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ORIGINAL ARTICLE

Down's Syndrome Screening in the First Trimester with Additional Serum Markers: Indian Parameters

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Abstract

Objective To derive a risk calculation algorithm suitable for use in India when screening for Down's syndrome using four first-trimester maternal serum markers either alone or with ultrasound nuchal translucency (NT).

Methods Stored maternal serum samples (− 20 °C) from 411 singleton unaffected pregnancies were retrieved and measured for pregnancy-associated plasma protein (PAPP-A), free β-human chorionic gonadotropin (hCG), placental growth factor and α-fetoprotein. Samples were taken at

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10–13 weeks' gestation. Equations were derived to express marker levels in multiples of the gestation-specific normal median, adjusted for maternal weight. Gaussian model parameters were derived and compared with six published non-Indian studies; NT parameters were derived from 27,647 women screened in India. On the basis of the maternal age distribution in 64,473 Indian women screened in 2016–2017, the model was used to predict test performance.

Results The model predicted a detection rate for a serum-only protocol of 80% for a 5% false-positive rate. Using a 1 in 250 at term Down's syndrome risk cut-off, the predicted detection rate was 78% and the false-positive rate was 4.1%. When NT was also included, the rates were 95% for 5% and 90% for 1.4%, respectively.

Conclusion First-trimester screening using four serum markers only can be carried out in India. Performance is expected to be similar to the second-trimester Quad test and will also facilitate early screening for preeclampsia and open spina bifida. A protocol of NT plus the four serum markers enhances the performance compared with NT, PAPP-A and free β-hCG.

Keywords First trimester · Screening · Down's syndrome · Prenatal diagnosis · PlGF · AFP

Introduction

The most widely used protocol for Down's syndrome screening in the first trimester is the 'Combined' test, comprising maternal serum pregnancy-associated plasma protein (PAPP)-A and free β-human chorionic gonadotropin (hCG) together with ultrasound nuchal translucency (NT). One multivariate Gaussian statistical model predicts that at 11 weeks' gestation it will yield a detection rate of 87% for a false-positive rate of 5%, with slightly lower performance at 12 and 13 weeks' gestation [1]. By comparison, the model predicted a detection rate for the second-trimester serum-only 'Quad' test of just 71%.

However, the performance of the Combined test is critically dependent on the availability of quality NT which accounts for 30% of the 87% detection rate. The model predicted a detection rate for maternal serum PAPP-A and free β-hCG alone of 57%. Three studies have considered the possibility of improving this by including two additional markers, placental growth factor (PlGF) and α-fetoprotein (AFP) [2–4]. They derived, for the four-marker serum-only test, a model that predicted detection rate of 66–82% suggesting a performance comparable with the second-trimester Quad test.

Screening for Down's syndrome in the first trimester has substantial advantages over second-trimester screening even when there is no increase in detection. It provides earlier reassurance, and if an affected pregnancy is diagnosed, termination can be carried out with greater safety, less psychological trauma and more discretion. Moreover, the four-marker serum-only test can be used in first-trimester screening for preeclampsia as well as spina bifida. Maternal serum PAPP-A and PlGF can detect about two-thirds of preeclampsia cases destined to present before 37 weeks' gestation [5]. Maternal serum AFP and free β-hCG together with ultrasound biparietal diameter (BPD) measurement can detect about two-thirds of open spina bifida [6].

Substantial numbers of pregnant women in India do not have access to quality NT. They would benefit from a first-trimester four-marker serum-only test, but there are no published Down's syndrome risk calculation algorithms suitable for this country. The minority for whom quality NT is available would also benefit from the additional serum markers. We have therefore carried out a study in India to derive the information needed to calculate risk in both circumstances.

Methods

Stored maternal serum samples from singleton unaffected pregnancies at 10–13 weeks' gestation were retrieved from storage and measured for the four markers. Equations were derived to express marker levels in multiples of the gestation-specific normal median (MoM), adjusted for maternal weight. Gestational age was determined on the basis of first-trimester foetal biometry. Gaussian model parameters, standard deviations and correlation coefficients were derived and compared with the literature. Parameters for Down's syndrome pregnancies were derived by meta-analysis from the literature. On the basis of the maternal age distribution in India, the model was used to predict the test performance.

Women participating in the Combined test screening programmes at three centres, Mediscan Systems (Chennai), Rainbow Hospitals (Hyderabad) and Fetal Care Centre (Kolkata), were recruited to have blood samples taken for the project. Five millilitres of sample was collected into a plain tube and refrigerated locally before being sent to the laboratory of Perkin Elmer Health Sciences (Chennai), a facility of PerkinElmer Inc., where they were centrifuged, aliquoted and frozen at − 20 °C. Samples from Mediscan Systems arrived within 1–2 h, whilst others took 1–1.5 days, and probes in the transport boxes indicated a temperature of 20–25 °C. Recruitment continued sequentially until 50 samples had been collected at each half week of gestation between 10 and 13 completed weeks of gestation, based on ultrasound.

