Preliminary Normal Reference Values of Nuchal Translucency Thickness in Taiwanese Fetuses at 11-14 Weeks of Gestation

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Background: To investigate normal reference values of nuchal translucency (NT) thickness in normal Taiwanese fetuses between 11 and 14 weeks of gestation.

Methods: A prospective study of ultrasound measurements of fetal NT and crown-rump length (CRL) at 11-14 weeks of gestation was conducted in 724 consecutive Taiwanese fetuses between 1998 and 2001. The relationship between NT and 5-mm intervals of the CRL of the fetus was analyzed. NT thickness was converted into multiple of median (MoM) values for the proper CRL. The estimated risk of trisomy 21 was calculated in combination with maternal age and NT MoM.

Results: NT thickness increased with increasing CRL and gestational week in the first trimester. The mean (median) of NT thickness at 11-14 weeks was 1.56 (1.50) mm. Values of NT logMoM showed a normal Gaussian distribution with a mean of -0.0062 and standard deviation of 0.1146. The overall frequency of NT thickness of >2.5 mm and >3.0 mm was 1.7% (12/724) and 0.7% (5/724), respectively. There were 18 (2.5%) of 724 normal fetuses with the eseimated risk of trisomy 21, based on maternal age and NT thickness higher than 1:300.

Conclusions: Because of weekly variations and racial differences in NT measurements, normal reference values should be established to convert NT thickness into MoM values for calculating the estimated risk of trisomy 21 in first-trimester NT screening.

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Key words: nuchal translucency, crown-rump length (CRL), multiple of median (MoM), Down's syndrome screening.

High-resolution sonography has enabled us to identify the thin translucent space, termed the nuchal translucency (NT), between the fetal echogenic skin and the soft tissues overlying the cervical spine.[1] Fetal NT has been proven to be an effective ultrasound marker for the screening of Down's syndrome[2-6] and other chromosomal abnormalities[7,8] in the first trimester. At present, NT measurement has been developed as a new screening program in the UK and Europe for the early detection of fetal chromosomal aberrations[1,9] and other congenital malformations[10,11] during the first

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trimester of pregnancy.

Initially, most studies used a fixed cutoff point of NT thickness of either ≥2.5 mm or of ≥3.0 mm to define an abnormal result for Down's syndrome screening between 10 and 14 weeks of gestation.[1,2,12-14] Currently, the assessment method of the NT screening program has changed from using a fixed cutoff point of NT thickness into a calculation of the estimated risk of trisomy 21 by a combination of a maternal age-specific risk with a NT-based likelihood ratio.[4,5] Several reports have documented that NT thickness increases with advancing crown-rump length (CRL) or gestational week.[3,5,13,15] Therefore, it is mandatory to establish normal reference values for NT thickness related to gestational age as a way of maternal serum screening for Down's syndrome.

Thilaganathan et al.[16] showed a small but significant difference in NT measurements between fetuses of different ethnic origins. Because of racial differences between Asians and Caucasians, it remains to be determined whether NT screening using statistical parameters from Caucasians is equally applicable in a Taiwanese population. However, limited data on NT measurements in the first trimester exist for Taiwanese pregnancies. The purpose of this study was to investigate and determine normal reference values for NT measurements related to CRL in Taiwanese fetuses during the first trimester.

METHODS

Between April 1998 and December 2001, a prospective observational study was conducted to investigate normal reference values of NT thickness in Taiwanese fetuses at 11-14 weeks of gestation. Demographic details and sonographic findings, including the number of fetuses, CRL, and NT thickness, were entered into a computer database at the time of scanning. The gestational age was determined by the last menstrual period (LMP). Those cases with an uncertain date for the LMP, multiple pregnancies, intrauterine fetal death, or fetal anomalies were excluded from this study. All fetuses in this study were followed up and examined carefully after birth.

