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Original Research Article

Correlation between maternal serum biochemical markers with karyotyping for prenatal screening of foetal chromosomal abnormalities

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ABSTRACT

Background: Prenatal screening for chromosomal abnormalities can be done by biochemical screening tests like dual marker test (DMT), triple marker test (TMT) and quadruple marker test (QMT). It is important to identify ideal screening test among them which best correlates with result of karyotyping which is confirmatory test of foetal chromosomal abnormalities. This helps to decrease need for invasive prenatal tests for foetal karyotyping. This study aims to evaluate sensitivity, specificity, diagnostic accuracy and correlation of DMT, TMT, and QMT with results of karyotyping.

Methods: Retrospective observational study was conducted in tertiary care maternity hospital over one year- 1st January 2015 to 31st December 2015. Women with singleton pregnancy undergoing DMT, TMT or QMT were included.

Results: Of the 529 women screened by biochemical marker tests, 462 (87.33%) were screen negative and 67 (12.66%) women were screen positive. In 56 women, it was false positive (83.58%) and in 11 women true positive (16.41%). In 461 women the test results were true negative (99.78%), but in one case, result was false negative (0.21%). 3/11 (27.27%)women with foetal chromosomal abnormalities were primigravidae. 4/11 (36.36%)women were below 35 years. DMT and QMT had higher sensitivity (both 100%) and specificity (90.00% and 93.18% respectively) than TMT (sensitivity 80% and specificity 82.61%). Positive likelihood ratio (LR+) was 1.00 in DMT. Diagnostic odds ratio was highest with DMT (DOR=115.11) and best correlated with karyotyping results (coefficient of correlation 0.4).

Conclusions: Universal screening of antenatal women, irrespective of their age and parity is suggested. DMT has highest diagnostic value and best correlation with the results of karyotyping. Hence the dual marker test can be considered to be better test for screening for aneuploidy.

Keywords: Chromosomal abnormalities, Correlation, Diagnostic accuracy, Dual marker test, Quadruple marker test, Sensitivity, Specificity, Triple marker test

INTRODUCTION

Chromosomal abnormalities give rise to pregnancy complications, many of which cause variable mental subnormalities and /or congenital birth defects in the foetus. The recognition of the foetus with chromosomal aberrations prompted a search for risk factors and markers which could be detected in-utero in early gestation. The practice of screening for aneuploidy commenced in 1960s when increased maternal age emerged as a risk factor for having foetus with congenital abnormalities.[1] Further research shed light on many biochemical markers which could be used for prenatal screening by indicating risk for chromosomal

abnormalities. In order to increase the sensitivity and specificity of these tests, multiple biochemical blood tests were clubbed and as a result dual marker test (DMT), triple marker test (TMT) and quadruple marker test (QMT) came in routine practice. The confirmation of chromosomal abnormalities is by karyotyping of the foetal cells from chorionic tissue or amniocytes cultured from amniotic fluid. Karyotyping mostly required invasive procedures like amniocentesis, chorionic villous biopsy to obtain foetal cells. The screening tests were helpful as they decreased the need for invasive prenatal testing.[2-5] At the same time, it was essential to identify an ideal screening test which best represented the confirmative test result.

To ascertain this, a retrospective data analysis was undertaken in a tertiary care hospital to evaluate and compare test results of all three: dual, triple and quadruple markers tests.

Dual marker test (DMT)

This is a first trimester screening test and can be performed between 10-14 weeks of gestation.[6] The markers for this test are pregnancy associated plasma protein-A (PAPP-A) and free beta human chorionic gonadotropin (hCG)[7]. The biochemical markers were converted into multiples of median (MoM). Statistical risk is calculated using a computerized program with PRISCA 5 software. It calculates the risk for trisomy 21, 18 and 13. Screen cut off were-for trisomy 21 - 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].

Triple marker test (TMT)

This is a second trimester test conducted between 15-20 weeks of pregnancy.[8,9] The biochemical markers used are alpha feto protein (AFP), (total) beta hCG and unconjugated serum estriol (UE3).[10] It calculates the risk for trisomy 21, trisomy 18 and open neural tube defects. The measured concentrations of these markers are converted in MoM.[11] Using MoM, statistical risk factor is calculated using the PRISCA 5 software. The cut off values are: for trisomy 21-1:250 [AFP ≤0.74, hCG≥2.06, uE3≤0.75] for trisomy 18-1:100 [AFP≤0.65, hCG≥0.36, uE3≤0.4]

Quadruple marker test (QMT)

This is also a second trimester biochemical screen test in 15 to 20 weeks of gestation.[9] The biochemical markers used are alpha foeto protein (AFP), (total) beta hCG and unconjugated serum estriol (UE3) and inhibin A. It calculates the risk for trisomy 21, trisomy 18 and open neural tube defects. The measured concentrations of these markers are converted in MoM. Using MoM, statistical risk factor is calculated using the PRISCA 5 software. The cut off values are: for trisomy 21-1:250 [AFP ≤0.74, hCG≥2.06, uE3≤0.75 + I-A≥1.77] for trisomy 18-1:100 [AFP≤0.65, hCG≥0.36, uE3</0.4].

