PATHOPHYSIOLOGY OF INCREASED FETAL NUCHAL TRANSLUCENCY THICKNESS

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SUMMARY

Increased fetal nuchal translucency thickness is associated with trisomy 13, trisomy 18, trisomy 21, Turner syndrome, other sex chromosome abnormalities, as well as many fetal anomalies and genetic syndromes. This article provides a comprehensive review of the cardinal proposed pathophysiology including altered composition of the extracellular matrix, abnormalities of the heart and great arteries, and disturbed or delayed lymphatic development. [*Taiwan J Obstet Gynecol* 2010;49(2):133–138]

Key Words: fetus, nuchal translucency, pathophysiology

Introduction

In the first trimester of pregnancy, the term *nuchal translucency* (NT) refers to the ultrasound finding of subcutaneous collection of fluid behind the fetal neck irrespective of whether the collection of fluid is septated and whether it is confined to the neck or envelopes the whole fetus [1]. Increased fetal NT thickness refers to the measurement of the vertical thickness in the midsagittal section of the fetus that is equal to or above the 95th centile of the reference range [2]. Fetal abnormalities are associated with the thickness of fetal NT rather than the appearance of fetal NT [1,3]. In 1998, the Fetal Medicine Foundation First Trimester Screening Group suggested that the optional gestational age for the measurement of fetal NT is 11–13 gestational weeks 6 days with the corresponding minimum fetal crown-rump length (CRL) of 45 mm and the maximum CRL of 84 mm, and that the 95th centile of NT increased linearly with fetal CRL from 2.1 mm at a CRL of 45 mm to 2.7 mm at a CRL of 84 mm, whereas the 99th centile did not change with CRL and it was approximately 3.5 mm [2].

Increased fetal NT thickness is associated with trisomy 13, trisomy 18, trisomy 21, Turner syndrome, other sex chromosome abnormalities, as well as many fetal anomalies and genetic syndromes [4–8].

Cardinal proposed mechanisms for the increase in NT thickness include altered composition of the extracellular matrix, abnormalities of the heart and great arteries, and disturbed or delayed lymphatic development [4,9,10].

Altered Composition of the Extracellular Matrix

Brand-Saberi et al [11,12] first observed changes in the extracellular matrix of the skin in trisomy 21 and trisomy 18 fetuses. In fetuses with trisomies 13, 18 and 21, there is overexpression of insoluble fibrils as a part of the framework of connective tissues particularly collagen type IV, laminin and collagen type VI, respectively,

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Accepted: September 21, 2009

leading to increase in NT thickness [13,14]. In addition to single dosage effects of genes on the extrachromosomes 13, 18 or 21, altered extracellular matrix may also cause an impairment of cell migration leading to maldevelopment of fetal organs, since many fetal organs such as heart, aortic arch, face, teeth, thymus and the enteric nervous system receive cell contributions from the neural crest [12,15,16].

von Kaisenberg et al [13] found that in the nuchal skin of human trisomy 13 fetuses, dermal fibroblasts were collagen type IV positive. The authors suggested that increased NT thickness in trisomy 13 fetuses is due to alteration in the composition of collagen type IV, which is a heterotrimer formed of two α-1 chains and one α-2 chain. The genes responsible for collagen type IV α-1 and α-2 chains, *COL4A1* (OMIM 120130) and *COL4A2* (OMIM 120090), respectively, are located on chromosome 13q34, and may be overexpressed in trisomy 13 fetuses. Collagen type IV is associated with laminin, entactin and heparan sulfate proteoglycans to form the sheet-like basement membranes that separate epithelium from connective tissue.

von Kaisenberg et al [13] found that in the nuchal skin of human trisomy 18 fetuses, dermal fibroblasts were laminin-positive, and the expression level in the basements was higher than that in normal controls. The genes responsible for laminin α-1 and α-3 chains, *LAMA1* (OMIM 150320) and *LAMA3* (OMIM 600805), respectively, are located on chromosome 18p11.31 and chromosome 18q11.2, respectively, and may be overexpressed in trisomy 18 fetuses. Laminin is a basement membrane protein composed of three nonidentical chains, A, B1 and B2, arranged in cross-shaped structure [17].