Ultrasound measurements were performed by 1 of the authors (J.J. Hsu) who is certificated by the Fetal Medicine Foundation in London. A curvilinear 5-MHz transabdominal transducer (Acuson 128XP/10, Acuson, Mountain View, CA, USA) was used for the NT and CRL measurements with data recording that allowed precision to 0.1 mm. Measurements of NT and CRL were performed according to the guidelines provided by the Fetal Medicine Foundation.[9] In our cases, NT thickness was measured on a magnified image (Fig. 1A), a view focused on the fetal neck area to clarify the NT margin, instead of using a standard image (Fig. 1B) in which the fetal image occupies about 3/4 of the screen as suggested by the Fetal Medicine Foundation. The maximal thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine was measured in an exact sagittal section when the fetus was in a sagittal neutral position. Care was taken to distinguish

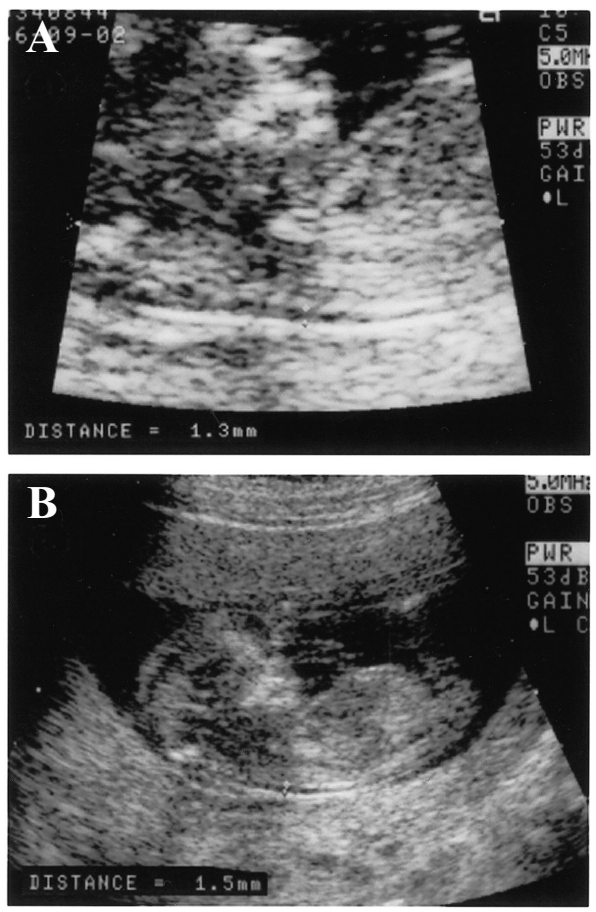

Fig. 1 Measurements of crown-rump length (CRL) and nuchal translucency (NT) thickness based on (A) a magnified image and (B) a standard image.

between fetal skin and the amnion membrane due to the similar appearance of both structures at this gestational age. Calipers were placed directly on the border of the echogenic to non-echogenic tissue ('on the line'). The images were subjected to regular internal auditing by the Fetal Medicine Foundation to verify the standardization and distribution of measurements.

We subdivided all cases into 7 categories according to 5-mm intervals of the CRL of the fetuses. CRL-specific medians for NT thickness were calculated by a weighted non-linear regression from the observed medians of each CRL category. To allow for weekly variations, the results of all data were converted into multiple of the median (MoM) values for the proper CRL. For each pregnancy, the estimated risk of trisomy 21 was calculated from the maternal age and gestational age-related prevalence of trisomy 21, which was then multiplied by the likelihood ratio from the NT measurement. The likelihood ratio is proportional to the degree of deviation in NT thickness from the normal-the thicker the nuchal translucency, the higher the risk of trisomy 21.[5] In this study, the estimated risk of trisomy 21 in each case was calculated using software provided by the Fetal Medicine Foundation.

Means and standard deviations (SD) were used for descriptive purposes. We used the Kolmogorov-Smirnov test to assess the normal distributions of the NT MoM values and used Pearson correlation coefficients to assess correlations among these various factors. A p value of ≤ 0.05 was considered statistically significant. Statistical analyses were performed using the statistical software package SPSS for Windows Release 9.0.0 (Chicago, IL).

