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Original Research Article

Correlation of maternal age and combined assessments on risk of chromosomal anomaly during prenatal screening

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ABSTRACT

Background: Prenatal detection of genetic abnormalities is one of the biggest challenges of current fetal medicine. Prenatal screening for chromosomal abnormalities can be done using biochemical tests. The screening is a risk estimation test and not a diagnostic test.
Methods: Statistical data treatment had been performed on a sample of 362 pregnant women for prenatal screening. This was a retrospective data analysis study undertaken at the National Reference Laboratory, Redcliffe Labs.
Results: Nine (2.48%) women out of 362 were screen positive for chromosomopathy. The point biserial correlation between variables (Free β-hCG - Free Beta Human Chorionic Gonadotrophin, PAPP-A- pregnancy associated plasma protein-A and NT-(nuchal translucency) amongst patients with positive and negative screen test was statistically significant. There was a positive correlation between positive screen for chromosomopathy and hCG, MoM, NT MoM whereas a negative correlation between them and PAPP-A. This study indicates that higher values of hCG and lower values of PAPP-A MoM as seen in the positive screen patients is associated with a significant risk of chromosomopathy. A positive correlation between age and screen positive cases was seen. The McNemar's test indicated a significant reduction in screen positive cases when biomarkers were added to screen for Trisomy 21 in women aged >35 years (n=86). 81 women eventually screened negative.
Conclusions: The analyses stresses on the importance of using state-of-the-art, prenatal noninvasive screening software to help provide a predictive outcome, individualized for that pregnant woman.

Keywords: Free β-hCG, PAPP-A, Age risk, Trisomy 21

INTRODUCTION

Prenatal care is an integral part of maternal, foetal, and neonatal health concept. High maternal and infant mortality at the beginning of the 20th century encouraged the formation of institutions for the provision of prenatal care, according to historical conclusions with the pyramid of old prenatal care.[1] 7.9 million births globally each year have significant birth defects, and 94% of these births take place in middle- and low-income countries, according to the March of Dimes (MOD) world report on birth defects.[2]

Birth abnormalities account for 7% of all neonatal mortality and 3.3 million under-five deaths, according to a joint World Health Organization (WHO) and MOD conference report.[2] Congenital abnormalities are one of the top 10 causes of newborn deaths in India. The prevalence of birth abnormalities in India ranges from 61 to 69.9 per 1000 live births.[3] According to mortality data from India, 16% of all birth defect fatalities among children were under the age of five in 2017. India accounts for 21% of birth defect-related early neonatal mortality cases globally.[4]

Early detection of congenital anomalies during pregnancy can prevent them. Regardless of maternal age or the likelihood of chromosomal abnormality, all pregnant patients should be informed about and given the option of prenatal screening (serum screening with or without NT ultrasound) as well as diagnostic testing (chorionic villus sampling [CVS] or amniocentesis) to determine whether they have a high or low risk of developing an aneuploid foetus with the aid of trisomy screening.[5]

In the modern world, first trimester non-invasive procedures are replacing invasive approaches. In many nations, prenatal trisomy screening based on the evaluation of biochemical markers in maternal serum has become a standard component of obstetric practice. Maternal serum Free β-hCG and PAPP-A have been proven to be useful amongst the various biochemical markers that have been studied. The purpose of first trimester maternal serum screening programmes is to identify women who will benefit from the testing and those who are at higher risk of having a baby with Down syndrome (DS), Patau syndrome, or Edward syndrome and other chromosomopathies.[6,7]

Screening helps to reduce invasive diagnostic procedures that further help to reduce the number of procedure related losses of normal fetus. If the screening risk is higher than the cut-off, then further testing is recommended for the patient. Obtaining a high detection rate (percentage of affected individuals) with a low false-positive rate is a challenge for screening tests. The objective is to provide screening tests with high detection rates (percentage of individuals affected) and low false-positive rates (proportion of unaffected individuals with a screen positive test).

First-trimester biochemical screening has a number of advantages over second-trimester biochemical screening, including the significant benefit of an earlier diagnosis for patients and clinicians, higher detection rates for foetal disorders (DS), such as 90%, or even higher, compared to 80% for the second-trimester quadruple test, and 70% for older triple screening test, and detection of the majority of major chromosome abnormalities other than T21 (DS).[8-10]

The screening is not a diagnostic test but a risk estimate test. A reported risk should be correlated and adjusted to the absence/presence of sonographic markers observed in the anomaly/malformation scan. An increased risk result does not necessarily indicate that the foetus is affected, and a low-risk result does not necessarily rule out a fetal malformation.

