

Original Articles

Does increased nuchal translucency indicate a fetal abnormality? : A retrospective study to clarify the clinical significance of nuchal translucency in Japan

¹Department of Obstetrics and Gynecology, Nagasaki University Graduated School of Medical Sciences, Nagasaki, Japan

Shigo Yoshida¹, Kiyonori Miura¹, *, Kentaro Yamasaki¹, Shoko Miura¹, Takako Shimada¹, Terumi Tanigawa¹, Atsushi Yoshida¹, Daisuke Nakayama¹, Hideaki Masuzaki¹

*Correspondence to: **Dr. Kiyonori Miura**, Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan.

Tel: +81-95-819-7363; Fax: +81-95-819-7365; e-mail: kiyonori@nagasaki-u.ac.jp

ABSTRACT

The results of a chromosomal test by genetic amniocentesis in 58 cases with an increased nuchal translucency (NT; ≥ 3 mm thickness) revealed 47 cases showing a normal karyotype (81%) and 11 cases (19%) showing an abnormal karyotype. However, the cases of a normal karyotype with increased NT also included those with fetal abnormalities. Among the 49 cases in which NT was observed during the 1st trimester and then subsequently disappeared, chromosomal abnormalities were observed in 5 cases, and fetal abnormalities other than chromosomal abnormalities were observed in 2 cases. Meanwhile, all 9 cases in which an increased NT remained or in which NT continued to increase in size during the 2nd trimester were diagnosed to have cystic hygroma, and chromosomal abnormalities were found in 6 cases (67%). It should be noted that the shape of increased NT includes NT with a notch (Notched NT) and NT without a notch (Smoothed NT). Among the 20 cases of Notched NT, chromosomal abnormalities were observed in 8 cases (40%), and cystic hygroma was observed in 9 cases (45%). On the other hand, among the 38 cases of Smoothed NT, chromosomal

abnormalities were observed in 3 cases (7.9%), but no cystic hygroma was observed.

Our results clarified that increased NT does not always indicate a fetal abnormality. Whether the thickness of NT should be measured as screening of fetal abnormalities remains controversial. However, an increased NT may be detected by chance, because a maternal-fetal medical examination using ultrasonography is usually performed in Japan. It is therefore considered to be extremely important to establish a system in which cases are referred to obstetricians who are licensed clinical genetic specialists in order to obtain appropriate genetic counseling whenever an increased NT is clinically observed.

INTRODUCTION

Nuchal translucency (NT) is a low intensity area observed in the fetal posterior cervical region upon ultrasonography at 11-14 weeks of gestation. NT itself is a finding inherent in all fetuses and it is not necessarily an abnormal finding.

The relationship between an increased NT and chromosomal abnormalities was first reported (Nicolaidis KH, *et al.* 1992). Since then, the relationship with diseases other than chromosomal abnormalities such as cardiac diseases, genetic diseases, and urinary system diseases has been described in various reports (Nicolaidis KH 1992, Souka AP *et al.* 2005, Westin M *et al.* 2006). However, while there are many reports on studies on the usefulness of NT measurements as a marker for chromosomal abnormalities, maternal age and the degree of increased NT vary in the medical literature, and the frequency of chromosomal abnormalities in cases of increased NT ranges from 11–88%, which also indicates differences, and therefore no consistent view has emerged so far (Pandya PP *et al.* 1995, Brambati B *et al.* 1995, Szabo J and Gellen J 1995).

It is known that even when an increased NT is observed in the 1st trimester, in most cases, it later spontaneously disappears. However, even in cases in which it has

later disappeared, the frequency of observing chromosomal abnormalities and other fetal abnormalities has been reported to be higher than that in the general population (Müller MA *et al.* 2004). In addition, there are cases in which the increased NT observed during the 1st trimester also remains into the 2nd trimester, and it is then diagnosed as cystic hygroma, however, it is difficult to diagnose this as cystic hygroma during the 1st trimester.

In Europe and the United States, a one-stop clinic for the assessment of risk (OSCAR) has been implemented, and a screening test according to maternal age with a serum marker for Down syndrome in addition to NT has been conducted. In addition, the measurement of NT has been used as one of the items of the screening test for Down syndrome (Bindra R *et al.* 2002).