RESULTS

The study population consisted of 808 singleton pregnancies attending for routine antenatal care or prenatal diagnosis. After excluding 45 cases with uncertain LMP or those lost to follow-up, 23 cases of twin pregnancies, and 16 cases with chromosomal abnormalities, a total of 724 singleton pregnancies was enrolled in this study. A significant correlation was found between CRL measurements and gestational age ($r=0.556$, $p<0.0001$). The ranges of gestational age, CRL, and maternal age were 11.2-13.9 weeks, 39.8-84.1 mm, and 18.5-42.8 years, respectively, in this study population.

Mean (SD) and median values of NT thickness for each category of CRL are shown in Table 1. Mean (SD) NT thickness and its MoM values were 1.56 (0.41) (median, 1.5) mm and 1.02 (0.28) (median, 0.99) MoM, respectively. We found that increased NT thickness was associated with increasing CRL of the fetus at 11-14 weeks of gestation (Table 1). From regression analysis on the observed medians of CRL and NT thickness in each CRL category, we found that the best fitting equation was log-quadratic (Table 2): $\log NT = -0.621139 + 0.019070\,CRL - 0.000101\,CRL^2$, ($r^2=0.998$). Table 3 demonstrates the percentiles of NT thickness according to 5-mm intervals of the CRL of fetuses. The median of NT measurements increased from 1.10 mm with a CRL < 50 mm to 1.75 mm in the category with a CRL ≥ 75 mm. Similarly, the 95th percentiles of NT measurements increased from 2.05 mm with a CRL < 50 mm to 2.56 mm in the category with a CRL ≥ 75 mm. Figure 2 depicts the distribution of

Table 1. Mean and Standard Deviation (SD) of Nuchal Translucency (NT) Thickness According to 5-mm Intervals of the Crown-Rump Length (CRL) of Fetuses

CRL (mm)		Maternal age (years)		CRL (mm)		NT (mm)		NT (MoM)	
category	n	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
< 50	35	30.3 (3.6)	29.8	45.7 (2.7)	46.1	1.26 (0.55)	1.10	1.15 (0.50)	1.03
50-55	51	30.5 (3.8)	30.5	52.8 (1.4)	53.3	1.40 (0.40)	1.30	1.10 (0.32)	1.07
55-60	121	30.7 (3.6)	31.0	57.7 (1.4)	57.9	1.46 (0.42)	1.40	1.05 (0.31)	1.01
60-65	133	30.7 (3.6)	30.6	62.4 (1.4)	62.3	1.58 (0.38)	1.50	1.06 (0.26)	1.02
65-70	187	31.0 (3.8)	30.9	67.2 (1.4)	67.1	1.61 (0.37)	1.60	1.01 (0.22)	0.98
70-75	114	30.8 (4.0)	30.6	71.9 (1.4)	71.5	1.66 (0.35)	1.70	0.96 (0.21)	0.96
> 75	83	31.2 (4.1)	31.1	78.4 (2.7)	78.3	1.71 (0.45)	1.75	0.92 (0.25)	0.90

Abbreviations: MoM: multiple of median.

NT thickness measurements for the relevant gestational weeks.

There was no significant correlation between maternal age and NT logMoM values (r = 0.006, $p = 0.873$). The NT logMoM values showed a log Gaussian distribution (D = 0.925 and $p = 0.359$) with a mean of -0.0062 and an SD of 0.1146. Table 4 shows the distribution of NT measurements at 5-mm

Table 2. Various Weighted Regression Equations for the Relationship between Nuchal Translucency (NT) Thickness and Crown-Rump Length (CRL) at 5-mm Intervals

Method*	β_0	β_1	β_2	r^2
Linear	0.23427	0.02013		0.97024
Quadratic	- 0.95953	0.05821	- 0.00030	0.99401
Log-linear	- 0.21951	0.00626		0.97093
Log-quadratic	- 0.62114	0.01907	- 0.00010	0.99878

