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Prediction of homozygous α-thalassemia-1 by nuchal translucency measurement at first trimester: is it possible?

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Summary

Purpose of investigation: To determine the performance of nuchal translucency (NT) measurement as a screening test for homozygous α-thalassemia-1 in at-risk pregnant women. *Materials and Methods*: Fetal NT thickness was measured at 11–13 weeks of gestation in 283 pregnancies at risk of homozygous α-thalassemia-1. The final diagnosis was confirmed by invasive procedures. The NT measurements were compared in affected fetuses and unaffected ones (normal or carriers). The sensitivity and specificity based on the cut-off of 95th percentile of normal NT ranges were calculated. *Results*: Out of a total of 283 pregnancies at risk of homozygous α-thalassemia-1, 65 were confirmed to be affected, and 218 were unaffected. The median NT value (median 2.6 mm, 95% CI: 2.357–2.880 mm) in affected cases was significantly higher than that in the unaffected cases (median 1.6 mm, 95% CI: 1.611–1.686 mm) ($p < 0.001$). Using receiver-operating characteristic (ROC) analysis, the increased NT yielded a detection rate of 52.3 % (95% CI: 39.54%–64.85%) at a 5% false positive rate. *Conclusion*: NT may be helpful in screening for fetal homozygous α-thalassemia-1 in clinical work.

Key words: Nuchal translucency; Homozygous α-thalassemia-1; Prenatal diagnosis; Screening; Ultrasonography.

Introduction

Homozygous α-thalassemia-1 (Hemoglobin Bart's disease) is the most common cause of fetal hydrops in Southeast Asia. Affected fetuses almost always die in utero or soon after birth. It is therefore important to make early prenatal diagnosis and timely terminate the affected pregnancy. Sonography is a helpful and cost-effective screening tool for detecting homozygous α-thalassemia-1 fetuses. Many studies have showed that combined with the fetal cardiothoracic ratio, middle cerebral artery peak systolic velocity (MCA-PSV) and placenta thickness, most of the fetuses with homozygous α-thalassemia-1 could be detected by ultrasound during the second trimester [1, 2]. In the first trimester, however, these sonographic markers may not work well because of the small size of fetus and unsatisfactory image quality [3,4].

Nuchal translucency (NT) measurement has been a routine examination in the first trimester, which can be successfully done in nearly all pregnancies, and has been widely used as a screening test for chromosomal and structural anomalies. Increased NT may be the consequence of fetal cardiac failure, as in the recipient fetus seen in twin-to-twin transfusion syndrome [5]. The fetus affected by homozygous α-thalassemia-1 suffers from severe anemia and may have cardiac changes even in the first trimester [6]. The purpose of this study was to assess the effectiveness of first trimester NT measurement in the prediction of homozygous α-thalassemia-1 among at-risk pregnancies.

Materials and Methods

This was a prospective study conducted at Guangzhou Women and Children Medical Center between January 2011 and August 2013. The study protocol was approved by the Ethics Committee of Guangzhou Women and Children Medical Center and informed consent was obtained from all pregnant women enrolled in this study. The entry criteria were as followed: 1) singleton pregnancy at 11^{+0} to 13^{+6} weeks determined by fetal crown-rump length (CRL); 2) both partners of the couple were ethnical Chinese; and 3) both partners of the couple were α-thalassemia-1 carriers. The exclusive criteria were as followed: 1) poor quality of ultrasonographic images and 2) fetal structural or chromosomal abnormalities.

Transabdominal first trimester scan was offered to all pregnancies before the invasive test. A system with RAB-8-D transducer was used. NT was measured according to the standards of the Fetal Medicine Foundation [www.fetalmedicine.com] by two qualified sonographers. An attempt was made to repeat NT measurement three times in each case and the mean of the measurements was recorded.

The distribution of NT in relation to CRL in pregnancies with known normal outcome was performed in this study. The reference range of CRL-dependent NT was constructed and a NT ≥ 95% of normal range was designated as increased. During the study period, 562 pregnant women attending the present center for first-trimester trisomy 21 screening with normal pregnancy outcomes were used as controls. The prenatal diagnosis of homozygous α-thalassemia-1 was confirmed by invasive procedures and DNA-based testing.