Karyotyping

This is performed on cells from chorion by chorionic villous sampling, an invasive procedure, is done under ultrasound guidance at 11 to 14 weeks of gestation. Karyotyping can also be done on foetal skin cells from amniocytes obtained by culture of amniotic fluid. The procedure of amniocentesis is also an invasive procedure done after 16 weeks under ultrasound guidance. Foetal prenatal karyotyping are the confirmatory tests for diagnosing foetal chromosomal abnormalities.

Objectives of present study were:

- Correlation between the dual, triple and quadruple marker test with prenatal karyotyping tests.
- To study the diagnostic accuracy, sensitivity and specificity of DMT, TMT, and QMT for diagnosing fetal chromosomal abnormalities.
- To study the demographics and pregnancy outcome in women having fetus with chromosomal abnormalities.

METHODS

It is retrospective observational study from 1st January 2015-31st December 2015 in a tertiary care Maternity Hospital.

Inclusion criteria

- Antenatal women with singleton pregnancy
- Gestational age between 11-20weeks.

Exclusion criteria

- Multifoetal gestation
- Women who register after 20weeks of gestation.
- Women who refuse biochemical screening test.

During the study period of 1st January 2015-31st December 2015, 529 women were screened for biochemical marker tests by DMT or TMT or QMT, according to the gestational age and affordability when they presented in antenatal department.

Of these 529 women, 462 women (87.33%) were screen negative indicating low risk for chromosomal abnormalities. 67 (12.66%) women were screen positive indicating increased risk. These 67 women underwent amniocentesis or chorionic villous biopsy after informed consent for karyotyping for confirmation. It is this cohort of women whose outcome was studied in detail. The results of the biochemical tests were compared with the results of karyotyping which is the confirmatory test and the data was statistically analysed.

Statistical analysis

Clinical characteristics were summarized in terms of frequencies and percentages for categorical variables and as minimum, maximum, range, mean±SD for continuous variables. We reported the observed diagnostic sensitivity, specificity and accuracy value which were calculated for outcome using standard formulas. The positive and negative predictive values for each outcome were derived from the cumulative event rates for each outcome at 95% confidence interval. Also, False Positive Rate, False Negative Rate, Likelihood Ratio for Positive and Likelihood Ratio for Negative were calculated based on outcome. Correlations were checked by using Phi correlation coefficient.

RESULTS

Of 529 women screened by biochemical marker tests, 462 (87.33%) were screen negative and 67 (12.66%) women were screen positive. In 56 women it was false positive (83.58%) and in 11 women true positive (16.41%). 461 women the test results were true negative (99.78%), but in one case, result was false negative (0.21%). The 67 women who were high risk for foetal aneuploidy on biochemical tests underwent invasive prenatal testing for karyotyping for confirmation.

Eleven women had foetal chromosomal abnormalities confirmed on prenatal foetal karyotyping. These were: 8 cases of foetal trisomy 21, 1 case of foetal trisomy of sex chromosome,1case of foetal karyotyping showing 46*inv (*) and one foetal karyotyping showed 46**inv (9) (p11q13). (* indicates unrevealed sex chromosomes. In the country in which study has been conducted, routine prenatal disclosure of foetal sex is not legal). Rest had normal foetal karyotyping. In the screen negative women (462), there was one case of neonatal Down's syndrome (trisomy 21) which was detected postnatal by karyotyping.

Data analysis was done for individual biochemical blood tests. The results of these are summarized in the following tabular forms.

Table 1: Data analysis of dual marker test result (DMT).

DMT	True results		Total
	+	-	
+	6	27	33
-	0	243	243
Total	6	270	276

Table 1 summarizes the data about DMT which was done on 276 women of which 243 women were screen negative. Twenty seven women were screen positive of which women 6 women had abnormal foetal karyotyping. So, true positive were 6/33 women (18.18%), true negative were 243/243 women (100%), false positive were 27/33 women (81.81%), false negative were 0/243 women (0%)

Table 2: Data analysis of triple marker test (TMT).

