

Assessment of Foetal Nuchal Translucency and its Relationship with Crown Rump Length in Normal Foetuses using Ultrasonography in a Subset of South Indian Population

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ABSTRACT

Introduction: Assessing Nuchal Translucency (NT) accurately is necessary in detection of various anomalies as described in the literature. Studies deriving reference range for NT in Indian population is not widely available.

Aim: To derive a normal reference range value of NT with respect to Crown Rump Length (CRL) by using ultrasound in South Indian population.

Materials and Methods: A cross-sectional study was conducted on 470 pregnant women from December 2018 to March 2020. Measurement of NT thickness and CRL was performed by ultrasound at 11-14 weeks of gestation. Association between quantitative explanatory and outcome variables was assessed by calculating Pearson correlation coefficient and the data was represented in a scatter diagram. The relationship between NT

thickness, CRL and gestational age was studied by using linear regression analysis. The Statistical Package for Social Sciences (SPSS) software version 22.0 was used for statistical analysis. The p-value less than 0.05 was considered statistically significant.

Results: Total sample of 470 pregnant females (mean age 25.626±3.82 years, 357 multipara, mean gestational age 12.60±1.36 weeks) was analysed. The mean CRL was 59.84±10.17 mm and mean NT thickness was 1.3±0.26 mm, respectively. The median gestational age was 12.4 weeks. The regression equation which showed relation between median NT thickness and CRL was described as follows: Expected NT thickness=-1.652+(0.050)×CRL mm (R² linear=0.995, p<0.0001).

Conclusion: The study provides normative data of NT thickness in normal foetus. This data can be used as reference to screen various chromosomal abnormalities between 11-14 weeks of gestation.

Keywords: Chromosomal, Foetal anomalies, Gestation, Prenatal

INTRODUCTION

Prenatal Ultrasound is widely used for detecting foetal chromosomal and structural anomalies during pregnancy. CRL measurement by ultrasound is the most sensitive predictor of gestational age in first trimester. CRL is measured in midsagittal section of foetus when oriented horizontally to the screen with sufficient magnification. The end points of crown and rump should be defined and measurement is done using electronic callipers with foetus in neutral position. "NT is a hypo-echoic region of sub-cutaneous fluid in the posterior aspect of neck at the level of the cervical spine, assessed at a period of 11-14 weeks of gestation". Elevated levels of NT increase the risk for structural and chromosomal abnormalities in foetus [1]. Previously, NT values >95th percentile for a given CRL was considered to be raised. Current advanced reports suggest that adverse outcomes are frequent if NT is more than the cut-off of 3.5 mm (i.e., R 3.5 mm)- corresponding to "99th percentile or more" [2,3]. The importance of assessing NT accurately is necessary in detection of various anomalies. The triple marker test includes NT, Beta-Human Chorionic Gonadotropin (β -hCG) and Pregnancy Associated Plasma Protein A (PAPP-A) and is done to classify the patient as high or low risk for chromosomal anomalies. Beta-HCG and PAPP-A are expensive for the major population of India. A proper NT assessment is the basic facility available for the common people at reasonable cost [2-4].

Several studies have brought to light the changes in reference range of NT in each ethnic groups [2,3]. A study deriving reference range in an Indian population is not widely available to the best of our knowledge and hence the aim of the present study was to derive a formula that enables to give the accurate value of NT for a given CRL in a selected south Indian population.

MATERIALS AND METHODS

This was a cross-sectional study conducted after obtaining ethical clearance in the Department of Radiodiagnosis, SRM Medical College Hospital and Research Centre, Chengalpattu, Tamil Nadu, India (letter No. 1468/IEC/2018). Convenient sampling technique was used. The study period was December 2018 to March 2020. Written informed consent was obtained from all the patients prior to the study procedure. The study was done using ultrasound machine Philips Affinity 30 with convex transducer, 2-6 MHZ. The sample size included was 470.

Inclusion criteria: All pregnant patients who came for antenatal visit in first trimester with CRL between 45 and 84 mm which corresponds to gestational age from 11 upto 14 weeks 6 days were included in the study.