On the other hand, the current situation in Japan is that ultrasonography is almost universally used as part of the normal maternal health checkup. Therefore, while the correlation between an increased NT and chromosomal abnormalities has been reported in Europe and the United States, NT measurements became widely used in Japan without a sufficient understanding of the clinical significance of such NT measurements

and how to accurately interpret such findings. It is therefore common for an increased NT to be identified by chance during ultrasonographic examinations, and a serious problem has recently been pointed out in which artificial abortion tends to often be selected when an increased NT is observed in a fetus, because sufficient genetic counseling has not been conducted.

Therefore, to clarify the clinical significance of NT in Japan, we studied the frequency of chromosomal abnormalities in cases of an increased NT, the frequency of fetal abnormalities in cases of a normal karyotype with an increased NT, the frequency of fetal abnormalities in cases with a disappearance of NT, and the relationship between the shape of NT and fetal abnormalities.

MATERIALS AND METHODS

Subject

To get the data of chromosomal karyotype, this study includes 171 pregnant women who got a genetic amniocentesis during 16 weeks of gestation between February 1998 and May 2007 at the Department of Obstetrics and Gynecology at

Nagasaki University Hospital. A breakdown thereof includes 102 cases with an advanced maternal age (35 or more years old), 58 cases with an NT thickness of at least 3 mm (increased NT), 5 cases with a history of delivering children with chromosomal abnormalities, and 6 other cases (4 cases of a request for a chromosomal test, 1 case with a history of delivery with Noonan syndrome, and 1 case with a history of delivering a child with a cardiac abnormality). All cases were managed at regional private clinics and introduced to Nagasaki University Hospital to get a genetic counseling. For all cases, genetic counseling was performed by obstetricians who were licensed clinical genetic specialists. Patient's consents before measuring NT were obtained and the thickness of NT was measured between the 11 and 14 weeks of gestation in 171 cases of single pregnancy.

NT measurement method by ultrasonography

NT was measured by an ultrasound specialist according to the measurement method stipulated by The Fetal Medicine Foundation (Nicolaidis KH 1999). NT thickness of 3 mm or more was defined as an increased NT, because the frequency of

abnormal karyotypes in fetuses with NT thickness of 3 mm or more at 11-14 weeks of gestation were higher than the respective number expected on the bases of maternal age (Pandya PP et al. 1995). Once fetuses with increased NT were detected 11-14 weeks of gestation, they were followed up as the cases of increased NT until the end of pregnancy. We measured the thickness of NT before a genetic amniocentesis at 16 weeks of gestation. We also performed a fetal screening of structural abnormalities by ultrasonography at 20 weeks of gestation. When increased NT at 11-14 weeks of gestation was not detected by visually or decreased to less than 3 mm at 16 weeks of gestation, those cases were defined as cases of NT disappearance.

Classification of the shape of increased NT

Those in which the NT had a smooth surface were defined and classified as smooth NT (s-NT group; Figure 1-a), and those in which the surface of the NT had a notch-like dent were classified as notch NT (n-NT group; Figure 1-b). The frequency of chromosomal abnormalities, and the frequency of cases diagnosed as cystic hygroma in the 2nd trimester were compared between the 2 groups.

Statistical analysis

For the statistical analysis, the Mann-Whitney U test was used. A value of $p < 0.05$ was determined to indicate a significant difference.

RESULT

Frequency of fetal chromosomal abnormalities in fetuses with Increased NT

As a result of genetic counseling at our hospital, among the 171 cases in which the subjects had undergone genetic amniocentesis, an increased NT was observed in 58 cases. Among the 58 cases with increased NT, chromosomal abnormalities were observed in 11 cases (19%) including 5 cases of trisomy 21, 4 cases of trisomy 18, 1 case of monosomy X, and 1 case of a mosaic (Figure 2).

Frequency of chromosomal abnormalities in fetuses demonstrating NT measuring less than 3 mm in size

The relationship between an increased NT and chromosomal abnormalities has

been reported, but some cases without an increased NT also have chromosomal abnormalities. Therefore, the frequency of fetal abnormalities was studied in 113 cases with an NT thickness of less than 3 mm. The indications for undergoing a chromosomal test were an advanced maternal age in 102 cases, a history of delivery with chromosomal abnormalities in 5 cases, and a history of delivery with Noonan syndrome in 1 case, a history of delivering a child with a cardiac abnormality in 1 case, and a request for a chromosomal test in 4 cases. Among these 113 cases, chromosomal abnormalities were observed in 6 cases (5%), including 4 cases of trisomy 21 and 2 cases of trisomy 18. One case of single atrium and single ventricle (1/107 cases; 0.9%) was detected in 107 fetuses with normal karyotype and normal NT.