TMT	True results		Total
	+	-	
+	4	20	24
-	1	95	96
Total	5	115	120

Table 2 summarizes the data regarding triple marker test which was performed on 120 women of which 96 women were screen negative. Hence true positive were 4/24 women (16.66%).

True negative were95/96 women (98.95%). But out of 96 screen negative women, one case was false negative (1.04%) diagnosed by neonatal karyotyping which showed trisomy 21. Twenty four women were screen positive, out of which, 4 women had abnormal foetal karyotyping. The false positive were 20/24 women (83.33%).

Table 3: Data analysis of quadruple marker test (QMT).

QMT	True results		Total
	+	-	
+	1	9	10
-	0	123	123
Total	1	132	133

Table 3 summarizes that the quadruple marker test was performed on 133 women of which 123 women were screen negative. Ten women were screen positive of which only one woman had abnormal karyotype. The true positive were 1/10 women (10%), true negative were 123/123 women (100%), false positive were 9/10 women (90%), false negative were 0/123 women (0%).

Table 4: Data analysis of all tests combined. (DMT, TMT, QMT).

Combined all tests	True results		Total
	+	-	
+	11	56	67
-	1	461	462
Total	12	517	529

Table 4 shows that biochemical screen tests were positive in 67 women of which in 56 women it was false positive and 11 women had abnormal karyotyping. This it was true positive in 11 women.

Four hundred and sixty two women were screened negative, however, in 461 women the test results were true negative, but in one case the results were false negative and this was detected by neonatal karyotyping.

Hence overall true positive were 11/67(16.41%), true negative were 461/462 women (99.78%), false positive were 56/67 women (83.58%) and false negative were 1/462 women (0.21%).

Table 5: Diagnosis test result.

Test	Sensitivity (%)	Specificity (%)	PPV (%) (95% C.I.)	NPV (%) (95% C.I.)	Accuracy (%)	Correlation
DMT	100.00	90.00	18.18 (8.61-34.39)	100.00 (98.44-99.99)	90.22	0.40*
TMT	80.00	82.61	16.67 (6.68-35.85)	98.96 (94.33-99.82)	82.50	0.31*
QMT	100.00	93.18	10.00 (1.79 40.42)	100.00 (96.97-100.00)	93.23	0.30*

(PPV: Positive predictive value, NPV: Negative predictive value. For interpreting the strength of associations range in only for 2 X 2 tables: <0.10 = weak, 0.11 – 0.30 = moderate, >0.31 = strong correlation. The range of correlation is between -1 to +1. *Positive Correlation is significant)

Table 5 shows that the correlation between DMT Test and TMT test with outcome have strong positive correlation, and correlation between QMT Test and outcome has moderate positive correlation. Also, test accuracy with DMT and QMT is high (90.22% and 93.23% respectively).

Table 6: Diagnostic test result.

Test	FPR (%)	FNR (%)	LR+ (%)	LR- (%)	Prevalence (%)	DOR
DMT	10.00	0.00	10.00	0.00	2.17	115.11
TMT	17.39	20.00	4.60	0.24	4.17	19.20
QMT	6.82	0.00	14.67	0.00	0.75	39.00

(FPR = False Positive Rate, FNR = False Negative Rate, LR+ =Likelihood Ratio for Positive, LR- = Likelihood Ratio for Negative, DOR = Diagnosis Odd Ratio, False positive rate= 1-specificity, False negative rate= 1-sensitivity)

Table 6 shows the false positive and false negative rates. Both DMT and QMT had nil false negative rates and 10% and 6.82% false positive rates respectively. Positive likelihood ratio (LR+) is the indicator for ruling-in diagnosis. The diagnostic tests have LR+ >10 and their positive result has a significant contribution to the diagnosis[12] (in our case for QMT is 14.67).

For calculation of Diagnosis odd ratio we added 0.5 in the all 2x2 table values to make non-zero, where we have no false negative positive value as in DMT and QMT.[13,14]

(The diagnostic odds ratio is undefined when the number of false negatives or false positives is zero – if both false negatives and false positives are zero, then the test is perfect, but if only one is, this ratio does not give a usable measure.[13,14]

In such cases, we add 0.5 to all cells in the contingency table, although this should not be seen as a correction as it introduces a bias to results.[13,14]

It is suggested that the adjustment is made to all contingency tables, even if there are no cells with zero entries).[13,14] The diagnostic odds ratio is highest for DMT which is 115.11 followed by QMT which is 39.00.

Table 7 suggests that in our study, diagnostic accuracy range of maternal serum prenatal biochemical tests is excellent as the area under the curve is 0.904.[15]

Table 7: Area under the curve.