Exclusion criteria: CRL less than 45 and more than 84 mm, multiple gestations and patients with foetal abnormalities during the scan were excluded from the study.

The crown rump length was measured in midsagittal section with foetus in neutral position with crown and rump clearly seen. Crown is the top of the head of foetus and rump is the bottom of torso.

Measurement of Nuchal Translucency (NT)

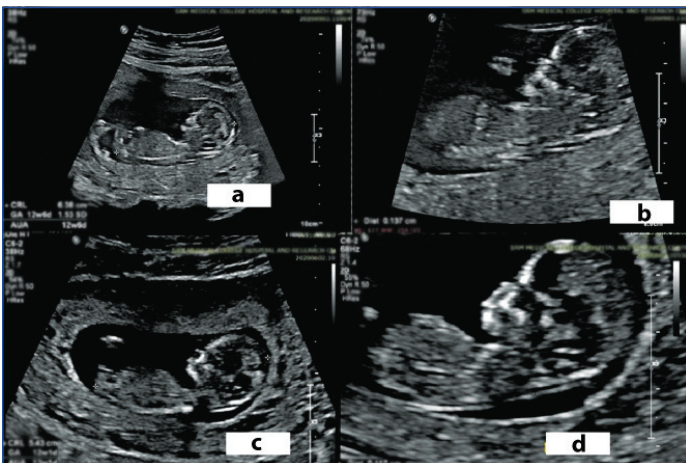
NT was measured when the foetus was in mid- sagittal imaging plane (the vertebral column facing the bottom of the screen). The measurement was done by an experienced radiologist with more than 10 years of experience in obstetric ultrasound practice. Following structures were seen to confirm correct mid-sagittal position [4]:

- Two tiny parallel echogenic lines: Tip of nose and Nasal bone (if not absent).
- Hard palate
- Diencephalon.

Magnification was done such that only foetal head and upper thorax included in the image; enabling 1 mm changes in measurement possible. The measurement was not taken when the foetal head was extended or flexed [5]. NT was measured when the foetus was floating free of the uterine wall i.e., amniotic fluid was seen between its back and uterus so as not to mistakenly measure the distance to the amniotic membrane or uterine wall [6]. Only lucency was measured (again differing from nuchal thickness) and the callipers were put inside the hyperechoic edges. The widest part of the translucency was measured [Table/Fig-1,2].



[Table/Fig-1]: Ultrasound image showing normal Nuchal Translucency (NT) measurement.



[Table/Fig-2]: Ultrasound image showing Nuchal Translucency (NT) measurement in a patient with 2(a) and 2(b) gestational age of 12 weeks 6 days, 2(c) and 2(d) gestational age of 12 weeks 1 day.

All foetuses were followed-up till birth. Pregnancy complications like eclampsia, pre-eclampsia and foetal complications like growth retardation assessed. Only normal foetuses after delivery and without any pregnancy complications were included in the study to assess the normal reference range of NT parameters. NT was considered as primary outcome variable. CRL, Gestational age, maternal age and parity of pregnant patients were considered as primary explanatory variables.

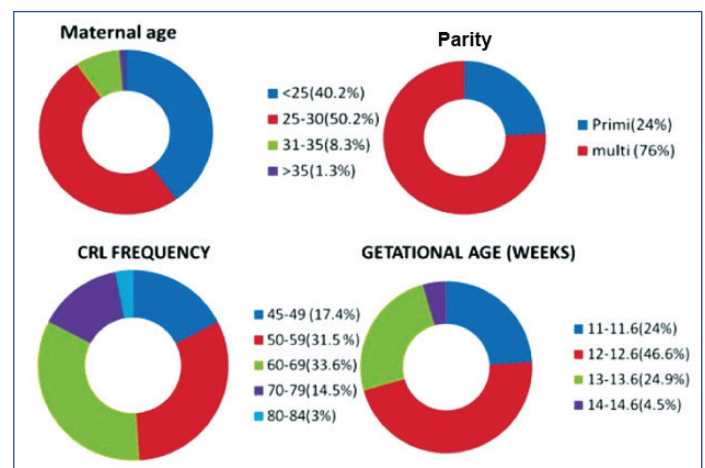
STATISTICAL ANALYSIS

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. All Quantitative variables were checked for normal distribution within each category of explanatory variable by using

