4). No exclusion was made for reasons other than the availability of transverse planes of the neck region on the videotapes or digital images. Fourteen fetuses had clearly recognizable round to oval translucencies in the antero-lateral region of the neck, next to the posteriorly located (increased) NT (Figure 4b). In six of these 14 fetuses chorionic villus sampling revealed a normal karyotype. Four had a trisomy 21 and in three cases a trisomy 18 was found. One fetus had an unbalanced translocation [46 XY, der(18)t(3;18)(q27;p11.1)]. The karyotype of the three fetuses in which the antero-lateral cavity could not be recognized as a separate entity, showed that all had Turner’s syndrome (45 XO). The bilateral septated posterior translucencies, common in Turner’s syndrome, however, were large, occupying the complete lateral and posterior neck region. In the 15 fetuses with a normal NT the antero-lateral region of the neck contained no translucent spaces (Figure 4a).

Pathomorphology of the nuchal region in human fetuses with increased NT

In two of these 17 fetuses with an increased NT detected by ultrasound (trisomy 18; NT 10.0 mm and trisomy 21; NT 4.2 mm) further examination was performed. A subepithelial mesenchymal cavity negative for LYVE-1 and 1A4 was found in the skin of the posterior nuchal area (Figure 5a,b). Surrounding this cavity, small arteries and veins as well as large lymphatic vessels were seen. The lymphatic vessels stained markedly with the LYVE-1 antibody (Figure 5a), while the venous endothelium was faintly positive. The various vessel types were also distinguished by staining with anti-α-smooth muscle actin (1A4) that defined the walls of small arteries and veins, but not the lymphatic vessels (Figure 5b). In the trisomy 21 fetus, large bilateral jugular lymphatic sacs, within the anterior region of the fetal neck, could be identified by staining with LYVE-1.
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Figure 3. Details of vascular neck structures in a normal 14 day gestation mouse embryo (a, b) and in a case of trisomy 16 of the same age (c, d). The LYVE-1 antibody stains the jugular lymphatic sac (a, c), which is extremely enlarged in trisomy 16 (c). Both the carotid artery (A) and the jugular vein (V) are negative for LYVE-1 and are positive for the smooth muscle cell marker 1A4, whereas the lymph vessel wall is, as expected negative for this smc marker. Bars = 10 µm.

Figure 4. Transverse plane ultrasound pictures through the neck of human fetuses with (a) normal NT (CRL 49 mm, gestational age 12+0 weeks, NT 1.2 mm) and (b) increased NT (CRL 60 mm, gestational age 12+2 weeks, NT 4.4 mm, trisomy 21). In (b) the enlarged bilateral jugular lymphatic sacs can be seen. J = jaw, S = spine, L = jugular lymphatic sac, NT = nuchal translucency.

Discussion

Background

A pathophysiological explanation for increased nuchal translucency, encompassing both normal outcome as well as the wide variety of malformations, has been lacking until now. However, many theories have been proposed as underlying mechanisms including temporary cardiac failure (Montenegro et al., 1997; Matias et al., 1999) overperfusion of the fetal neck due to narrowing of the aortic isthmus (Hyett et al., 1995a) and venous compression (Maymon et al., 1999; Brown and Nicolaides, 2000). The mechanisms leading to these, mainly physiologically based, defects remain obscure.

The theory of temporary cardiac failure has been based on the finding of abnormal blood velocity waveforms in the ductus venosus (Montenegro et al., 1997; Matias et al., 1998, 1999) and has been explained by an impaired atrial contraction of the human fetal heart. Cardiac abnormalities have been proposed as a contributing factor in this process of cardiac failure, while fluid accumulation has been compared with the situation of oedema in right ventricular failure in adults (Montenegro et al., 1997; Matias et al., 1998). However, it
leads to increased NT have been based on the finding of an increased expression of type VI collagen in the nuchal skin of trisomy 21 fetuses (von Kaisenberg et al., 1998a). Type VI collagen is, however, decreased in trisomy 18 fetuses with an enlarged NT (von Kaisenberg et al., 1998a). An increase of hyaluronan in the nuchal skin was found in fetuses with trisomy 21, whereas in trisomy 18, 13 and Turner’s syndrome the amount was similar to normal controls (Bohlandt et al., 2000). Thus, all the fetuses examined in these studies had an increased NT, but the changes reported in the extracellular matrix did not show the same pattern in the different types of trisomies (von Kaisenberg et al., 1998a). Furthermore, the changes found in the extracellular matrix do not explain why the fluid accumulation is located regionally in the nuchal skin and do not explain the transient nature of the increased NT.