When a sufficient number of samples had been collected, they were retrieved from storage and tested for PAPP-A, free β-hCG, PlGF and AFP in Perkin Elmer Health Sciences using Delfia Xpress™ assays.

For each serum marker, normal gestation-specific median curves were derived from the median concentration in each half-week group against median gestation in days weighted for the number in the group. The best-fitting curves were used to express results in MoMs. Maternal weight correction curves were derived by dividing the samples into eight weight groups and carrying out regression of median MoM on median weight, weighted by the numbers in each group. There were too few smokers (six) and diabetics (two) to adjust for these potential covariables.

The standard deviations of $\log_{10}$ MoM were estimated from the 90th–10th centile range divided by 2.563; the *r*-values were estimated directly, after excluding outliers exceeding three standard deviations from the median.

Multivariate log Gaussian modelling was used to predict test performance [1]. Numerical integration was used whereby the theoretical range is divided into a number of equal sections, thus forming a 'grid' in multidimensional space. The Gaussian distributions are then used for calculating each section: the proportion of Down's syndrome and unaffected pregnancies in the section and the likelihood ratio. These values are then applied to the maternal age distribution to derive a distribution of Down's syndrome risk values. At each maternal age, the number of Down's syndrome and unaffected pregnancies was estimated from an age-specific risk curve [1]. The results were summed over the theoretical range to compute detection rates for various fixed false-positive rates.

The serum marker distribution parameters for unaffected pregnancies were derived from the analysed samples. The corresponding values and the mean for Down's syndrome pregnancies were derived from a meta-analysis of the three studies of the four-marker serum-only protocol together with three studies that investigated combinations of the four markers and NT, ductus venosus or foetal heart rate [7] or inhibin-A [8, 9]. The average of each parameter was calculated weighted for the number of cases.

Performance was also predicted for an 'Enhanced' Combined test whereby maternal serum PlGF and AFP are added to the Combined test. Assuming that the four biochemical markers are independent of NT, risk is calculated by applying a likelihood ratio (LR) from the NT MoM to the risk from the four serum marker MoMs and maternal age. A log Gaussian model was used for LR based on a published Down's syndrome mean of 2.10 MoM at 12 weeks' gestation [1] with standard deviation tailored to the standard deviation of $\log_{10}$ MoM in 27,647 results from Mediscan Systems.

The maternal age distribution observed in 64,473 women maternal serum screening samples was tested at the Perkin Elmer Health Sciences laboratory between July 2016 and February 2017.

Results

A total of 415 samples were measured, of which four were excluded because the pregnancy was subsequently found to be twins. Table 1 shows the curves used to calculate MoMs and, where appropriate, for calculating weight correction. The normal median data were best-fitted by log-linear equations except for PAPP-A which required a quadratic equation. There was no statistically significant effect of maternal weight on PlGF; PAPP-A data were best-fitted by an inverse, free β-hCG by a log-linear and AFP by a quadratic equation.

The parameters derived from the samples are shown in Table 2 and compared with parameters from the six published studies which have also assessed the four-marker test alone or in other combinations. There was no material difference in the standard deviations compared with the other studies. There were statistically significant correlations between PAPP-A and both free β-hCG and PlGF which were also statistically significant in the other studies. There was a statistically significant correlation between free β-hCG and PlGF, but this was not found consistently in the other studies. Smaller non-significant correlations were found between the other markers, and this was inconsistent across the studies. There were differences in magnitude of the correlations for all combinations. However, this is to be expected as the confidence intervals on *r*-values are generally wide.

Table 1 Median and weight correction equations

	Type*	*A*	*B*	*C*
Median				
PAPP-A	Quadratic	40,158.1	− 1091.22	7.60518
Free β-hCG	Log-linear	3.05575	− 0.01571	–
PlGF	Log-linear	0.81799	0.01025	–
AFP	Log-linear	− 0.45832	0.01827	–
Weight correction				
PAPP-A	Inverse	− 0.35250	84.1341	–
Free β-hCG	Log-linear	0.41761	− 0.00703	–
PlGF	None			
AFP	Quadratic	4.49232	− 0.09793	0.000663

Log-linear = 10^{A+Bx}; quadratic = $A + Bx + Cx^2$; inverse = $A + B/x$; x = days or kg

Table 2 Unaffected serum parameters compared with six published studies [2–4, 9–11]