One of the most significant etiological variables linked to any human genetic illness is the association between advancing maternal age and trisomy, which has been known for more than 50 years. With regards to specifics, the probability of trisomy in a clinically confirmed pregnancy increases from roughly 2-3% for women in their twenties to an incredible 30% or more for those in their forties. Consequently, chromosomal segregation mistakes are the primary barrier to a successful pregnancy as women near the end of their reproductive years.[11]

The likelihood of chromosomal aberrations is increased when age is factored into the equation, either separately or in conjunction with the findings of serum tests. The maternal serum variables are also influenced by gestational age, maternal weight, ethnicity, smoking, in vitro fertilization, parity and diabetes, the background risk for each being calculated and then included in the algorithm with NT and maternal age.[12,13]

However, in contrast, several other studies show that despite maternal age being a major determinant for the risk of DS, nevertheless, frequency of infants with DS who were born to women aged >35 years is not very high that is, they were not aligned with age risk.[14-16] Studies to correlate between the dual markers test with risk of chromosomal defects are scarce in India. Factors like biochemical marker values, USG details and individual maternal details may in some cases negate the influence of age risk alone. To evaluate these objectives a retrospective study was undertaken to evaluate laboratory data from 362 pregnant women

METHODS

This research design, a retrospective data analysis, was undertaken at the National Reference Laboratory, Redcliffe Labs, Noida. A cohort of women (n=362) participated in the present clinical study. Ssdw 6.3 is a software aimed for prenatal detection of trisomies and other aneuploidies, preeclampsia and was used for data analysis.

Maternal details like date of birth, weight, height, LMP, pregnancy type (natural conception or assisted), number of fetuses, history of previous congenital anomaly, correction factors (race, smoking and diabetic status) were mentioned during sample collection. The most recent ultrasonography report having details like (NT and presence/absence of nasal bone were mentioned. More specific details like abnormal ductal flow, tricuspid regurgitation, echogenic cardiac foci, nuchal fold thickness, ventriculomegaly, echogenic bowel, pielectasis, short femur, absent-hippo nasal bone single choroid plexus cyst and single umbilical artery, where available in USG details were also used for risk calculation in the ssdw 6.3 software.

It is a web browser-based application which is configurable with user defined biochemical and ultrasound markers, correction factors, units of measurement, cut-off levels, truncation limits, and population parameters. Fetal NT together with maternal serum free β-hCG and PAPP-A were measured at 11 weeks to 13±6 weeks of gestation. This was performed by ECLIA (Electrochemi-luminescence immunoassay on Roche automated analyzer (Cobas 8000).

The influence of these variables was examined using the data with multivariate linear regression correlation

analysis performed to ascertain the effects of hCG MoM, and PAPP-A MoM on the likelihood of women having positive screen for chromosomal defects. In order to calculate the risk, the biochemical indicators were converted into multiples of median (MoM) with factors including gestational age, maternal weight, multiple gestations, assisted reproductive techniques, ultrasound, smoking history from the past, and T21.

Statistical risk was calculated using a computerized program with ssdw6.3 software. Statistical risk factor calculation for Trisomy 21 (Down's syndrome), Trisomy 18 (Edward syndrome) and Trisomy 13 (Patau syndrome) had been done using fetal medicine foundation (FMF) approved assays using Roche Cobas Analyser. It calculates the risk for trisomy 21, 18 and 13. Screen cut offs used were T21-1:250 [free beta hCG ≥1.98, PAPP-A≤0.43] and for trisomy 18/13-1:100 [free beta hCG≤0.5, PAPP-A≤0.4] based on ACOG 2007 guidelines.

The first objective of the present study was to ascertain the values of hCG MoM, and PAPP-A MoM on the likelihood that participants having positive screen for chromosomal defects. The second objective was to analyze high risk cases based on age with screen positive cases.

Statistical analysis

Point biserial correlation and logistic regression analysis was performed to analyze the association of hCG MoM, and PAPP-A MoM in positive screen women for risk of chromosomal defects. Linear-by-Linear association test and computed McNemar's test were used to analyses high risk women based on age with screen positive cases. A p-value of ≤0.05 indicates statistical significance.