visual inspection of histograms and normality Q-Q plots. Shapiro-wilk test was also conducted to assess normal distribution. Shapiro wilk test p-value of >0.05 was considered as normal distribution. For normally distributed Quantitative parameters, the mean values were compared between study groups using Analysis of Variance (ANOVA). Post-hoc test was performed to check the inter group comparisons. Association between quantitative explanatory and outcome variables was assessed by calculating pearson correlation coefficient and the data was represented in a scatter diagram. Linear regression analysis was done. Regression coefficient, along with its 95% CI and p-values were calculated. Categorical outcomes were compared between study groups using Chi-square test/Fisher's-Exact test. The p-value <0.05 was considered statistically significant. International Business Management (IBM) SPSS version 22.0 was used for statistical analysis.

RESULTS

A total of 470 patients presenting in Department of Radiodiagnosis during the study time period were enrolled for the study. Maternal age distribution is given in [Table/Fig-3]. Among study population, the mean of Maternal Age was 25.626 ± 3.82 years. According to parity, 357 (76%) of them were Multipara and 113 of them were Primigravida [Table/Fig-3]. The mean of Gestational age calculated according to LMP was 12.60 ± 1.36 weeks. However, since LMP is not considered the best parameter for assessment of gestational age in modern era as majority of patients have irregular periods and few had forgotten their last menstrual period. Hence, gestational age is better assessed using CRL which is the best indicator of foetal growth in first trimester. CRL distribution is given in [Table/Fig-3]. Among the study population, the mean of CRL was 59.84 ± 10.17 mm and median was 60 mm. The gestational age distribution according to CRL is given in [Table/Fig-3]. The mean Gestational age according to CRL was 12.35 ± 1.02 weeks. The median gestational age according to LMP and CRL was 12.4 weeks. Among the study population, the mean heart rate was 160.67 ± 10.99 bpm and mean cervical length was 3.77 ± 0.50 cm.

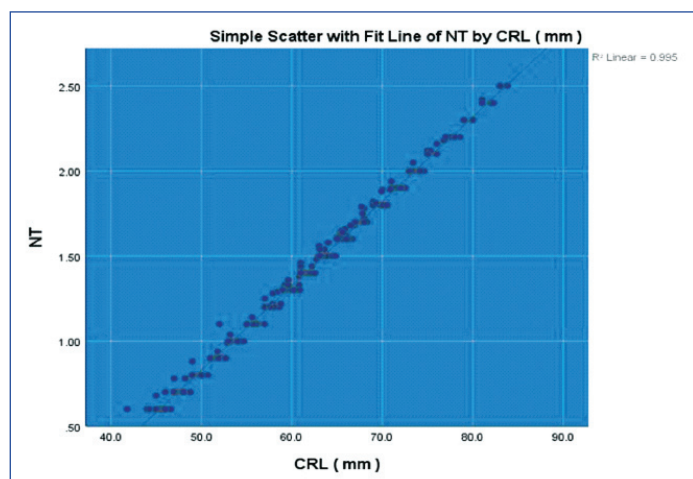


[Table/Fig-3]: Maternal age, parity, CRL distribution and gestational age distribution according to CRL.

The distribution of NT in relation to CRL according to simple linear regression is shown in the scattered plot diagram [Table/Fig-4]. NT thickness increased with CRL estimates of gestational age. The regression equation which shows relation between median NT thickness and CRL was described as follows: Expected NT thickness = $-1.652 + (0.050) \times \text{CRL mm}$.