Parameter	Current study	Donalson et al. [2]	Johnson et al. [3]	Wright et al. [7]	Huang et al. [4]	Palomaki et al. [8]	Carmichael et al. [9]
SD (log_{10} MoM)							
PAPP-A	0.248	NK	0.262	0.235*	0.240	0.238	0.251
Free β-hCG	0.263	NK	0.252	0.256*	0.269	0.268	0.242
PlGF	0.147	0.147	0.168	0.171	0.167	0.144	0.171
AFP	0.207	0.183	0.195	0.188	0.172	0.178	0.187
R-value							
PAPP-A & free β-hCG	$0.177^{\square}$	0.216	0.043	NK	0.274	0.258	0.191
PAPP-A & PlGF	$0.312^{!}$	0.120	0.302	0.325	0.264	0.267	0.251
PAPP-A & AFP	0.030	− 0.087	− 0.076	− 0.031	− 0.100	− 0.118	0.088
Free β-hCG & PlGF	0.131^{+}	0.070	0.085	0.130	0.086	0.209	0.090
Free β-hCG & AFP	0.039	− 0.010	− 0.021	0.007	− 0.051	− 0.071	− 0.024
PlGF & AFP	− 0.028	− 0.087	− 0.076	− 0.102	− 0.100	− 0.035	− 0.045

*Calculated from the log inter-quartile range divided by 1.35

Current study only: $^{+}P<0.01$; $^{\square}P < 0.0005$; $^{!}P < 0.0001$

The six published studies included a total of 603 Down's syndrome cases although one study did not report the means and standard deviation for PAPP-A and free β-hCG and the correlation between them [2] and one study only tested a subset for PlGF and AFP and also did not report the correlation between PAPP-A and free β-hCG [7]. Table 3 shows the Down's syndrome parameters and the number of cases used to derive each of them. The log_{10} standard deviation of NT MoM was 0.0878, and the tailored value for Down's syndrome pregnancies was 0.2230.

Table 3 Down's syndrome serum parameters: weighted average from six studies [2–4, 9–11]

Parameter	Cases	Value
Mean (MoM)		
PAPP-A	603	0.503
Free β-hCG	603	2.029
PlGF	530	0.655
AFP	457	0.776
SD (log_{10} MoM)		
PAPP-A	603	0.278
Free β-hCG	603	0.258
PlGF	530	0.162
AFP	457	0.173
R-value		
PAPP-A & free β-hCG	300	0.123
PAPP-A & PlGF	530	0.164
PAPP-A & AFP	457	0.059
Free β-hCG & PlGF	530	− 0.005
Free β-hCG & AFP	457	− 0.087
PlGF & AFP	457	− 0.044

Figure 1 shows the maternal age distribution in single years of age. The median age was 28, and 10% of women were age of 35 or more.

Table 4 shows for the four-marker serum-only test the model that predicted test detection rates for three fixed false-positive rates (3%, 5% and 7%) as well as the false-positive rates for three fixed detection rates (65%, 75% and 85%). Using a fixed 1 in 250 term Down's syndrome risk cut-off, the predicted detection rate was 78% and the false-positive rate was 4.1%.

The Enhanced Combined test has a model that predicted the detection rate of 95% for a 5% false-positive rate. Using a fixed 1 in 250 term risk cut-off, the predicted detection and false-positive rates were 90% and 1.4%, respectively. By comparison, a standard Combined test has a predicted 91% detection rate for 5% false-positive rate, and using the 1 in 250 risk cut-off, the rates are 85% and 1.8%, respectively.

Discussion

Our study provides all the information required for an algorithm that can be used to interpret a first-trimester four-marker serum-only protocol or an Enhanced Combined test in India. Using such an algorithm, modelling predicts that the screening performance of the serum-only test is comparable with the second-trimester Quad test. And the Enhanced Combined test has a superior performance to a standard Combined test. The same cut-off risk of 1 in 250

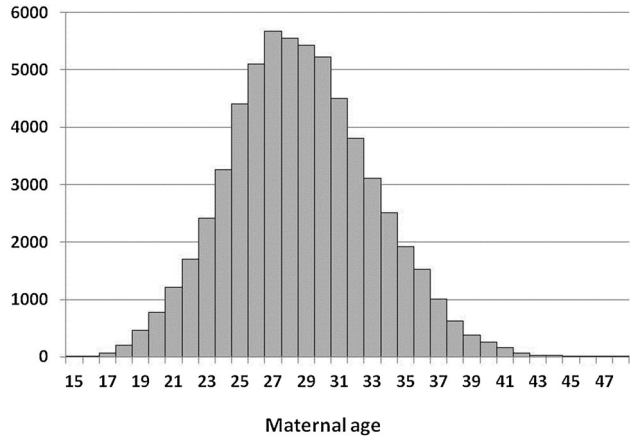


Fig. 1 Distribution of maternal ages

Table 4 Model that predicted the performance: four-marker serum-only test

	Detection rate (%)	False-positive rate
Fixed false-positive rate	74	3.0
	80	5.0
	84	7.0
Fixed detection rate	65	1.5
	75	3.2
	85	7.4

at term used by other screening protocols in India could be maintained for the new tests.