Pathomorphological findings in the nuchal region
The posterior subepithelial mesenchymal cavity observed in this study of human fetuses with increased NT and trisomy 16 mouse embryos is most likely the anatomic substrate for the ultrasonographic human increased NT. This local morphological abnormality does not contain blood cells and does not resemble an artery or vein. As the lining of the cavity is negative for IA4, CD34 and LYVE-1 staining, both in mouse embryos and human fetuses, the cavity is probably the result of coalescence of oedematous mesenchymal spaces, and is not due to an enlargement of an existing vascular structure. This is supported by the study of Chitayat in which no endothelial lining of the NT cavity in non-Turner’s cases was found (Chitayat et al., 1989). Hence our conclusion is, that the NT measurement, employed in many countries as screening method for chromosomal abnormalities, is in fact the measurement of mesenchymal oedema in the fetal neck.

Pathomorphology of the lymphatic changes
The wide bilateral antero-lateral spaces, which were positive for LYVE-1, equate to the bilateral jugular lymphatic sacs described by Sabin (Sabin, 1909). These jugular lymphatic sacs are during normal development derived from early veins, which thereafter become isolated and relatively large and subsequently reorganize into lymph nodes and reconnect to the jugular vein (Sabin, 1909). This process is completed in human embryos of 10 weeks gestational age. The 14 fetuses with an increased NT all showed large oval translucencies in the posterior neck region on ultrasound and were all similar to normal controls (Bohlandt et al., 2000). Thus, all the fetuses examined in this study of human fetuses with increased NT and trisomy 16 mouse embryos is most likely the anatomic substrate for the ultrasonographic human increased NT. This local morphological abnormality does not contain blood cells and does not resemble an artery or vein. As the lining of the cavity is negative for IA4, CD34 and LYVE-1 staining, both in mouse embryos and human fetuses, the cavity is probably the result of coalescence of oedematous mesenchymal spaces, and is not due to an enlargement of an existing vascular structure.

Figure 5. (a) Nuchal skin from a trisomy 18 fetus of 14 weeks gestational age, showing the non-staining posterior mesenchymal cavity (C). The markedly stained lymphatic vessel (L) and a less well stained vein (V) with the LYVE-1 antibody. (b) Anti-α actin staining showing the small arteries and venous wall (V). The posterior mesenchymal cavity (C) is negative. Bars = 50 μm.
lymphatic sacs in 14 human fetuses with increased NT by ultrasound.

The fact that these distended jugular lymphatic sacs were found both in fetuses with different types of trisomies as well as in fetuses with a normal outcome, suggests their role in the development of an increased NT. Most probably the distended jugular lymphatic sacs are similar to what has been described as ‘lateral neck cysts’ (Achiron et al., 1995). Differences in time of detection and definition of the increased NT do not allow full correlation of our data with those of the latter group.

The strong correlation of the persistence of the lymphatic jugular sacs with increased NT suggests the following hypothetical mechanism for the development of the increased NT. At differentiation of the jugular lymphatic sacs from the early venous system, there is a delay in remodelling of the endothelial system. This leads to bilateral jugular lymphatic sac distension. It remains to be investigated whether the enlargement is solely the result of a delay of reconnection to the venous system or whether the increased overall expression of the hyaluronic acid receptor (LYVE-1) in the numerous lymphatic vessels might also play a role. Hyaluronic acid is known to bind large amounts of water, leading to increased lymph fluid formation. The latter resulting in increased size of the jugular lymphatic sacs and accumulation of mesenchymal lymph oedema with subsequent formation of an enlarged nuchal cavity. Once this process has started, it might retrogradely affect cardiac function and this results in abnormal Doppler blood flow velocity waveforms (Montenegro et al., 1997; Matias et al., 1998). Our hypothesis could link the various theories that have been proposed up until now.