The Mean NT in women according to gestational age is given in [Table/Fig-5]. Taking gestational age 11-11.6 weeks as base line, the mean difference of NT in gestational age 12-12.6 weeks, 13-13.6 weeks and 14-14.6 weeks was statistically significant

(p-value <0.05) [Table/Fig-5]. Among the study population, the mean NT was 1.3±0.26 mm.



[Table/Fig-4]: Scatter plot diagram showing relationship between Nuchal Translucency (NT) and Crown Rump Length (CRL).

Gestational age (weeks)	NT (Mean±SD) (mm)	Mean difference (mm)	95% CI		p-value
			Lower	Upper	
11-11.6 (Base line)	0.733±0.13				
12-12.6	1.29±0.21	-0.560	-0.615	-0.504	<0.001
13-13.6	1.84±0.16	-1.114	-1.178	-1.051	<0.001
14-14.6	2.31±0.21	-1.581	-1.695	-1.467	<0.001

[Table/Fig-5]: Comparison of mean NT across Gestational Age (N=470). ANOVA test used. p-value <0.05 statistically significant, p-value <0.001 statistically highly significant

The difference in the proportion for maternal age between groups of CRL was statistically not significant (p=0.349). The Mean NT in women according to maternal age is given in [Table/Fig-6].

Maternal age	NT (Mean±SD) (mm)	Mean difference (mm)	95% CI		p-value
			Lower	Upper	
<25 years (Baseline)	1.30±0.48				
25-30 years	1.38±0.47	-0.084	-0.205	0.037	0.279
31-35 years	1.32±0.47	-0.0246	-0.238	0.189	0.991
>35 years	1.67±0.46	-0.372	-0.885	0.139	0.2409

[Table/Fig-6]: Comparison of mean Maternal Age with NT in study population (N=470). ANOVA test used. p-value <0.05 statistically significant, p-value >0.05 statistically not significant

We calculated the 95th percentile of NT with respect to CRL at an interval of 5 mm and it was found to be 0.8 mm, 1.1 mm, 1.33 mm, 1.6 mm, 1.82 mm, 2.1 mm and 2.3 mm for CRL <50 mm, 51-55 mm, 56-60 mm, 61-65 mm, 66-70 mm, 71-75 mm and 76-80 mm respectively. The 5th percentile of NT value for CRL <50 mm, 51-55 mm, 56-60 mm, 61-65 mm, 66-70 mm, 71-75 mm, 76-80 mm and >80 mm were 0.6 mm, 0.9 mm, 1.1 mm, 1.3 mm, 1.6 mm, 1.8 mm, 2.1 mm and 2.4 mm, respectively. The Mean NT in the present study increased from 0.667±0.080 mm when CRL was <49.99 mm to 2.40±0.08 mm when CRL was 85 mm. The Mean NT (mm) in women with CRL <49.99 mm was 0.667±0.080 mm, it was 1.07±0.16 in women with CRL 50-59.9 mm, 1.54±0.15 mm in women with CRL 60-69.9 mm, 1.99±0.15 mm in women with CRL 70-79.9 mm and 2.40±0.08 mm in women with CRL >=80 mm.

Taking CRL <49.99 mm as baseline, the mean difference of NT score in CRL ranges 50-59.9 mm, 60-69.9 mm, 70-79.9 mm and ≥80 mm was statistically significant (p-value <0.05) [Table/Fig-7].

DISCUSSION

NT is one of the most important parameter used in first trimester to detect various chromosomal anomalies. Most of the studies have used the recommended definition for NT thickness by the Foetal

CRL mm	NT (mm) (Mean±SD)	Mean difference (mm)	95% CI		p-value
			Lower	Upper	
<49.99 (Base line)	0.667±0.080				
50-59.9	1.07±0.16	-0.404	-0.459	-0.350	<0.001
60-69.9	1.54±0.15	-0.879	-0.933	-0.825	<0.001
70-79.9	1.99±0.15	-1.327	-1.391	-1.262	<0.001
≥80	2.40±0.08	-1.740	-1.855	-1.626	<0.001

[Table/Fig-7]: Comparison of mean NT across the CRL (mm) (N=470). ANOVA test used. p-value <0.05 statistically significant, p-value <0.001 statistically highly significant

Medicine Foundation (i.e., 2.5-3 mm) [5-7], whereas recent study reported that using NT thickness as a continuous variable was more appropriate than using a single cut-off value for the foetal NT and consequently, the outcomes of its increased values and screening programs [8].

