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ORIGINAL ARTICLE

The First Trimester Combined Screening Test in the Indian Population: Insights from a Cohort of 27,647 Pregnancies

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Abstract

Objective To analyse the distribution and determinants of the first trimester screening risk for the detection of trisomy 21 in the population in and around Chennai, a south Indian metropolitan city.

Methods A cross-sectional analysis of 27,647 singleton pregnancies that underwent the first trimester combined screening test (FTS) was carried out. For the screen positive cases, karyotype reports or postnatal phenotype outcome were available. The distribution of the various components of risk assessment in the screen positive cases and screen negative cases formed the main outcome measures.

Results Of the 27,647 cases, 4.6% (1270) of cases had unossified nasal bone; 1.8% (499/27,647) had risk more than 1:250 (screen positive). Fifteen (3.2%) of the screen positive cases had confirmed Down syndrome while 46 had termination of pregnancy and 8 had fetal loss.

Conclusions With the current screening protocol, the screen positive rate and the odds of being affected given a positive result (OAPR) for a threshold of 1:250 are 1.8% and 1 in 25 respectively.

Keywords Trisomy 21 · Down syndrome screening · India · First trimester combined screening test · Nuchal translucency · Prenatal screening

Introduction

According to the most recent sample registration system (SRS) survey figures, the birth rate of India stands at an average of 21 per 1000 population [1]. The annual burden of births with chromosomal abnormalities expected from this country with an estimated population [2] of 1.3 billion is enormous and has been variously estimated to be around 23,000–29,000 [3] to 37,000 [4], based on different assumed background prevalence rates. Currently there are no 'complete-ascertainment' postnatal surveys indicating the actual burden of Down syndrome and other chromosomal abnormalities at birth. The primary focus of the health care domain of the country has long been the mother rather than the fetus. Consequently, there are no state—sponsored Down's syndrome screening programs for antenatal women. Compared to the developed nations, the practice of antenatal screening for Down syndrome has 'caught up' rather late in India and is still largely confined to urban and semi-urban populations.

In Chennai and its sub-urban locations, antenatal screening for Down's syndrome using the first trimester combined screening test [FTS, incorporating maternal age, fetal nuchal translucency (NT), maternal serum free β-hCG and pregnancy associated plasma protein-A (PAPP-A)] was offered from 2005 by Mediscan Systems—a tertiary level fetal medicine center that caters to the states of Tamilnadu and Pondicherry, and to the neighboring districts from the states of Andhra Pradesh and Karnataka. At present, the availability FTS in the nation is widespread

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with the increase in the number of NT operators and laboratories offering biochemical markers assay. Studies on the performance of NT and biochemical markers across different ethnicities have not consistently ruled out the effect of ethnicity on the performance of the FTS. Also the studies have been conducted in a multi-ethnic population and the proportion of Indian fetuses is rather small. The performance of first trimester screening (FTS) program in the native Indian population has not been critically evaluated so far. Therefore, the authors designed this cross-sectional study to understand the determinants and the distribution of the FTS risk in the detection of Down syndrome in Indian population.

Material and Methods

This was a retrospective cross-sectional analysis spanning 42 months from January 2013 through June 2016. All women with singleton pregnancies who underwent the combined screening test were included in the analysis. A broad search using query terms "first trimester" and "singleton" was carried out from the ultrasound electronic database, Sonocare™ (Medialogic Solutions Private Ltd, Chennai, India). In the final data set, only those fetuses with a pre-determined minimum mandatory data were included.