RESULTS

Characteristics of the sample population

The study included a sample of 362 pregnant women. The mean age of the study population was 30.59±5.83. From the sample of 362 woman screened by biochemical marker tests, 353 (97.51%) were screen negative and 9 (2.48%) women were screen positive. (7 were positive for trisomy 21, 2 for trisomy 12/18). This was based on MOM Cut off values (ACOG 2007).

The mean Crown-rump length (CRL) was 61.98±9.08, with NT values as 1.40±0.44. The free beta hCG (human chorionic gonadotropin) expressed as MoM values was 1.33±0.95. The PAPP-A (MoM) values are 1.33±0.87. The median with 25th, 50th and 75th percentiles are as given in Table 1.

Comparison of variables between patients with positive and negative screen test for chromosomopathy

A point biserial correlation was computed to assess the linear relationship between Screen results for chromosomopathy with CRL, hCG MoM, PAPP-A MoM, and NT MoM.

Further comparison of variables between patients with positive and negative screen test for chromosomopathy was done with point biserial correlation. Except for the age and Crown-rump length (CRL) all the other parameters of hCG, PAPP-A, NT were statistically significant.

Point biserial correlation with the hCG MoM, PAPP-A MoM and NT MoM was 0.167, -0.172 and 0.357 respectively which was statistically significant.

There was a positive correlation between positive screen for chromosomopathy and hCG MoM (p=0.001). Whereas there was negative correlation between positive screen for chromosomopathy and PAPP-A MoM (p=0.001) (Table 2).

Further comparison of variables between patients with positive and negative screen test for chromosomopathy was done. The mean beta HCG MOM was comparatively higher in positive screen than negative screen patients and difference was significant.

Similarly, the mean NT MOM value was statistically higher in positive screen than negative screen patients. We found that the PAPP-A MoM values were significantly lower in positive screen than negative screen patients

A logistic regression was performed to ascertain the effects of hCG MoM, PAPP-A MoM, NT MoM on the likelihood that participants have positive screen for chromosomal defects. The Logistic regression analysis models used Cox and Snell R square and Nagelkerke R square. The logistic regression model was statistically significant, $\chi^2(3)=70.8$, $p<0.001$. The model explained 85% (Nagelkerke R^2) of the variance in chromosomal defects and correctly classified 99.4% of cases. Increasing hCG MoM and NT mm was associated with an increased likelihood of exhibiting chromosomal defects but decreasing PAPP-A MoM was associated with an increased likelihood of exhibiting chromosomal defects. (Table 3 and Figure 1).

Correlation between the high-risk pregnant women (based on age) with screen positive cases

The number of screen positive were 5 from the age group of 35-39 years, 3 from women less than <35 years, and 1 from age group of >40 years. This study shows a correlation of age and screen positive cases by Linear-by-Linear association test estimated p value as statistically significant value of 0.032. Maximum screen negative women 238 (98.8%) were from the age group of the <35 years.

McNemar test was done to establish correlation of positive and negative risk of chromosomopathy based on age risk and screen test results. Age above of 35 with probability 1/250 was considered as high risk. Out of a total of 86

cases (23.84%) considered high risk by age alone, only 5 (1.4%) were tested screen positive. Out of 276 women considered low risk by age, 4 (1.1%) was screen positive. McNemar test showed that the two proportions between age risk and scan results were different, p≤0.001 (2 sided), (Table 4 and Figure 2).

Table 1: Characteristics of the sample population.

Parameters	Mean	SD	Median	25 percentiles	50 percentiles	75 percentiles
Age (Years)	30.59	5.83	29.50	26.00	29.50	36.00
Crown-rump length (CRL) (mm)	61.98	9.08	62.00	56.00	62.00	68.00
NT mm	1.40	0.44	1.31	1.10	1.31	1.60
free beta hCG (human chorionic gonadotropin) (IU/L)	48.46	39.43	34.82	24.15	34.82	58.30
Pregnancy-related protein like A (PAPP-A) (IU/L)	4884.73	3918.45	3820.0	2153.75	3820.0	6312.5
NT MoM	0.89	0.27	0.86	0.72	0.86	1.03
hCG MoM	1.33	0.95	1.06	0.68	1.06	1.74
PAPP-A MoM	1.33	0.87	1.18	0.67	1.18	1.70

Table 2: Comparison of variables between patients with positive and negative screen test for chromosomopathy.