The current restricted availability in India of quality ultrasound NT could be overcome, to a great extent, by the introduction of a first-trimester four-marker serum-only test. For centres already carrying out a second-trimester Quad test, this could be readily achieved. The cost of implementing a first-trimester four-marker serum-only test will be no different to that of a second-trimester Quad test and yields the benefits of early diagnosis and reassurance, as well as facilitating early screening for preeclampsia and open spina bifida. Protocols involving the ultrasound measurement on NT are much more expensive; in a Canadian study, the unit cost of an NT scan was 4.4-fold greater than a four-marker serum test [3]. For those centres already carrying out a standard Combined test, the measurement of PlGF and AFP on the same automatic equipment used for PAPP-A and free β-hCG is unlikely to considerably increase costs.

For the serum-only test, the model predicted a Down's syndrome detection rate of 78% for a fixed 4.1% false-positive rate. In England, Donalson et al. [2] carried out a study based on stored serum samples from 92 Down's syndrome cases and 522 unaffected matched controls; the predicted detection rate was 71, 69 and 66% at 11, 12 and 13 weeks' gestation for a 5% false-positive rate. Two case–control studies were carried out in Canada. Johnson et al. [3] tested 90 cases and 1607 controls predicting a detection rate of 74%, whilst Huang et al. [4] tested 137 cases and 684 controls predicting a detection rate of 82%.

Unlike the predicted second-trimester Quad test detection rate of 71% for a 5% false-positive cited above [1], the estimates for the first-trimester serum-only test in the three case–control studies and the current analysis are somewhat inflated by 'viability' bias since cases were identified from prospective screening. The bias arises because of the high intrauterine fatality rate for Down's syndrome so that a proportion of affected pregnancies which were detected and terminated would not have been viable. Nevertheless, the magnitude of the bias is likely to be smaller enough to conclude that performance is at least as good as the Quad test.

For all Down's syndrome screening protocols, both the detection and false-positive rates are determined by the cut-off risk. In a given protocol, the detection rate can be increased but only at the expense of an increased false-positive rate. In general, when two protocols are being compared, it is best to either fix the false-positive rate and compare detection rates or fix the detection rate and compare false-positive rates. Fixing the cut-off risk will, in general, mean that neither detection nor false-positive rates will remain the same. Nevertheless, in the current analysis using a fixed 1 in 250 term cut-off risk will result in predicted performance for the serum-only protocol similar to either fixing detection or false-positive rates to that expected for the second-trimester Quad test.

All women included in our study had first-trimester ultrasound biometry, and MoMs were calculated from gestational ages calculated on the basis of these measurements. Therefore, to achieve in practice the performance predicted here for the serum only test it will be necessary to have reasonably accurate gestational dating. In localities with insufficient resources to perform an early crown-rump length measurement on *all* women, high performance can still be achieved if only those with uncertain menstrual dates are scanned. Alternatively, only women with positive test results could have ultrasound dating and risk revision.

For the Enhanced Combined test, a model predicted a Down's syndrome detection rate of 90% for a 1.4% false-positive rate, which was much better performance than the Combined test where the rates were 85% and 1.8%, respectively. A specialist centre that also routinely determines an additional ultrasound marker, say nasal bone, would also benefit. Modelling shows that the rates for the Enhanced Combined test would be 95% and 0.8% compared with 92% and 1.1% for the Combined test. In addition to these advantages in performance, the additional

markers provide: a safety net for occasional atypical results for one or more markers; preeclampsia screening; and the detection of some spina bifida cases in the first trimester.

For localities with insufficient ultrasound resources for routine Enhanced Combined testing, a 'contingent' protocol might be considered. This would involve routine four-marker serum-only testing; however, the next step for those with positive or borderline results would not be invasive prenatal diagnosis but ultrasound marker determination and risk modification.

In conclusion, first-trimester screening using four serum markers only can be carried out in India. Performance is expected to be similar to the second-trimester Quad test and will also facilitate early screening for preeclampsia and open spina bifida. A protocol of NT plus the four serum markers enhances the performance compared with a standard Combined test.

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Compliance with Ethical Standards

Conflict of interest Howard Cuckle is a paid consultant of PerkinElmer Inc. All other authors declare that they have no conflict of interest.

Ethical Approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent Informed consent was obtained from all patients for being included in the study.

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