From 2005, first trimester screening has been offered by a combination of maternal age, fetal nuchal translucency (NT), maternal serum free beta-hCG, and PAPP-A. The nasal bone was routinely included in the risk assessment from the year 2013; while reporting nasal bone status, the term 'unossified nasal bone' was used instead of absent/hypoplastic nasal bone as the latter term was perceived by the clinicians and parents as a 'malformation'. All NT images were reviewed and approved by FMF—certified operators. In addition, an internal audit was performed regularly using the 'NT—audit' module of the Sonocare™ software that would permit visualisation of the trend and spread of each operator's measurement. The protocol followed for the first trimester screening test in singletons was—firstly, documentation of maternal medical history, review of relevant medical records, a pretest counseling explaining the screening rather than diagnostic nature of the test, and finally a systematic evaluation of the fetus and placenta using the 'rule of 2' protocol (provided as supplementary information) developed in authors' institute. For women who would be 38 years or more at the time of their estimated delivery date [considered as advanced maternal age (AMA) in the screening program], the option of direct invasive testing was also offered. Similarly, women were given the options of direct invasive testing or combined screening test when the NT was greater than or equal to 3.5 mm ("Increased NT"). In those women where the fetus showed a major malformation, an individualized counseling including the option of direct testing for karyotyping was provided. For all other pregnancies, a venous blood sample was collected for free beta-hCG and PAPP-A if the crown-rump length fell between 45 and 84 mm. From January 2015, the blood sampling switched over to dried blood spot (DBS) method. The laboratory had suitably adjusted the Multiples of Median (MoM) derivation from DBS such that the final cut-off threshold remained unchanged. All biochemical testings and risk calculations were done by PerkinElmer laboratory, Chennai, using the auto-Delfia equipment and LifeCycle™ software. Prior to January 2015, venous blood samples were analysed using Delfia Xpress equipment. The laboratory uses the MoM for NT, free beta-hCG, and PAPP-A based on the crown-rump length (CRL) for the calculation of the risks. For maternal age-based risk assessment, the actual age of the donor at the time of egg collection was used in cases of oocyte donor in-vitro fertilization (IVF) pregnancy. All age-based risks were based on age at term and were calculated by the laboratory as part of the risk calculation.

The laboratory reports 'risk at term' and a cut-off of 1:250 at term is set as screen positive. For all results that reported a risk of more than 1:250 (screen positive results), a post-test counseling letter explaining the meaning of the test result and the available diagnostic options was provided to the referring clinician and the patient. A formal post-test counseling was given to couples who sought the same. The need for confirmation of the aneuploidy through direct invasive testing was stressed upon. A chorionic villus sampling (CVS) or amniocentesis was performed as per the gestational age at which the willing couples returned to the authors. For all procedures, genetic counseling was provided before the procedure and after the availability of the karyotype results.

From 2015, the non-invasive prenatal test (NIS, PerkinElmer) using massively parallel shotgun sequencing technology was made available through the same laboratory to couples on a case to case basis, such as for screen positive women; women in whom the final combined test risk was greater than the a priori risk (*e.g.*, a final risk of 1 in 300 for a 30-year-old whose age based risk is 1 in 800); and other special circumstances such as parents' request *etc*. For all screen positive pregnancies who declined further testing, the pregnancy outcome was obtained from the patients themselves or their treating physician through telephonic conversation. Ascertainment was by proxy—*i.e.*, only those who reported outcome after the baby had been seen by a pediatrician during the vaccine visits were included.

The statistical analysis was done using SPSS version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for

Windows, Version 20.0. Armonk, NY: IBM Corp.). The distribution of the population parameters was analysed visually by histogram and scatter diagram; means and proportions were used appropriately. The odds of being affected given a positive result (OAPR) was calculated as the ratio of affected pregnancies (Down syndrome) to unaffected pregnancies (normal babies, with known KT or phenotype).

Results

The authors identified 31,350 singleton pregnancies that were sonographically examined at 11–14 wk from January 2013 through June 2016. In 891 cases, there was a structural defect and these were excluded; in 27,647 cases, the minimum mandatory data was complete. The baseline characteristics of the population are presented in Table 1. In 326 pregnancies (about 1% of the cases), the NT was at 99th centile or more for the period of gestation and in about 1270 cases (4.6%), the NB was unossified. The distribution of CRL and NT are presented in Figs. 1 and 2. Additionally, fifty-seven women underwent direct invasive testing without undergoing combined screening test: 36 for increased NT and 21 for advanced maternal age (AMA, age more than 38 years at term). Among these 57 cases, there were six cases of Down syndrome (5 out of 36 increased NT and 1 out of 21 AMA procedures).

The distribution of the final adjusted risk (posterior risk) for all those who underwent the combined screening is presented in Table 2. In 499 cases (1.8%), the final risk was 1:250 or greater; and in these cases the karyotype of the fetus, or the clinical assessment of the phenotype of the child was available. Some of these cases had opted for termination of pregnancy, had miscarriage or were still ongoing pregnancies at the time of writing this paper. There were 15 cases of Down's syndrome within this screen positive group. Of these, 10 were identified antenatally; 5 were detected postnatally as the parents declined invasive testing. The OAPR (Odds of being affected given a positive result) in this cohort is 1 in 25. The clinical/karyotype outcome of these screen positive cases is provided in Table 3. Table 4 compares the risk-component distribution amongst the screen positive cases between fetuses with Down syndrome and with normal karyotype. For the purpose of comparison, NT more than 1.5 MoM, free β-hCG more than 2 MoM and PAPP-A less than 0.5 MoM were taken as abnormal values.