Data were analyzed using the statistical software SPSS version 16.0. Graphpad 5.0 was used to draw graphs. In order to establish normative data of NT thickness, the relationship between fetal NT thickness and CRL was analyzed with the linear regression

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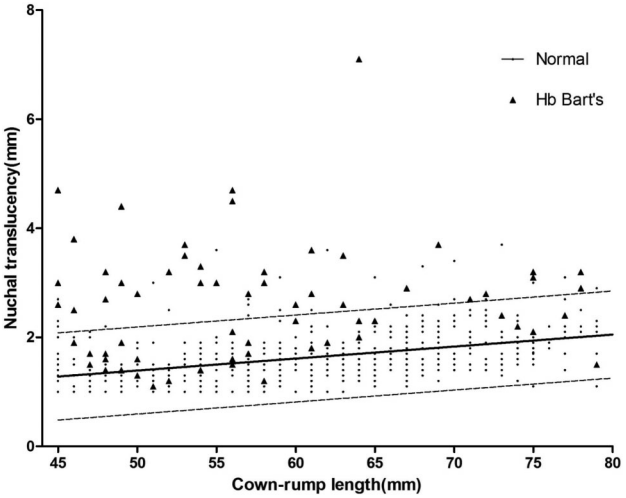

Figure 1. — Variation of NT thickness in 562 normal fetuses (·) and 65 fetuses with homozygous α-thalassemia-1 (▲) based on the crown-rump length. The regression lines shows the 5th, 50th, and 95th centile values of the NT thickness.

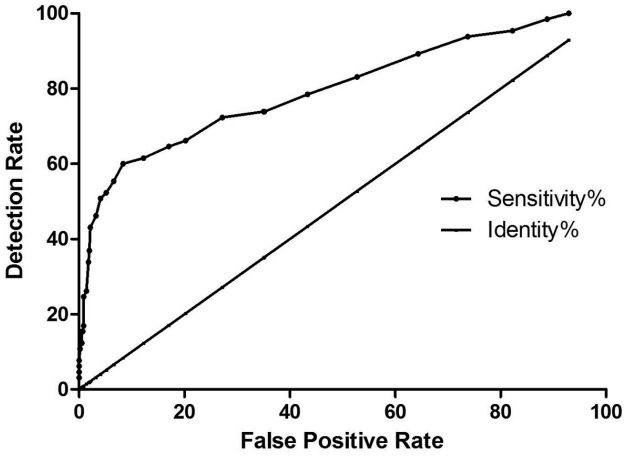

Figure 2. — ROC curve of NT thickness in predicting homozygous α-thalassemia-1 at 11-14 weeks of gestation. The best cutoff point is 2.55 mm, AUC 0.808, 95% CI: 0.742–0.873.

method. According to the regression equation, the expected the 5th, 25th, 50th, 75th, 95th percentile values of NT thickness were obtained for a given CRL. The normality of measurements at each week of gestation was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk's W-tests, and p value of < 0.05 was considered statistically significant. Receiver-operating characteristic (ROC) analysis was constructed to estimate the detection rate (DR) and false-positive rate (FPR) of predictive markers at different cutoff points for each marker. Estimated area under the curve (AUC) was compared with the value of 0.500 that represented the nondiscrimination between cases and controls. Intra- and inter-observer reproducibility was analyzed by construction of Bland-Altman plot [7].

Results

During the study period, 288 pregnant women at risk of homozygous α-thalassemia-1 were recruited; five of these cases were excluded from analysis (two with fetal structural anomalies and three with fetal trisomies). Of 283 at-risk pregnancies, 65 (23.0%) proved to have affected fetuses (homozygous α-thalassemia-1) and 218 (77.0%) had unaffected fetuses (normal or carriers).

In 562 control pregnancies, the mean maternal age was 29 (range 18–43) years, and the mean CRL was 62 (range, 45–80) mm. NT thickness increased with increasing CRL expressed in the following equation: NT = 0.282 + 0.022CRL (R = 0.442, $p < 0.001$, SD = 0.406) (Figure 1). The percentile values of the NT thickness based on the ten-mm CRL interval is shown in Table 1.