Test variable: Screening test and outcome.			
Area	Significance value at 5%	95% confidence interval	
		Lower boundary	Upper boundary
0.904	0.000	0.812	0.996

Area diagnostic accuracy Range: 0.9 – 1.0 excellent, 0.8 - 0.9 very good, 0.7 - 0.8 good, 0.6 - 0.7 sufficient, 0.5 - 0.6 bad, <0.5 test not useful.

Table 8 summarizes the demographics of women with foetal chromosomal abnormalities. It shows that 4 out of 11 women (36.36%) were of age below 35 years. 3/11 (27.27%) women were primigravidae.

7 women had their nuchal translucency scan done at 11-13.6 weeks of gestation, however, only 3 out of 7 women had nuchal translucency ≥3.0 mm and 2 out of 7 women had absent nasal bone which is an important soft tissue marker for aneuploidy. The women with foetal karyotype 46**inv (9) (p11q13) was investigated further and the

maternal karyotyping revealed 46XXinv (9) (p11q13). Hence the pregnancy was allowed to carry till term and the women delivered a male child with no obvious anomalies at birth. Rest of the women which were confirmed with abnormal foetal karyotype underwent MTP (medical termination of pregnancy).

Table 8. Summary of demographics and outcome in women with foetal chromosomal abnormalities.

Age	o/h	DMT, TMT, QMT	High risk	Risk factor	NT (mm)	NB	Anomaly scan	Karyotyping	Out-come
34	G2P1L1	TMT	T21	1:125	2.0	Ns	Echogenic focus	T21	MTP
38	G2A1	DMT	T21/ 18/13	1:5, 1:40, 1:21	5.5	Ns	-	T21	MTP
38	G1	TMT	T21	1:56	-	-	-	T21	MTP
38	G3P1L1 NND1	DMT	T21/ 18	1:20, 1:55	3.0	S	-	T21	MTP
26	G2P1L1	DMT	T21	1:63	2.2	S	-	T21	MTP
42	G4P1L1A2	QMT	T21	1:50	-	-	-	T21	MTP
31	G3P2L2	TMT	T21	1:108	-	-	N	T21	MTP
27	G1	TMT	T21	1:56	-	-	N	T21	MTP
36	G3P2L1D1	DMT	T21	1:199	0.6	S	N	46**inv(9) (p11q13)	LSCS
28	G1	DMT	T21/ 18/13	1:50, 1:50	3.8	S	-	Trisomy of sex chromosome	MTP
36	G2P1L1	DMT	T21	1:150	1.0	S	-	46*inv(*)	MTP

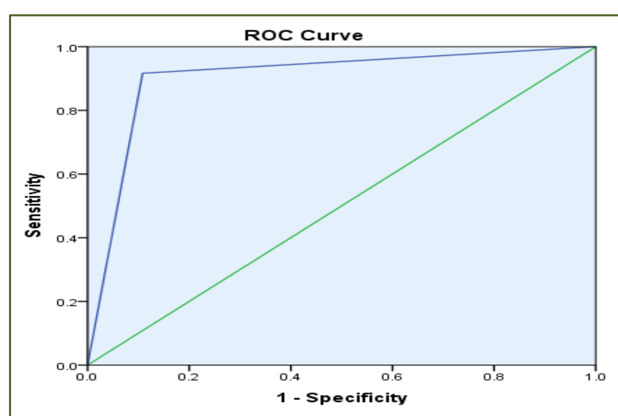

Figure 1: Receiver operating characteristic (ROC) curves for the maternal serum biochemical markers.

DISCUSSION

All three biochemical tests: dual marker test, triple marker test, and quadruple marker test show positive correlation with true results, that is, the result of karyotyping. The dual marker test and the triple marker test reveal strong correlation (coefficient of correlation 0.4 and 0.31 respectively) whereas the quadruple marker test has moderate correlation (coefficient of correlation 0.30). All three tests have high sensitivity and specificity but DMT and QMT showed high sensitivity (both 100%) than TMT (80%). The specificity is higher with DMT (90%) and QMT (93.18%). The positive likelihood ratio (LR+) is higher in QMT. The diagnostic odds ratio (115.11) is highest with DMT. The diagnostic accuracy is also high with DMT (90.22%).

In the cohort of women with abnormal karyotyping, 36.36% women were not elderly (age below 35 years). This is almost 1/3rd of the women in the cohort of women with foetal chromosomal abnormality. Also, 27.27% women with foetal chromosomal abnormalities were primigravida. This suggests that in more than 1/4th cases, the chromosomal abnormalities were in the first conceived foetus. Hence, it is prudent to offer universal screening to antenatal women, irrespective of their age and parity. This can help to detect more number of cases.