Various studies have demonstrated a homogeneous overexpression of genes encoding α-1 and α-2 chains of collagen type VI and superoxide dismutase (SOD) in the nuchal skin of trisomy 21 fetuses [13,14,18]. The genes responsible for collagen type VI α-1 and α-2 chains, *COL6A1* (OMIM 120220) and *COL6A2* (OMIM 120240), respectively, are located on chromosome 21q22.3, and the gene responsible for SOD, *SOD1* (OMIM 147450) is located on chromosome 21q22.1. Collagen type VI is a component of microfibrillar structures that localize close to cells, nerves, blood vessels and large collagen fibrils, and have an anchoring action [19]. SOD-1 is a major cytoplasmic antioxidant enzyme that metabolizes superoxide radicals to molecular oxygen and hydrogen peroxide to provide a defense against oxygen toxicity [20]. Böhlandt et al [21] investigated the formation of an interstitial edema and found a large amount of hyaluronan in the skin of fetuses with trisomy 21. Collagen type VI binds to hyaluronan [22], and SOD protects against free radical-mediated degradation of hyaluronan. Böhlandt et al [21] suggested that the increased amount of hyaluronan in the skin of trisomy 21 fetuses is due to decreased degradation of hyaluronan. Additionally, high concentrations of hyaluronan have been shown to impair the migration of neural crest cells [23,24], and the persisting atrioventricular canal specific for Down syndrome is caused by defects in migratory cell populations [25].

In fetuses with Turner syndrome, von Kaisenberg et al [26] found that biglycan was underexpressed, and chondroitin-6-sulfate and chondroitin-4-sulfate proteoglycans were increased. The gene responsible for biglycan, *BGN* (OMIM 301870), is located on chromosome Xq28 near the second pseudoautosomal region. Geerkens et al [27] found that in 45,X Turner syndrome, the BGN expression level was reduced but in patients with additional sex chromosomes, the BGN expression level was increased. The authors suggested that BGN is subject to X inactivation but is transcribed like an X-Y homologous gene. BGN is a small proteoglycan that may function in connective tissue metabolism by binding to collagen fibrils and TGF-β, and may promote neuronal survival [28]. BGN is localized in the cartilage, aorta, and mineral compartment of bones [26]. Xu et al [28] found that Bgn-deficient mice had deficiency of a non-collagenous extracellular matrix protein leading to an osteoporosis-like phenotype in mice. von Kaisenberg et al [26] suggested that the narrowing of the aortic isthmus in Turner syndrome is a result of decreased BGN or a result of abnormal migration of cells from the neural crest, and that the consequent increased impedance to flow in the aortic arch leads to overperfusion of the head and neck and increased NT thickness. The authors also suggested that increased levels of chondroitin-6-sulfate and chondroitin-4-sulfate proteoglycans may bind large amounts of water to form swelling of the skin.

Souka et al [4] reported an association between increased NT thickness and a wide range of rare genetic syndromes and skeletal dysplasias. To date, there are at least 30 reports of increased NT thickness associated with skeletal dysplasias such as achondrogenesis, osteogenesis imperfecta, hypophosphatasia, thanatophoric dysplasia, short-rib polydactyly syndrome, diastrophic dysplasia, Robinow syndrome, achondroplasia, Jarcho-Levin syndrome, cleidocranial dysplasia, campomelic dysplasia, Jeune syndrome, and ectrodactyly-ectodermal dysplasia-clefting syndrome [29]. Possible mechanisms for the association between increased NT thickness and skeletal dysplasias include a narrow thorax with mediastinal compression, reduced fetal movements, and alteration of the extracellular

matrix, especially in skeletal dysplasias with collagen defects [6,29].

Abnormalities of the Heart and Great Arteries

In a study of the heart and great arteries in 60 fetuses with trisomy 21, 29 with trisomy 18, 17 with trisomy 13 and six with Turner syndrome, all diagnosed by chorionic villus sampling (CVS) because of a high risk identified by a combination of maternal age and fetal NT thickness at 10–14 gestational weeks, Hyett et al [30] found that an atrioventricular or ventricular septal defect for trisomy 21, ventricular septal defects and/or polyvalvular abnormalities for trisomy 18, atrioventricular or ventricular septal defects, valvular abnormalities and either narrowing of the isthmus or truncus arteriosus for trisomy 13, and severe narrowing of the whole aortic arch for Turner syndrome as the most common cardiac lesions. The authors also found that significant narrowing of the aortic isthmus was noted in trisomies 13, 18 and 21 and Turner syndrome compared with the normal fetuses, and the degree of narrowing was significantly greater in fetuses with high NT thickness. Hyett et al [30] suggested that narrowing of the aortic isthmus may be the basis of increased NT thickness in trisomies 13, 18 and 21, and Turner syndrome.