Fetal abnormalities in fetuses with a normal karyotype and an increased NT

As various fetal abnormalities other than chromosomal abnormalities have been reported for cases with an increased NT, we looked into which of the possible causes of an increased NT were present. The screening of structural abnormalities by ultrasonography was performed at 20 weeks of gestation. As a result of the amniotic

fluid chromosomal test, among the 58 cases with an NT measuring at least 3 mm in size, 47 cases (81%) demonstrated a normal karyotype. Among the 47 normal karyotype cases with an NT measuring at least 3 mm in size, fetal abnormalities were observed in 5 cases (5/47 cases; 10.6%), including 1 case of cardiac abnormality, 2 cases of fetal hydrops, 1 case of fetal pleural effusion, and 1 case of diaphragmatic hernia (Figure 2).

Outcomes in the cases of an increased NT that later disappeared

It is known that most cases of an increased NT observed in the 1st trimester mostly disappear as pregnancy progresses. We therefore investigated the frequency of fetal abnormalities in the cases in which NT later disappeared. The average of NT thickness in the cases of an increased NT that later disappeared was 4.1 ± 1.4 (mm).

Among the 58 cases in which an increased NT was observed in the 1st trimester, 49 cases (84%) showed a disappearance of NT in the 2nd trimester. In 5 of these cases, chromosomal abnormalities (3 cases of trisomy 21 and 2 cases of trisomy 18) were observed, and in 2 cases, congenital abnormalities (1 case each of diaphragmatic hernia, single atrium and single ventricle) were observed (Figure 3).

Subsequent outcomes in the cases of an increased NT that did not later disappear

Meanwhile, among the 58 cases in which NT was observed in the 1st trimester, 9 cases (16%) did not show a disappearance of NT even during the 2nd trimester. All of these cases were diagnosed to have cystic hygroma, and chromosomal abnormalities were observed in 6 (67%) of the 9 cases. The breakdown thereof includes 2 cases of trisomy 21, 3 cases of trisomy 18, and 1 case of monosomy X (Figure 3). The average of NT thickness in 9 cases of cystic hygroma was 6.7 ± 2.5 (mm).

Relationship between the shape of NT and fetal abnormalities

The average of NT thickness in 38 cases of s-NT was 3.9 ± 1.4 (mm), while that in 20 cases of n-NT was 5.5 ± 2.2 (mm). There was significant difference between two groups ($p<0.0006$). All nine cases that diagnosed as cystic hygroma colli in the 2nd trimester demonstrated an increased NT with a notch in the 1st trimester. Maymon *et al.* reported a notch to be observed in 62% of the NT cases that showed an increased NT in the 1st trimester and which were later diagnosed to have Down syndrome (Maymon

R *et al.* 2001). It was thus indicated that NT with a notch is a marker more closely related to Down syndrome. As described above, because cystic hygroma has a high frequency of being accompanied by chromosomal abnormalities, we analyzed the relationship between NT with a notch observed in the 1st trimester and chromosomal abnormalities as well as cystic hygroma, and studied whether NT with a notch observed in the 1st trimester can thus be regarded as a marker for fetal abnormalities and cystic hygroma.

As a result of classifying 58 cases of an increased NT according to the presence or absence of a notch, the s-NT group included 38 cases while the n-NT group included 20 cases (Figure 4). In the s-NT group, 3 cases had chromosomal abnormalities (7.9%; 2 cases of trisomy 21, 1 case of trisomy 18) and in the n-NT group, 8 cases (40%) had chromosomal abnormalities. The frequency of chromosomal abnormalities in the n-NT group was significantly higher than that in the s-NT group ($p=0.0027$). Among the 8 cases in the n-NT group with chromosomal abnormalities indicated, 6 cases were diagnosed to have cystic hygroma in the 2nd trimester, with 2 cases of trisomy 21, 3 cases of trisomy 18, and 1 case of monosomy X. The remaining 2 cases were diagnosed

as 1 case each of mosaic (mos 47,XXY [52]/46,XY[8]) and 18 trisomy. In addition, 9 cases of cystic hygroma in the 2nd trimester were classified as belonging to the n-NT group, but none were classified as belonging to the s-NT group ($p=0.0001$).