Parameters	Negative screen, (n=353)		Positive screen, (n=9)		F value	P value
	Mean	SD	Mean	SD		
Crown-rump length (CRL) (mm)	61.94	9.03	63.73	11.40	0.34	0.559
NT (mm)	1.38	0.39	2.34	0.93	48.24	<0.001
free beta hCG (human chorionic gonadotropin) (IU/L)	47.21	38.11	97.23	59.18	14.66	<0.001
Pregnancy-related protein like A (PAPP-A) (IU/L)	4977.03	3922.70	1264.89	830.07	8.03	0.005
hCG MoM	1.31	0.93	2.32	1.05	10.28	0.001
PAPP-A MoM	1.35	0.87	0.39	0.13	10.99	0.001
NT MoM	0.88	0.24	1.50	0.61	52.73	<0.001

Table 3: Logistic regression analysis for prediction of positive screen test for chromosomal defects.

Variables	B (Coefficient for constant)	S.E.	Wald (chi Square test)	Df (Degree of freedom)	Sig.	Exp(B) (Exponentiation of B coefficient)	95% C. I. for EXP (B)	
							Lower	Upper
hCG MoM	3.323	1.47	5.078	1	0.024	27.748	1.541	499.501
PAPP-A MoM	-16.589	7.41	5.003	1	0.025	<0.001	0.001	0.128
NT (mm)	9.926	4.42	5.031	1	0.025	20448.9	3.499	119493595.8
Constant	-11.709	5.67	4.264	1	0.039	<0.001		

Table 4: McNemar Test of positive and negative risk of chromosomopathy between age risk and screen test results.

Parameters			Screen result		Total	(McNemar test) p value
			Negative	Positive		
Age risk	High risk	Count	81	5	86	<0.001
		% of total	22.4	1.4	23.8	
	Low risk	Count	272	4	276	
		% of total	75.1	1.1	76.2	
Total		Count	353	9	362	
		% of total	97.5	2.5	100	

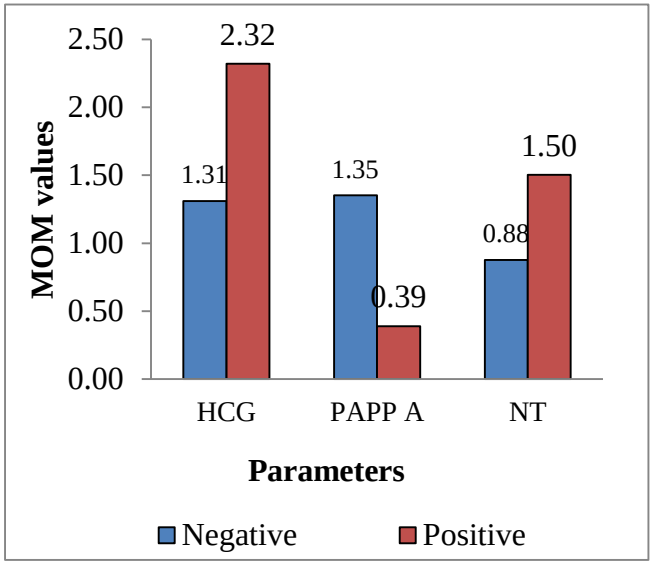

Figure 1: Comparison of MOM value in positive and negative screen individuals.

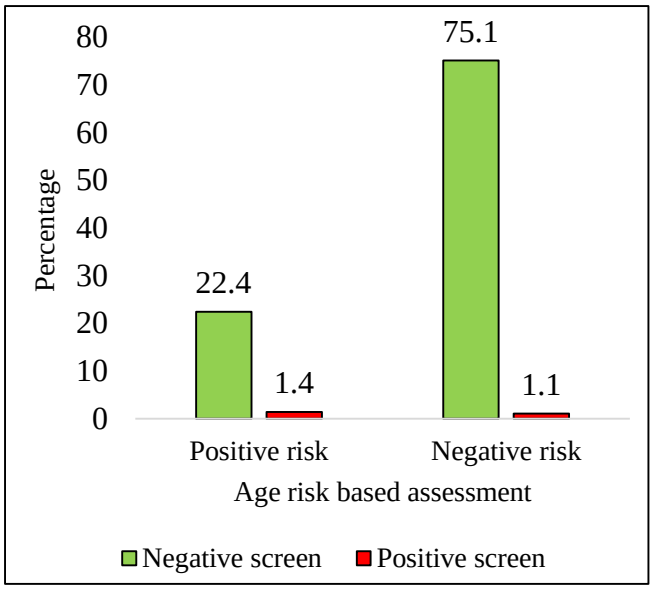

Figure 2: Comparison of positive and negative risk of chromosomopathy between age risk and screen test results.