*$NT = \beta_0 + \beta_1 \times CRL$ or $NT = \beta_0 + \beta_1 \times CRL + \beta_2 \times CRL^2$

Table 3. Percentiles of Nuchal Translucency Thickness According to 5-mm Intervals of the Crown-Rump Length (CRL) of Fetuses

CRL (mm)	Percentiles of nuchal translucency thickness						
category	5	10	25	50	75	90	95
< 50	0.60	0.66	0.90	1.10	1.50	1.98	2.05
50-55	0.80	1.00	1.10	1.30	1.70	2.00	2.10
55-60	0.90	1.00	1.20	1.40	1.70	1.98	2.10
60-65	1.10	1.10	1.30	1.50	1.80	2.06	2.20
65-70	1.00	1.18	1.30	1.60	1.80	2.10	2.25
70-75	1.00	1.20	1.45	1.70	1.85	2.10	2.30
> 75	1.00	1.25	1.50	1.75	1.90	2.16	2.56

Table 4. Frequency of Nuchal Translucency (NT) Thickness of ≥ 2.5 mm or ≥ 3.0 mm According to 5-mm Intervals of the Crown-Rump Length (CRL) of Fetuses

CRL (mm)		NT thickness ≥2.5 mm		NT thickness ≥3.0 mm	
category	n	n	%	n	%
< 50	35	2	5.7	1	2.6
50-55	51	0	0.0	0	0.0
55-60	121	3	2.5	1	1.0
60-65	133	2	1.5	1	1.0
65-70	187	1	0.5	0	0.0
70-75	114	0	0.0	0	0.0
> 75	83	4	4.8	2	1.8
Total	724	12	1.7	5	0.7

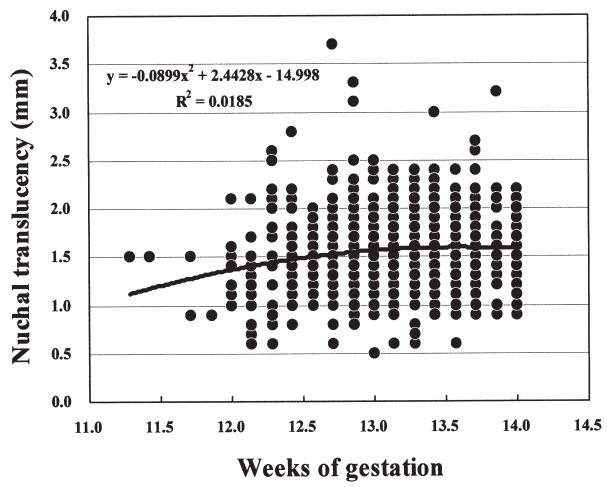

Fig. 2 Distribution of nuchal translucency thickness of normal Taiwanese fetuses measured at relevant gestational weeks.

Table 5. Frequency of Estimated Risk of Trisomy 21 for Various Cutoff Values According to 5-mm intervals of Crown-Rump Length (CRL) of Fetuses

		Estimated risk of trisomy 21*											
CRL (mm)		≥1:100		≥1:200		≥1:300		≥1:400		≥1:500		≥1:600	
category	n	n	%	n	%	n	%	n	%	n	%	n	%
< 50	35	2	5.7	2	5.7	3	8.6	3	8.6	3	8.6	3	8.6
50-55	51	0	0.0	0	0.0	1	2.0	1	2.0	1	2.0	1	2.0
55-60	121	1	0.8	2	1.7	3	2.5	4	3.3	4	3.3	6	5.0
60-65	133	2	1.5	3	2.3	3	2.3	4	3.0	5	3.8	5	3.8
65-70	187	0	0.0	1	0.5	1	0.5	1	0.5	7	3.7	11	5.9
70-75	114	0	0.0	2	1.8	2	1.8	3	2.6	4	3.5	5	4.4
> 75	83	2	2.4	3	3.6	5	6.0	5	6.0	5	6.0	5	6.0
Total	724	7	1.0	13	1.8	18	2.5	21	2.9	29	4.0	36	5.0