NT was successfully measured in all 283 pregnancies of this study. The mean maternal age was 28 (range, 20–42) years and the mean CRL was 57 (range, 45–79) mm. In the 65 affected fetuses, 58 (89.2%) had a NT measurement above the median value of the normal range, and 34 (52.3%) with a NT above the 95th centile of the normal range (Figure 1). The NT median value (median 2.6 mm, 95% CI: 2.357–2.880 mm) of affected fetuses was significantly higher than that of unaffected (median 1.6 mm, 95% CI: 1.611–1.686 mm) ($p < 0.001$). The ROC curve was then obtained using several values for the threshold. As shown in Figure 2, the increased NT yielded a detection rate of 52.3 % (95% CI: 39.54%–64.85%) at a 5% false positive rate with likelihood ratio 12.8. The AUC was 0.808 (95 CI: 0.742–0.873) ($p < 0.0001$) (Figure 2). The specificity was 95.9% (95% CI: 93.93%–97.39 %).

Table 1. — *Percentile values of fetal NT thickness for ten-mm CRL interval in 562 normal fetuses at first trimester.*

CRL (mm)	No. of fetuses	NT thickness (mm) (percentile)				
		5th	25th	50th	75th	95th
45-54	138	1.0	1.1	1.3	1.5	2.1
55-64	196	1.0	1.3	1.5	1.8	2.2
65-74	180	1.1	1.5	1.8	2.1	2.6
75-80	48	1.1	1.7	2.0	2.2	3.0

Discussion

Fetuses affected by homozygous α-thalassemia-1 develop severe anemia in early fetal life [8, 9], and have to compensate by dilating the cardiac chamber and increasing cardiac output in response to the anemia and hypoxia [10]. Since increased NT has been associated with fetal cardiac function [11], it is possible that fetuses with homozygous α-thalassemia-1 present with an increased NT. The present results confirmed this hypothesis that the median NT value in affected fetuses was significantly higher than that in the unaffected ones. Using a cut-off of 95th centile according to the

corresponding CRL, the authors demonstrated a 52.3% sensitivity of NT measurement for detection of homozygous α-thalassemia-1 with a 5% false positive rate. This sensitivity was much lower compared to that of fetal cardiothoracic ratio which has a high accuracy in prediction of affected pregnancies [1, 2]. However, sonographic cardiac assessment is not part of routine investigations at the first trimester. In the present authors' practice guidelines for performance of first-trimester fetal ultrasound scan, the purpose is to detect gross fetal abnormalities and measure the NT thickness that offers first-trimester aneuploidy screening. A first trimester cardiothoracic ratio measurement is beyond their real practical ability. Indeed, a previous study by Lam *et al.* described only a 19% detection rate of NT in prediction of homozygous α-thalassemia-1 [12]. The finding of a relatively higher detection rate in the present study might be due to several issues. There was a larger sample size including more cases in earlier gestational age. Additionally, compared with that study performed more than ten years previously, ultrasound equipment and performers' skills had improved greatly in practice. However, both studies found a very high positive predictive value. Therefore, it is necessary to re-assess the screening ability of NT for homozygous α-thalassemia-1 at the first trimester. In the present study, only half of the fetuses with homozygous α-thalassemia-1 were found with increased NT, compromising its conventional use in prediction of homozygous α-thalassemia-1 as the role of fetal cardiothoracic ratio. However, one remarkable advantage of NT measurement over other markers is that it possesses an intact training and measuring protocol which could guarantee the repeatability and to minimize the intra- and inter-observer difference [13, 14]. For those at-risk women with a normal NT, they can choose to receive follow-up by repeat scans. Other sonographic markers such as cardiothoracic ratio and MCA-PSV will be used in subsequent investigations [15].

The present authors acknowledge that this study had some limitations. One limitation was that the results were applied only to fetuses at risk (25% having an affected fetus), not to the general population with a low prevalence of homozygous α-thalassemia-1. Another limitation was that NT measurements also require some training; thus, it may not always be practical in general practice in community hospitals.

Conclusion

In summary, the present study showed that NT measurement at the first trimester should be taken into account as a screening tool for fetal homozygous α-thalassemia-1 in at-risk pregnancies. A fetus with an increased NT suggests a high risk of being affected. Therefore CVS was recommended to the women with an increased NT. For those with a normal NT, the women would be given the option of follow-up by repeat ultrasound scans, followed by invasive testing only in cases with abnormal findings. The cases with normal sonographic scans were followed with routine prenatal care, and saved the invasive testing. With this strategy, invasive procedures were avoided in at least three out of four at-risk pregnancies, and were limited to the few pregnancies identified to be at high risk by ultrasound.

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