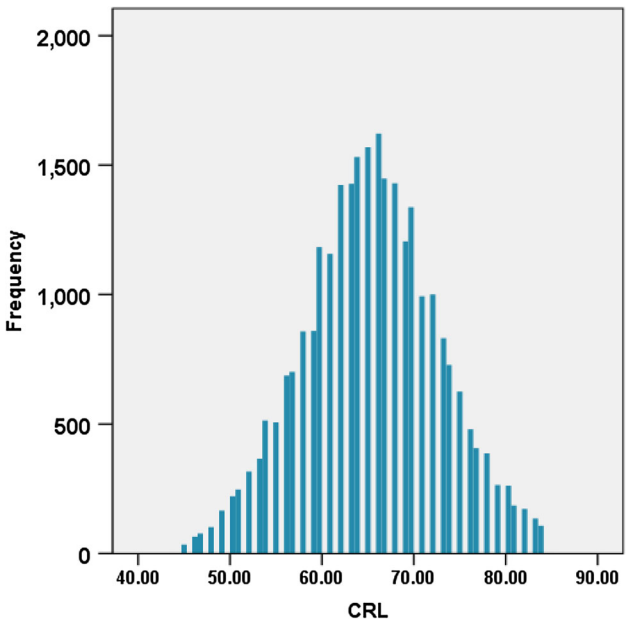

Fig. 1 Frequency distribution of the crown-rump length (CRL) of the fetuses

Table 1 Baseline characteristics of 27,647 singleton pregnancies

	Number
Total pregnancies studied	27,647
Conception, assisted n (%)	2533 (9)
Age, mean (SD), years	28.27 (4.4)
Maternal BMI, mean (SD)	26.2 (2.9)
Median gestational age at scan, completed week, (range)	12 (11–14)
Crown-rump length at scan, mean (SD, range) in millimeters	65.4 (7.4, 44–85)
Nuchal translucency (NT), mean (SD) in millimeters	1.7 (0.6)
NT multiples of median, median (IQR)	1.05 (0.92–1.2)
NT > 99th centile, n (%)	326 (1.02)
NB status, n (%)	
Unossified	1270 (4.6)
Indeterminate	354 (1.3)