*: Estimated risk of trisomy 21: risk as a combination of maternal age and nuchal translucency thickness.

intervals of the CRL of fetuses. The overall frequency of NT measurements ≥2.5 mm and ≥3.0 mm was 1.7% (12/724) and 0.7% (5/724), respectively. In the category with CRL <50 mm, 5.7% and 2.6% of the study group had NT measurements ≥2.5 mm or ≥3.0 mm, respectively. At a CRL ≥75 mm, 4.8% and 1.8% of the study group had NT measurements ≥2.5 mm or ≥3.0 mm, respectively. The estimated risk of trisomy 21, based on maternal age and NT thickness, was 1 in 300 or higher in 18 (2.5%) fetuses of this study population (Table 5).

DISCUSSION

Little is known of the physiological basis which can explain increased in NT thickness in either some of normal and abnormal fetuses during the first trimester of pregnancy. It may represent an accumulation of fluid associated with overperfusion for protecting the developing fetal neural structures.[17] The placenta grows rapidly at the end of the first trimester with a consequent increase in circulating blood volume in parallel with that of fetal circulation.[18] Between 9 and 12 weeks of gestation, the bony components over the posterior neck are not fully differentiated, leading to the formation of transient physiological edema at the level of the nuchal fold to protect intracranial organs from the risk of overperfusion.[17]

In addition, increased NT is recognized in fetuses with cardiovascular malformations. There is a strong association between increased NT and Down's syndrome in humans and animals.[19] Moreover, it is well known that Down's syndrome is often accompanied by a great incidence of cardiovascular malformations.[20] Fetuses with normal karyotypes, but affected by cardiovascular malformations, do show increased NT in ultrasound examinations during the first trimester.[10] In particularly, there is an association between the degree of nuchal edema and the severity of the cardiovascular disease.[17]

Our study confirms previous reports which found that fetal NT thickness appears to increase with gestational age.[3,5,13,15] Therefore, it is not appropriate to use a fixed cutoff point for NT thickness regardless of the gestational age in NT screening for Down's syndrome. When determining a given increase in NT thickness, it is essential to take the gestational age or the CRL into account. Measuring the CRL is commonly used to calculate the gestational age during the first trimester. There are 2 reasons to consider using CRL instead of gestational age for NT screening. First, it is common for pregnant women to be uncertain of the date of the LMP; and second, it is convenient to measure the CRL of the fetus at the same time that the NT thickness is measured.

An increased false-positive rate (NT thickness ≥2.5 mm or ≥3 mm) with increasing gestational age was noted in previous reports.[3,13,21] However, various frequencies of increased NT thickness in different categories of CRL were found in the present study. This finding confirms that the concept of a fixed cutoff point is not clinically valid. In addition, the false-positive rate in our result (1.7%) was far lower than those of Jou et al.[21] (6.3%), Scott et al.[13] (5.5%), and Pandya et al.[12] (5.7%) who used a cutoff point of 2.5 mm. This may be because we measured NT thickness on a magnified image on which the NT margin could be clearly identified and the thickness accurately measured. Differences of even a few decimal millimeter can influence the results of the NT-based likelihood ratio and the estimated risk of trisomy 21. Thus, accurate measurement of NT thickness is mandatory for the assessment of risk of trisomy 21 by ultrasound. Although a suitable uniform protocol has been established by the Fetal Medicine Foundation,[9] it seems better to use a magnified image in order to accurately measure NT thickness.