Hyett et al [30,31] suggested that increased NT thickness at 10–14 gestational weeks may act as a screening marker for major cardiac defects. The prevalence of congenital heart defects increases with the increase in NT thickness. Ghi et al [32] found that the prevalence of major heart defects increased from 2.4% (10 of 416) to 2.6% (8 of 306), 3.1% (12 of 384), 8.3% (13 of 157), 19.0% (8 of 42) and 64.3% (9 of 14), with NT thickness of 2.5–2.9 mm, 3.0–3.4 mm, 3.5–4.4 mm, 4.5–6.4 mm, 6.5–8.4 mm, and ≥ 8.5 mm, respectively. In a meta-analysis of an association between increased NT thickness and major cardiac defects, Hyett [33] found that the prevalence of major cardiac defects in fetuses with NT > 2.5 mm (approximately ≥ 95th centile) was 1.7%. Atzei et al [34] found that the prevalence of major cardiac defects increased from 0.49% to 0.87%, 1.82%, 3.52%, 6.44% and 12.67%, with NT thickness of < median, median to < 95th centile, ≥ 95th centile to 3.4 mm, 3.5–4.4 mm, 4.5–5.4 mm and ≥ 5.5 mm, respectively. Makrydimas et al [35] found that NT thickness ≥ 3.5 mm occurred in 97 of 397 (24.4%) chromosomally normal fetuses and in 14 of 240 (5.8%) of chromosomally abnormal cases. Simpson et al [36] suggested that three out of every 100 patients referred for fetal echocardiography with NT thickness of ≥ 99th centile will have a major cardiac anomaly. In a meta-analysis of prenatal screening for serious congenital heart defects using NT thickness, Wald et al [37] found that the estimated detection rate was 52% (95% confidence interval, 42–71) for a 5% false-positive rate, and concluded that prenatal screening congenital heart defects using NT thickness is likely to be effective.

However, there is lack of evidence of an etiologic link of structural cardiac defects or cardiac failure to increased NT thickness [38–40]. Huggon et al [38] studied the myocardial performance index and atrioventricular valve ratios of the peak early diastolic velocity (E wave) to peak late diastolic velocity (A wave) for both sides of the heart by Doppler echocardiography in 159 normal control fetuses, 199 otherwise normal fetuses but with increased NT ≥ 4 mm, 142 fetuses with trisomy 21, 58 fetuses with trisomy 18, 19 fetuses with trisomy 13, 37 fetuses with Turner syndrome, and 24 fetuses with isolated heart defects at 11–14 gestational weeks. They found no evidence to support a hypothesis for cardiac dysfunction in the genesis of increased NT thickness. Haak et al [40] additionally found no difference in intracardiac flow velocities between fetuses with normal and those with increased NT thickness, and hypothesized that a coexisting developmental process, linking both enlargement of the NT as well as cardiovascular malformations might be the common pathophysiologic pathway.

Disturbed or Delayed Lymphatic Development

van der Putte [41] found marked malformations of the lymphatic system in seven spontaneously aborted fetuses with assumed Turner syndrome, and suggested that this pathologic process is essentially a generalized hypoplasia and partial agenesis of the lymphatic system, which ceases to extend peripherally at an early embryonic stage. Byrne et al [42] observed large cystic hygromas, generalized edema and edematous chorionic villi in fetuses with 45,X or monosomy X, and suggested impaired lymphatic drainage of the jugular lymphatic sacs in Turner syndrome. In a study of lymphatic abnormalities in fetuses with cystic hygroma, Chitayat et al [43] found that in the non-45,X fetuses (two with 47,XX, +21, one with 47,XX, +13, four with 46,XY and one with lethal multiple pterygium syndrome), the lymphatic vessels were dilated and increased in number; while in the 45,X fetuses, the lymphatic vessels were hypoplastic. von Kaisenberg et al [44] investigated the distribution of lymphatic vessels in nuchal skin tissue from fetuses with Turner syndrome compared with

fetuses carrying trisomies 21, 18 and 13 and chromosomally normal controls by immunohistochemistry. They found that in Turner syndrome, there was lymphatic hypoplasia, which was completely different from fetuses with trisomies who had evenly distributed lymphatic vessels throughout the dermis and subdermis.