Allred SK et al., conducted a meta-analysis including 126 studies in the year 2017, according to which they found that there are small but statistically significant differences in NT measurement among women of different ethnicities. In a predominantly Caucasian population, it was found that correcting NT MoM (Multiple of the median) for racial differences may only have small impact on overall screening performance but significant impact on an individual woman's result. However, since NT is an influential marker in risk estimation compared with other screening markers, screening programs may be considered based on local requirements and the ethnic composition of their screening population. The results of the study by Allred SK et al., provided useful information for genetic counselling also [9]. Chen M et al., in the year 2002 conducted study on difference in NT values with 16,981 pregnancies according to ethnicity and concluded that the median NT MoM (95% CI) of the Filipinos was 1.07 mm (1.04-1.11). This was significantly higher than that of the Chinese, 1.01 mm (1.01- 1.02); other Asians (Indians, Pakistanis and Nepalese), 0.96 mm (0.94- 0.99), and Caucasians, 0.98 mm (0.93-1.06) (p<0.05), respectively; Mann-Whitney U-test). Even though the NT measurements had significant differences they were told to be clinically insignificant [10].

Thilaganathan B et al., in the year 1998, have investigated the possible role of ethnicity on NT and concluded that the differences reported could not have a significant impact in this regard in 1944 women [11]. Ethnic differences in NT measurements especially when it is used for screening of Down syndrome are not clinically significant according to few studies [10-12]. Using ethnic-specific reference values of NT thickness can help us in the first trimester screening programs when they are integrated with other ultrasonographic and biochemical measurements [9].

However, only one Indian study conducted by Kumar M et al., is available for the same to the best of our knowledge and the results of this study almost correlate with that previous study. The study established normal range of NT between CRL of 45 mm to 84mm in Indian population with 400 patients. It provided detailed assessment of NT at 5mm CRL intervals providing 5th, 50th and 95th centile and SD for each interval. The mean and median age of women in their study was 25.9 and 25 years, respectively; with more than half of women in their study were primipara [13]. Among the present study population, the mean and median of maternal age was 25.626±3.82 years and 25 years, respectively; more than half of the present study population was multipara. The mean CRL of their study was 63 mm (range: 40.1- 84.4 mm), corresponding to gestational age of 12 weeks and 5 days (range: 10 weeks 4 days to 15 weeks 3 days) in their study. The mean CRL of the present study was 59.84±10.17 mm (range: 44-85 mm) corresponding to gestational age of 12.35±1.02 weeks (range: 11-13.6). The average NT of this study by Kumar M et al., was 1.3±0.3 mm (range: 0.2-2.5 mm). The average NT of the present study was 1.3±0.26 mm (range: 0.6-2.5 mm). Both the previous study and the present study

conclude the previous reports that the foetal NT thickness appears to increase with gestational age. Hence, a fixed cut-off point should not be used for NT thickness. The 95th percentiles of NT for various study population in this study is given in [Table/Fig-8] [14-19].

Author, Year	Sample size	Study population	95 th centiles
Sun Q et al., 2012 [14]	1790	China	1.84-2.35 mm
Hasegawa J et al., 2013 [15]	970	Japan	2.1-3.2 mm
Chung JH et al., 2004 [16]	2577	Korea	2.14-2.3 mm
Sharifzadeh M et al., 1995 [17]	1614	Iranian	1.8-2.35 mm
Araujo Júnior E et al., 2014 [18]	1420	Brazil	1.57-2.10 mm
Kor-Anantakul O et al., 2011 [19]	6347	Thailand	1.00-2.90 mm
Present Study	470	South Indian	0.8-2.3 mm

[Table/Fig-8]: The 95th percentiles of NT for various study population [14-19].