Whitlow and Economides[22] demonstrated that the optimal time to examine fetal anatomy and measure NT thickness in the first trimester is 13 weeks. The success rate of visualizing the complete fetal anatomy is increased progressively from 10 to 14 weeks. At 12-13 weeks, the complete anatomy could be clearly visualized in 96%-98% of fetuses.[22,23] The success rate of measuring NT was similar at 10-13 weeks but significantly less at 14 weeks. Braithwaite et al.[23] reported that the NT increases to a maximum at 13 weeks+2 days of gestation. A steady decrease and then disappearance of the NT was found after 14 weeks of gestation. This may account for the necessity to measure NT before 14 weeks of gestation. In addition, increased NT thickness in affected and unaffected fetuses may either resolve or evolve into nuchal edema in the second

trimester. Thus, the resolution of NT by the second trimester does not reduce the risk for fetal trisomy and should not falsely reassure clinicians or patients.[24]

Currently, it is common to use the concept of MoM to express the relationship between NT thickness and gestational age in NT screening for Down's syndrome.[5,25,26] Since there is no significant difference in CRL between Asian and Caucasian populations,[27] expressing the NT measurements in MoM may eliminate differences due to race and institution.[21,26] Wide variations in results of NT screening may be attributed to different statistical methods as well as race-specific distributions. The report by Thilaganathan et al.[16] challenged every center to establish their own database for the NT screening for Down's syndrome.

A new method for assessment risk of trisomy 21 uses a combination of maternal age-specific risk and the MoM value of the NT thickness measurement. A cutoff for estimated trisomy 21 risk of 1 in 300 has been suggested for NT screening in the first trimester.[5] Snijders et al. revealed that 8.3% of normal pregnancies and 82.2% of those with trisomy 21 had an estimated trisomy 21 risk of 1 in 300 or higher. Therefore, for a cutoff value of estimated trisomy 21 risk of 1 in 300, the sensitivity was 82.2% and the false-positive rate was 8.3%.[5] However, our population showed that only 2.5% of the normal pregnancies had an estimated trisomy 21 risk of 1 in 300 or higher. This may be attributed to racial differences and to taking the NT measurement from the magnified image.

Measurement of NT thickness has been documented as an effective method of first-trimester screening for fetal chromosomal abnormalities. However, there are limited data dealing with the NT thickness in Taiwanese populations. Our data may provide preliminary information to perform first-trimester NT screening for Down's syndrome in Taiwan. We recommended that one should use the estimated risk of trisomy 21, instead of a fixed cutoff point of NT thickness, based on center-specific median MoM values derived from CRL. Nevertheless, NT screening for Down's syndrome should be applied under expert training and internal auditing in the future.

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台灣胎兒在懷孕11-14週之頸部透明帶厚度之初步正常參考值

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背 景： 探討台灣正常胎兒在懷孕11至14週之頸部透明帶厚度的正常參考值。

方 法： 從1998年到2001年，前瞻性研究在懷孕11至14週使用超音波測量724名正常胎兒的頸部透明帶厚度。使用回歸分析頸部透明帶厚度和每5 mm間距頭臀徑的相關性。頸部透明帶厚度依相對的頭臀徑迴歸後的標準中位值再換算成中位值倍數值 (MoM)。母親年齡和頸部透明帶厚度合併計算三染色體21的評估危險機率。

結 果： 懷孕初期之頸部透明帶厚度會隨著頭臀徑和懷孕週數的增加而增加。懷孕11-14週的頸部透明帶厚度平均值 (中位值) 是1.56 (1.50) 毫米。頸部透明帶厚度中位值倍數值的對數值呈正常常態分布，其平均值是-0.0062，而標準誤差是0.1146。本研究族群裡的頸部透明帶厚度大於等於2.5 mm和大於等於3 mm的比例，則分別是1.7% (12/724) 和0.7% (5/724)。使用母親年齡和頸部透明帶厚度合併計算三染色體21的評估危險機率超過1:300的有18名(2.5%)。

結 論： 由於頸部透明帶的每週中位值不同和人種差異，如要發展懷孕初期頸部透明帶厚度篩檢，應先建立頸部透明帶厚度之正常參考值，將其轉換成中位值倍數值以用於計算三染色體21的評估危險機率。

(長庚醫誌 2003;26:12-9)

關鍵字： 頸部透明帶，頭臀徑，中位值倍數值，唐氏症篩檢。

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