With continued development, the lymphatic sacs are remodelled into lymph nodes and the excess of fluid drains away. This explains the transient nature of the increased NT. The fact that the lymphatic distension presents at an earlier developmental stage (ED 13) in trisomy 16 mouse embryos than the occurrence of the nuchal oedema (ED 14), suggests that the lymphatic distension as a secondary phenomenon to the increased NT is less likely.

It remains to be investigated where the disturbance of embryonic lymphangiogenesis has its common denominator in the wide variety of malformations with increased NT. The increased size of the lymphatic vessels, which cells contain the hyaluronan receptor LYVE-1, may lead to increased lymphatic fluid formation that may be additionally responsible for the oedema. A link to the lymphangiogenesis promoting growth factor VEGF-C and its receptor VEGFR-3 (Wiltig et al., 1999; Veikkola et al., 2001) also needs further study as they might provide a link to a disturbed timing of angiogenesis. The high incidence of cardiac abnormalities including aortic arch malformations, in which abnormal endothelial developmental processes play a role, would support this theory. This might be reflected in the high incidence of heart (Hyett et al., 1995b, 1997a) and aortic malformations (Hyett et al., 1995a) in which endothelial developmental pathophysiologic processes play a role (Hyett et al., 1996). Disturbance of the timing and differentiation of endothelial development could be the common factor underlying lymphatic abnormalities, the cardiac malformations as well as the haemodynamic changes in fetuses with increased NT.

In Turner’s syndrome the mechanism leading to the increased NT is also related to abnormal development of the jugular lymphatic sacs. We hypothesize that the enlarged lymphatic jugular sacs become partly organized and do not find a proper connection into the jugular veins. This leads to the formation of a large amount of fluid in the posterior neck region, which is not transient and is called ‘hygroma colli’. This hypothesis is supported by the study of Chitayat, in which the walls of the cavities of a cystic hygroma of four fetuses with monosomy X were lined by endothelial cells (Chitayat et al., 1989). These posterior cavities are therefore most probably the enlarged lymphatic sacs that extend to the posterior neck region. The disturbance of lymphatic development in cases with Turner’s syndrome is described by Von Kaisenberg, in which lymphatic vessel hypoplasia was found in the upper dermis of the skin of the Turner’s fetuses (von Kaisenberg et al., 1999).

The current study has, however, several limitations. Firstly, our animal model solely provides morphological data. The value of the link between animal and human development is a frequently encountered problem in developmental research. Normal and even abnormal human embryos, however, are seldom available for research purposes. Pregnancies are often terminated with suction curettage, making the material useless for histological examination. Therefore, animal models are necessary for this kind of research. Secondly, the ultrasound data are the result of a retrospective study. Although no pre-selection was made on NT thickness, there are several cases with an extremely large NT. These oval anterior located translucencies should be studied in a prospective manner in the future and, if possible, pathomorphological examination should be performed on these fetuses, in case of termination of pregnancy.

In conclusion, the posterior subepithelial mesenchymal cavities observed in this study of human fetuses with increased NT and trisomy 16 mouse embryos is likely to be the anatomic substrate for the ultrasonographic nuchal translucency. Thus, the nuchal translucency measurement is in fact the measurement of mesenchymal oedema in the fetal neck. Furthermore we demonstrated a strong association of jugular lymphatic sac dilatation and increased nuchal oedema in human fetuses as well as in trisomy 16 mouse embryos. The fact that these distended jugular lymphatic sacs were found both in fetuses with different types of trisomies as well as in fetuses with a normal outcome, suggests their role in the development of an increased NT.

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References


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