DISCUSSION

Prenatal detection of genetic abnormalities is one of the great challenges of current fetal medicine. Prenatal screening for chromosomal abnormalities can be done by biochemical screening test like dual marker test, triple marker test and quadruple marker test. The screening is a risk estimation test and not a diagnostic test. An increased risk result does not mean that the fetus is affected, and a low risk does not mean that the fetus is unaffected, reported risk must be correlated and adjusted to the absence/presence of sonographic markers observed in the anomaly/malformation scan. But they do guide to further investigations for accurate detection.

An extensive examination of biochemical indicators, clinical variables, and ultrasound markers is part of a first trimester risk assessment report. This study is an attempt to strengthen the evidence for the above. Retrospective data analysis of test findings for the dual markers along with NT was done at a diagnostic laboratory to understand the relationship between them and increased risk.

The risk for age and biochemical screening of PAPP-A and Free β-hCG is detected at a rate of 70%, but the risk for maternal age and foetal NT is detected at a rate of 75-80%. Trisomy 21 is more likely to be detected in 85-95% of cases when age-related risk markers NT, PAPP-A, and Free β-hCG are combined.[12-14]

In this study very few i.e., 9 (2.43%) women tested screen positive from a total of 362 woman screened for biochemical markers (7 were positive for trisomy 21, 2 for trisomy 12/18). The mean age of study population was 30.59±5.83. The highest number of screen positive results was 5 from the age group of 35-39 years. There seems to be a statistically significant correlation between age and screen positivity.

The point biserial correlation performed between the various variables with positive and negative screen test for chromosomopathy showed that except for Crown-rump length (CRL) all the other parameters like Free β-hCG, PAPP-A and NT were statistically significant. There was a positive correlation between positive screen for chromosomopathy and Free β-hCG MoM values. There was a negative correlation between positive screen for chromosomopathy and PAPP-A MoM values. This study indicates that higher values of Free β-hCG and lower values of PAPP-A MoM as seen in the positive screen patients is associated with a significant risk of chromosomopathy.

This is well documented from various studies in literature that decreased levels of PAPP-A before the 14th week of gestation is associated with an increased risk for T 21and T18 and increased levels of Free β-hCG are associated with an increased risk of Down syndrome.[12,13,17]

The logistic regression model to ascertain the effects of Free β-hCG MoM, and PAPP-A MoM on the likelihood that participants having positive screen for chromosomal defects showed statistical significance. This is in similar lines to the above finding by point biserial correlation as in the both the biochemical parameters and NT.

The findings highlight the significance of employing a prenatal screening tool that allows for the correlation of PAPP-A, Free β-hCG to calculate the final risk of Down syndrome and other chromosomopathy.

The computed McNemar's test value indicated a significant reduction in Screen positive cases when biomarkers were added to screen for T21 in women aged >35 years. A total number of women aged >35 years were screened (n=86) as high risk for T21 on the basis of age

alone. On addition of biochemical parameters, USG details and other maternal details, and risk assessment by ssdw6.3 software, 81 (22.4%) screened negative for T21 This is correlates with some studies showing similar results. This negates the belief that advanced maternal age is the only predisposing factor for the risk of Down Syndrome in a fetus.[14-16,18]

Limitations

Since this was a retrospective study with screening dual marker tests, it was not possible to prospectively follow up the screen positive cases to analyze their outcome at birth.

CONCLUSION

The findings of this study, which characterized pregnant women as screen positive or negative, on the basis of Free β-hCG and PAPP-A MoM levels, correlated well with those published in the literature, and underscored the significance of adopting dual marker risk assessment as a practical prenatal screening method. The ssdw6.3 software's exact approach of dual maker analysis and interpretation eliminates the need for human changes.

Advanced maternal age is a known a risk factor for T21. However, when biochemical measures were added, a statistically significant majority of this age group screened negative. This substantiates that biochemical markers, USG details, and advanced software-based risk assessment reduce the overall risk to a significant degree negating the influence of age risk alone.

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