DISCUSSION

Among the 58 cases with increased NT, 11 cases (19%) had chromosomal abnormalities. The frequencies of chromosomal abnormalities in cases with an increased NT varies in reports by different authors, but it was 19.2% and 16.2% respectively in a study on 11,315 cases (Kagan KO *et al.* 2006) and a study on 1,015 cases (Pandya PP *et al.* 1995), and our study results were also quite similar. Indeed, the frequency of accompanying chromosomal abnormalities is high in cases with increased NT, but conversely, 81% of the cases with increased NT demonstrate a normal karyotype. This means that most cases are normal karyotype fetuses despite an increased NT being observed. The presence or absence of chromosomal abnormalities can not be determined according to NT thickness, since performing NT measurements is only a screening test, and it is therefore necessary to be aware that a chromosomal test is

also required in order to verify a diagnosis of chromosomal abnormalities.

Among 113 cases with NT measuring less than 3 mm in size, chromosomal abnormalities were observed in 6 cases (5%). Since the 113 cases included 102 cases (90%) of mothers with advanced maternal age (i.e. over 35 years of age) and all 6 cases with chromosomal abnormalities were included in such advanced maternal age cases, it is believed that chromosomal abnormalities cannot be ruled out, even if the thickness of NT is less than 3 mm, and also, the frequency of chromosomal abnormalities is also more significantly affected by the maternal age than by an increased NT.

As for the outcome of the 47 cases that had an increased NT but demonstrated a normal karyotype, fetal abnormalities were observed in 5 cases (11%). The breakdown thereof includes 2 cases of fetal hydrops, and 1 case each of cardiac disease, fetal pleural effusion, and diaphragmatic hernia. The causes of the incidence of increased NT that have been reported include chromosomal abnormalities, heart failure due to cardiovascular and great vessel anomalies, diaphragmatic hernia, venostasis in the head and neck, abnormal development and outflow obstruction of the lymph system, renal and urinary system, nervous system, and genetic disease, and such. Various causes were

observed in our study as well. As most instances of NT disappear during the 2nd trimester despite having previously been observed, NT is thus considered to be a transient physiological finding of the skin during the 1st trimester, and because it cannot be explained by any single mechanism, it should therefore be used instead as a collective term in transient findings. Therefore, regarding the outcome after NT has been detected, NT disappears during the 2nd trimester of pregnancy in some cases, while conversely it continues to grow in other cases.

We therefore studied the outcome of 49 cases in which an increased NT was observed in the 1st trimester and it thereafter disappeared during the 2nd trimester. Among these cases, chromosomal abnormalities (3 cases of trisomy 21 and 2 cases of trisomy 18) were observed in 5 cases, and congenital abnormalities (1 case each of diaphragmatic hernia, single atrium and single ventricle) were observed in 2 cases. It was therefore indicated that it is not a finding that rules out disease, even when a previously observed increased NT has already disappeared, and it is therefore necessary to carefully follow up such cases with an increased NT.

The relationship between cystic hygroma and chromosomal abnormalities has

been reported, but it is difficult to differentiate NT from cystic hygroma in the 1st trimester. In addition, no consensus diagnostic criteria have yet been established for cystic hygroma in the 1st trimester. It is relatively easy to diagnose so-called multilocular septated cystic hygroma that has septal walls in the fetal posterior cervical region; however, it is very difficult to differentiate non-septated cystic hygroma without septal walls and an increased NT (Pistorius *et al.* 2005). In our study, the frequency of chromosomal abnormalities in the n-NT group was significantly higher than that in the s-NT group ($p=0.0027$). In addition, the 9 cases that were subsequently diagnosed to have cystic hygroma were all classified as n-NT, and none were classified as s-NT ($p=0.0001$). A relationship between NT with a notch and Down syndrome has been indicated (Maymon R *et al.* 2001) and the cases with cystic hygroma had a high frequency of chromosomal abnormalities (67%) in our study, thus suggesting a positive relationship between NT with a notch and cystic hygroma. The increased NT observed in the 1st trimester includes the initial findings of cystic hygroma in the 2nd trimester, and it was indicated that such cases tend to have a notch in NT. The average of NT thickness in nine cases of cystic hygroma was significantly increased comparing to that