BMI Body mass index, *NB* Nasal bone

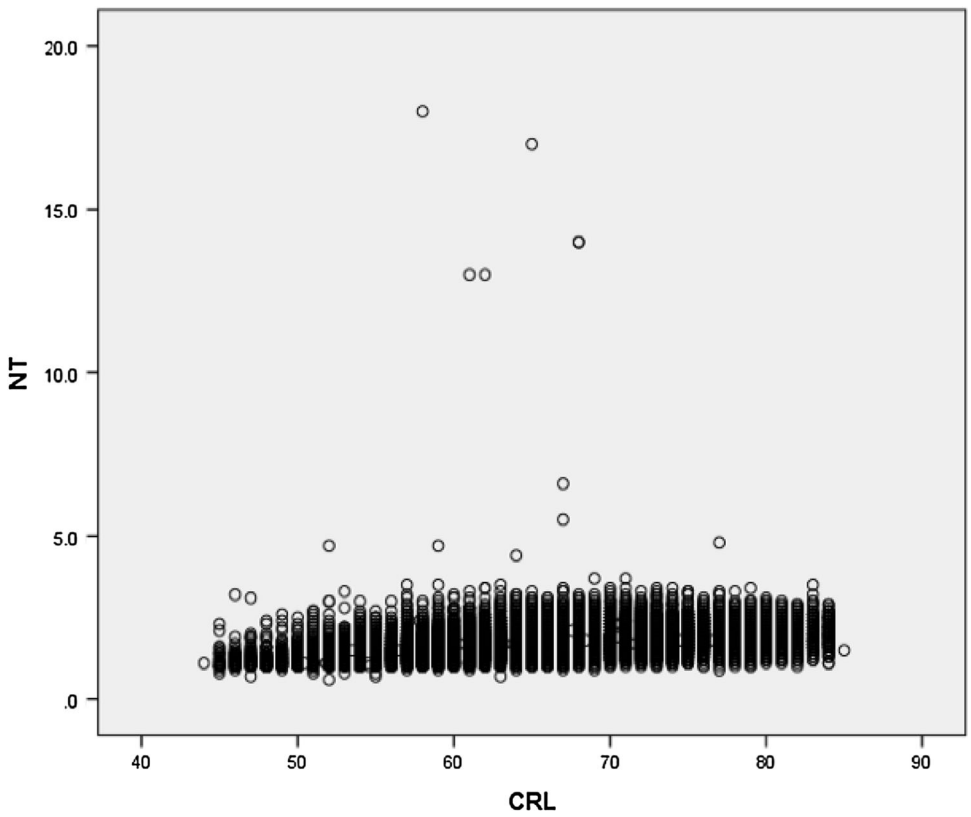

Fig. 2 The distribution of NT against the CRL in the study cohort

Table 2 Distribution of the final adjusted risk in the population

Screen status	Risk stratum	Frequency, n (%)	Cumulative percent	Down syndrome (n)
Screen positive				
	Greater than 1 in 50	152 (0.5)	0.5	7
	1 in 51–100	93 (0.3)	0.9	4
	1 in 101–250	254 (0.9)	1.8	4
Screen negative				
	1 in 251–500	353 (1.3)	3.1	Data not available
	1 in 501–1000	545 (2.0)	5.1	
	Less than 1 in 1000	26,250 (94.9)	100.0	
	Total	27,647		

Table 3 Clinical/karyotype outcome of screen positive cases

Outcome	Frequency	Percent (rounded off)
Normal KT/normal phenotype child	391	78.3
Trisomy 21	15	3.0
Fetus, other aneuploidy	2	0.4
Miscarriage/fetal loss	8	1.6
Termination of pregnancy	46	9.2
Ongoing pregnancy	14	2.8
Lost to follow-up	23	4.6
Total	499	100
OAPR	15:391 or 1 in 25	

KT Karyotype, *OAPR* Odds of being affected given a positive result

Table 4 Distribution of risk and risk determinants between Down syndrome and normal babies in screen positive pregnancies (final risk of 1:250 or more)

Risk/Risk determinant	Normal babies (n = 391)	Down syndrome (n = 15)
Maternal age-based risk, mean	1 in 783	1 in 708
Final adjusted risk (based on USG markers and serum analytes), mean	1 in 113	1 in 70
Cases with normal NT and NB		
Only HCG > 2 MoM	3	0
Only PAPP-A < 0.5 MoM	4	0
Both HCG > 2 MoM and PAPP-A < 0.5 MoM	6	0
Cases with normal HCG and PAPP-A		
Only NT > 1.5 MoM	24	1
Only unossified NB	25	0
Both NT > 1.5 MoM and UNB	131	5
Cases with any one USG markers abnormal AND any one serum analyte abnormality	198	9
Total	391	15

HCG Human chorionic gonadotropin, *MoM* Multiples of median, *NB* Nasal bone, *NT* Nuchal translucency, *PAPP-A* Pregnancy associated plasma protein-A, *UNB* Unossified nasal bone, *USG* Ultrasonography

Discussion

In this analysis of 27,647 singleton pregnancies that underwent a first trimester screening at a single tertiary level fetal medicine center in Chennai, India, authors have presented the various characteristics of the population. The mean maternal age of the examined women is 28 years. The NT distribution with the fetal CRL was also along the expected lines from other studies that have reported such a distribution from Asian fetuses [5, 6]. A detailed analysis of the distribution of the NT among the fetuses is being written up as a separate paper. Previous studies have not consistently indicated whether NT distribution among normal fetuses is dependent on the ethnic origin of the fetus [5, 6]. However, there may be subtle differences between the distribution of the serum analytes between mothers of different ethnic groups [7]. The break-up of abnormal risk components (Table 4) reiterates the fact that detection rate is highest when all the factors are used. Isolated abnormalities of biochemical or ultrasound parameters are not sensitive markers.

The rate of unossified nasal bone (absent nasal bone) in the study population was 4.6%. To authors' knowledge, this is the largest data set from Indian fetuses reporting the proportion of unossified nasal bone in the first trimester. Studies done in multi-ethnic population have pointed to a slightly higher rate of unossified nasal bone and this overestimation may due to the smaller numbers of Indians in those studies [8].