Among this study population, the mean of maternal age was 25.626±3.82 years. However, on evaluation of correlation of NT with maternal age, it was found to be statistically insignificant ($p>0.05$). According to the study conducted by Sun Q et al., on 2012, it was found that NT thickness had no relationship with maternal age ($p>0.05$), similar to the present study [14].

Correlation between NT and CRL: Among the present study population, the Regression equation was: $NT = -1.652 + (0.050) \times CRL$ mm. There was strong positive linear correlation between CRL and NT and there was statistically significant relationship between two variables. (R^2 value=0.995, p -value <0.001). The lower limit and upper limit of NT according to the regression equation in the present study was 0.6 mm and 2.5 mm, respectively. According to the study conducted by Karki S et al., in 211 pregnant women on 2013, the regression equation which shows relation between median NT thickness and CRL was described as follows: $expected\ NT\ thickness = 0.013CRL + 0.725$, ($R^2 = 0.258$, $p < 0.001$) [20]. There was strong positive correlation between the two variables as in the present study. The lower limit and upper limit of NT according to the regression equation in this study was 1.24 mm and 1.83 mm, respectively. The lower limit values being slightly higher than this study and while upper limit slightly lower in comparison.

According to the study conducted by Hasegawa J et al., in 970 cases, the formula of median NT thickness = $0.0229\ CRL\ (mm) + 0.1714$ ($R^2 = 0.96$). This study also concludes the strong positive correlation between the two variables just like the present study. The lower limit and upper limit of NT according to the regression equation in this study was 1.51 mm and 2.56 mm, respectively [15]. The lower limit being higher while upper limit being almost same as compared to the present study.

According to the study conducted by Chung JH et al., on 2004, on 2577 fetuses, NT thickness increased with CRL. The regression equation relating median NT thickness to CRL: $expected\ NT\ thickness\ (mm) = 0.437 + 0.01969 \times CRL\ (mm)$ ($R^2 = 0.127$, $p < 0.001$). This study has included a CRL range of 40-92 mm and hence the lower limit and upper limit of this study was 1.22 mm and 2.25 mm respectively [16]. The lower limit being higher and the upper limit being slightly lower than the present study.

Even though every study is indicating the strong positive correlation between CRL and NT, the variations in upper and lower limit for each population is again emphasising the variations with ethnicity [Table/Fig-9] [13,14,16,17,19,20].

All the studies reviewed here emphasised the importance of using a reference range for NT instead of a single cut-off value. Secondly, by comparing each study we can clearly understand that NT is strongly related to CRL and that it has difference in reference ranges with ethnicity.

Author, Year	Sample size	Study population	Mean CRL (mm)	Mean NT (mm)
Kumar M et al., 2017 [13]	400	Indian	63	1.3±0.3
Sun Q et al., 2012 [14]	1790	Kunming, China	59.6±9.2	1.7±0.5
Karki S et al., 2013 [20]	211	Kathmandu, Nepal	63.67±13.48	1.55±0.35
Chung JH et al., 2004 [16]	2577	Korean	60.16±9.67	1.62±0.5
Sharifzadeh M et al., 1995 [17]	1614	Iranian	59.35±8.35	1.3±0.54
Kor-Anantakul O et al., 2011 [19]	6347	Thailand	64.93±10.75	1.3±0.74
Present Study	470	South Indian	59.84±10.17	1.3±0.26

[Table/Fig-9]: Mean CRL and NT for various study populations [13,14,16,17,19,20].

Limitation(s)

The 95th percentile of NT for CRL >80 mm was not calculated due to less number of cases with the CRL in that range. A huge sample size would have been able to solve the same.

CONCLUSION(S)

There was strong positive linear correlation between NT, CRL and Gestational age. There was no significant correlation for NT with maternal age. A separate reference range should be followed for each ethnic origin instead of "a single cut-off value" as established in this study which would enable correct evaluation of chromosomal abnormalities.

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