On studying the pathomorphology of the nuchal region in normal and trisomy 16 mouse embryos and in human fetuses with increased NT thickness, and the ultrasonography of the nuchal region in normal and abnormal human fetuses, Haak et al [45] found a mesenchyme-lined cavity within the posterior nuchal region, bilaterally enlarged jugular LYVE-1 positive lymphatic sacs, and the persistence of jugular lymphatic sacs by ultrasound in 14 human fetuses with increased NT thickness. The authors concluded that mesenchymal edema in the presence of distended jugular lymphatic sacs is the cause of increased NT thickness, and the delayed organization and connection of these lymphatic sacs to the venous circulation can explain the transient nature of NT thickness. Haak et al [45] suggested that disturbance in timing of endothelial differentiation is a common denominator in the origin of NT thickness such a disturbance links cardiovascular abnormalities and hemodynamic abnormalities in the pathogenesis of increased NT thickness. Castelli et al [46] hypothesized that the echo-free image of NT is likely the superficial recesses of the jugular lymphatic sacs placed in the nuchal soft tissues by their light and scanning electron microscopes study of NT in a normal fetus. The hypothesis that an increased NT is caused by abnormal jugular lymphatic development was further tested by using the trisomy 16 mouse model [47]. Gittenberger-de Groot et al [47] concluded that abnormal jugular lymphatic sacs are associated with the development of nuchal edema (NE), and a disturbance of lymphangiogenesis is the basis for increased NT thickness.

Bekker et al [48] found a significant difference in the prevalence of jugular lymphatic sacs between fetuses with enlarged NT and the normal controls, and a significant association between increased NT and distended jugular sacs on first-trimester ultrasound, and suggested a disturbance in lymphangiogenesis as the pathophysiology of increased NT thickness. Bekker et al [49] further observed a disturbed venous-lymphatic phenotype in aneuploid human fetuses and mouse embryos with enlarged jugular sacs and NE with the associated findings of absent or diminished expression of the lymphatic markers in the enlarged jugular sacs, as well as abnormal endothelial differentiation, which provides a link to the cardiovascular anomalies associated with NE. An impaired neural migration signaling, in addition to abnormal endothelial development, causes abnormal migration of neural crest cells leading to aortic arch anomalies and cardiovascular anomalies [50–54]. Bekker et al [49] suggested that a disturbed venous-lymphatic differentiation is the common process leading to increased NT thickness regardless of karyotype. Bekker et al [49] additionally found that distended jugular lymphatic sacs were visible in 91.9% of fetuses with increased NT in which NT expression preceded jugular lymphatic sac enlargement, and that aneuploid fetuses had a more disturbed lymphangiogenesis.

Bekker et al [49] demonstrated diminished expression of lymphatic marker Prox-1 and podoplanin, and the presence of blood vessel characteristics such as vascular endothelial growth factor (VEGF)-A and neuropilin-1 in the lymphatic endothelial cells of the enlarged jugular lymphatic sacs with NE. They also found aberrant smooth muscle cells surrounding the enlarged jugular lymphatic sac with NE. de Mooij et al [55] additionally demonstrated an increased expression of Sonic hedgehog (Shh), VEGF-A and platelet-derived growth factor (PDGF)-B, and a decreased expression of forkhead transcription factor FOXC2 in the lymphatic endothelial cells of the jugular lymphatic sacs of the trisomic fetuses. The authors hypothesized that increased Shh and VEGF-A expression in human trisomic fetuses with enlarged jugular sacs and NE is correlated with an aberrant lymphatic differentiation, and increased PDGF-B expression induces smooth muscle cells recruitment and/or differentiation. Mutations in FOXC2 are responsible for the hereditary lymphedema-distichiasis syndrome [56]. FOXC2 normally suppresses the expression of PDGF-B in the lymphatic endothelial cells and inhibits smooth muscle cells attraction and proliferation [55,57,58]. de Mooij et al [55] suggested that increased fetal NT thickness in trisomic fetuses may be due to a loss of lymphatic identity and a shift towards a blood vessel wall phenotype.

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