in the cases of an increased NT that later disappeared (6.7 ± 2.5 (mm) vs 4.1 ± 1.7 (mm), respectively, $p=0.002$). There was also significantly difference of NT thickness between the s-NT group and the n-NT group ($p<0.0006$). Further large-scale study will clarify the association between NT thickness in the 1st trimester and disappearance of increased NT in the 2nd trimester, or cystic hygroma in the 2nd trimester. The association between fetal abnormalities and disappearance or the shape of NT in fetuses with normal karyotype is interesting, though the sample numbers in the present study are too small to give sufficient strength to the analysis. Further study regarding this association should be performed in the future.

In Europe and the United States, a screening system for chromosomal abnormalities has been established that integrates and determines the maternal age and maternal serum marker test results in addition to NT measurements while also calculating the probability of Down syndrome, and NT measurements are handled in a similar way to such screening markers as other maternal serum markers. Therefore, clinically fetal chromosomal abnormalities and fetal structural abnormalities are never

evaluated by NT measurements alone.

Conversely, in Japan, maternal serum markers were introduced as a screening test for Down syndrome in the early 1990s, and it became widely used because of its simplicity without fully understanding that this test is only a screening test. Because a system of genetic counseling had at that time not yet been developed, the explanation of the test results was therefore insufficient, and a problem occurred in which couples sometimes elected to have an artificial abortion before undergoing genetic amniocentesis, which would thus verify such a diagnosis. Therefore, a negative view on the implementation of maternal serum markers was issued by the Health Sciences Council in 1999 in Japan.

In addition, regarding NT measurements, a similar problem to that of the maternal serum markers has recently been identified. In Japan, NT can be easily measured because fetal ultrasonography is normally performed as part of the regular maternal health checkup, and therefore these measurements alone became widely used before the clinical significance of such NT measurement was fully understood. As a result, we are now struggling with the interpretation and explanation of NT in Japan. In addition, because NT can be observed in images and is indicated as a numeric value, the anxiety

that parents feel may be greater than that for the results of serum markers. The parents of a fetus with an increased NT may elect to have an artificial abortion without pursuing a confirmation of diagnosis via amniotic fluid testing before sufficiently understanding the significance of NT.

In conclusion, our results clarified that increased NT does not always indicate a fetal abnormality. As NT measurement is only a screening test and not a verification of any diagnosis, normal fetuses with increased NT should therefore not be artificially aborted.

A genetic counseling including the information of both NT and genetic amniocentesis should be performed in all cases before and after measuring NT. Whether the thickness of NT should be measured as screening of fetal abnormalities remains controversial in Japan. However, an increased NT may be detected by chance, because a maternal-fetal medical examination using ultrasonography is usually performed in Japan. It is therefore considered to be extremely important to establish a system that all cases of increased NT are referred to obstetricians who are licensed clinical genetic specialists in order to obtain appropriate genetic counseling of NT.

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FIGURES LEGENDS

Figure 1: Classification according to the shape of NT

a) NT with a smooth surface was classified as smoothed NT and b) NT with a notch (an arrow) was classified as notched NT. NT: nuchal translucency

Figure 2: NT thickness, fetal chromosomal abnormalities and fetal structural abnormalities

A flow chart is shown for the 58 cases with an increased NT of at least 3 mm observed in the 1st trimester and the 113 cases with an NT measuring less than 3 mm in size.

Figure 3: Outcome of NT observed in the 1st trimester

The outcome of 58 cases with an increased NT measuring at least a 3 mm in thickness in the 1st trimester is described in the flow chart. In the 2nd trimester, increased NT disappeared in 49 cases (84%), but it remained in 9 cases (16%) who were later diagnosed to have cystic hygroma. NT: nuchal translucency, CDH: congenital diaphragmatic hernia, SASV: single atrium and single ventricle

Figure 4: Relationship between NT patterns and fetal abnormalities

the s-NT group included 38 cases while the n-NT group included 20 cases. In the s-NT group, 3 cases (7.9%) had chromosomal abnormalities. In the n-NT group, 8 cases (40%) had chromosomal abnormalities. The frequency of chromosomal abnormalities in the n-NT group was significantly higher than that in the s-NT group ($p=0.0027$). s-NT; smooth nuchal translucency, n-NT; notched nuchal translucency