The screen positive rate with a threshold of 1: 250 was about 1.8%. This proportion is less than the oft-quoted 4–5% in papers from Europe [9]. This difference may be attributable to, firstly, the screening protocol that was employed, secondly to a difference in the distribution of the risk—determinants in the study population and finally, a possible difference in the prevalence of Down syndrome. In screening for fetal chromosomal abnormalities, the authors offer the combined test only if there are no major fetal abnormalities. The anatomical survey protocol at authors' institution in the first trimester is quite exhaustive and during the same period 891 singleton fetuses with defects were detected and in these pregnancies the combined screening was not offered. Spencer et al. have suggested that the serum analytes' distribution among Asians may be different from that of Caucasians and may lead to a marginally lower screen positive rate [7]. Venous blood sampling may lead to higher free-βhCG values, especially during summer and this may elevate the false positive rate [10]. This is a particularly significant problem in countries like India where transportation logistics may not be optimum leading to exposure of the samples to a higher temperature. The DBS method has been claimed to circumvent this problem [11, 12], at least partially. Although the absolute recovery of PAPP-A and free-βhCG is different between DBS and venous samples, the two methods have been shown to have a consistent correlation. This allows for appropriate adjustments in deriving the MoM values such that the risk cut-off need not be altered. In the estimation of the Down syndrome burden in a population, it is universally assumed that the maternal age related risk is the same across different ethnicities. However, until there are hard data on the actual prevalence of Down syndrome in India, the possibility of a different pattern of prevalence cannot be totally ruled out, however unlikely this may appear with the current scientific knowledge.

In assessing the performance of a prenatal screening test for conditions such as Down syndrome, the odds of being

affected given a positive result (OAPR) is the key measure for parents to decide whether to undergo the diagnostic invasive testing. The OAPR in the screening strategies discussed above is 1 in 25 for the combined screening test protocol. The reported OAPR for the combined test for a fixed 85% detection rate in the multicentric UK study (SURUSS [13]) is 1 in 22.

Previous studies on Down syndrome prevalence from India have concentrated on cases presenting to Genetic Clinics or Genetic laboratories. These may not reflect the true prevalence at birth or during pregnancy on a population scale. Among screen positive women, about 9% underwent termination of pregnancy (ToP) without further confirmatory testing. This is an area of concern and clearly represents misconceptions about the nature of the screening results, both among the referring clinicians and the patients. Partly, the confusion is due to the general concept that a high NT indicates the possibility of a multitude of problems for the fetus, some of which may not be apparent prenatally. Clinicians often use 'availability heuristic' to judge the outcome of a screen positive pregnancy and may equate screen positivity to 'high NT'. Awareness among Indian population is also far from ideal with only about 59% of patients being aware of Down syndrome, in a prospective study among 745 women [14]. In authors' institute, all patients who are referred for a first trimester screening are provided with an information leaflet that explains the principles behind screening and the difference between the screening test and the diagnostic invasive test. In those cases, that test screen positive, a detailed Q&A style counseling letter explaining the meaning of the test result is sent to the patient and the referring physician along with the report.

Non availability of the clinical/karyotypic outcome of the entire cohort is an important limitation of the study. The Fetal Medicine Foundation [15] suggests that the FTS performs in such a way that about 84% of all Down syndrome babies would be segregated into the >1 in 50 group, about 15% segregated in the group with risk between 1 in 51 and 1000, and only about 1% in the group with risk of less than 1 in 1000. The SURUSS study estimated an 85% detection rate for a screen positive rate of 4.2% for the first trimester screening test at a screen positive cut-off of 1 in 250. Assuming the screening test performs similar to published results, given that the distribution of the components is comparable, one can assume that 90% of the expected Down syndrome fetuses is captured within the screen positive group (n = 21 Down syndrome fetuses). With the allowance of 1 or 2 cases among the terminations/miscarriages/lost to follow-ups, this would mean a total burden of about 23 or 24 Down syndrome in this cohort which is much less than that expected on the basis of the maternal age distribution (about 54, correcting for the in-utero lethality rate of 30%) [16]. Another limitation that is not directly related to the study is the lack of authentic birth prevalence data for Down syndrome in the population. Therefore, the findings of this study provide a strong ground for initiating population based estimation of the birth prevalence of Down syndrome in the country. This would require substantial funding and collaboration between major research groups across the nation.

Compliance with Ethical Standards

Conflict of Interest SS is the clinical director and SMJ is a laboratory consultant at PerkinElmer Laboratory, Chennai.

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