McDuffie R S et al used triple marker test for screening for Down's syndrome with 1: 295 as cut off in 6,474 women.[11] They had initial screen positive rate for Down's syndrome as 7.1% and 75% detection rate.[11]

Alldred SK et al reviewed both first and second trimester serum biochemical markers for screening for Down's syndrome in the Cochrane database review.[6,9] They conducted a meta analysis of 59 studies which involved 3,41,261 pregnancies.[9] They showed the sensitivity of triple marker as 61% (95% CI) at 5% false positive rate and sensitivity of quadruple marker as 83% (95% CI) at 5% false positive rate.[9] The diagnostic odds ratio (DOR) at 95% CI for triple marker test was 71 and for quadruple marker test was 50.[9]

Alldred SK et al studied first trimester biochemical markers by conducting a meta analysis of 56 studies involving 2,04,759 pregnancies which were again screened with different biochemical markers.[6] For cut off of 1:250, the sensitivity of dual marker test was 73% (95%CI) and specificity was 93% (95% CI).[6] They concluded that maternal age and double marker test having a combination of PAPP-A and free beta hCG, significantly outperformed either individual markers tests.[6] These results are similar to present study which also showed dual marker test to be better.

CONCLUSION

Since the foetal chromosomal abnormalities are not limited to elderly women (36.36% women with foetal chromosomal abnormalities were not elderly) or women with bad obstetric history (27.27% women with foetal chromosomal abnormalities were primigravidae), it is suggested that screening for chromosomal abnormalities be offered in all antenatal women irrespective of age and parity.

The biochemical tests, dual marker, triple marker and quadruple marker have shown high sensitivity and specificity; however, quadruple marker and dual marker test have higher specificity (90% and 93.18% respectively). The dual marker test best correlates (coefficient of correlation is 0.40) with the confirmative test, which is: karyotyping. Dual marker test has highest diagnostic odds ratio (115.11). The dual marker test is done in first trimester, so abnormalities can be diagnosed early in gestation and provides enough time for confirmative test which is important as in some countries, there is a gestational age limit of for medical termination of pregnancies. Hence the dual marker test can be considered to be better than triple and quadruple marker test for screening for aneuploidy.

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REFERENCES

1. Avătăjitei MC, Moscalu M, Martiniuc V, Onofriescu M. Performance of different methods of estimating risk screening for chromosomal anomalies.Rev Med Chir Soc Med Nat Iasi. 2012 Apr-Jun;116(2):515-22.
2. Kjaergaard S, Hahnemann JM, Skibsted L, Jensen LN, Sperling L, Zingenberg H et al. Prenatal diagnosis of chromosome aberrations after implementation of screening for Down's syndrome Ugeskr Laeger. 2008 Mar;170(14):1152-6.
3. Ekelund CK, Jørgensen FS, Petersen OB, Sundberg K, Tabor A. Impact of a new national screening policy for Down’s syndrome in Denmark: population based cohort study. BMJ. 2008;337:a2547.
4. Haddow JE, Palomaki GE, Knight GJ, Cunningham GC, Lustig LS, Boyd PA. Reducing the need for amniocentesis in women 35 years of age or older with serum markers for screening. N Engl J Med. 1994 Apr;330(16):1114-8.
5. Summers AM, Langlois S, Wyatt P, Wilson RD. Prenatal screening for fetal aneuploidy. J Obstet Gynecol Can. 2007 Feb;29(2):146-79.
6. Alldred SK, Takwoingi Y, Guo B, Pennant M, Deeks JJ, Neilson JP, Alfirevic Z. First trimester serum tests for Down's syndrome screening. Cochrane Database Syst Rev. 2015;(11):CD011975.
7. Wald NJ, Kennard A, Hackshaw AK. First trimester serum screening for Down's syndrome. Prenat Diagn. 1995 Dec;15(13):1227-40.
8. Huderer-Duric K, Skrablin S, Kuvacic I, Sonicki Z, Rubala D, Suchanek E. The triple-marker test in predicting fetal aneuploidy: a compromise between sensitivity and specificity. Eur J Obstet Gynecol Reprod Biol. 2000 Jan;88(1):49-55.
9. Alldred SK, Deeks JJ, Guo B, Neilson JP, Alfirevic Z. Second trimester serum tests for Down's Syndrome screening. Cochrane Database Syst Rev. 2012 Jun 13;(6):CD009925.
10. Wald NJ, Kennard A. Prenatal biochemical screening for Down's syndrome and neural tube defects. Curr Opin Obstet Gynecol. 1992 Apr;4(2):302-7.
11. McDuffie RS Jr, Haverkamp AD, Stark CF, Haverkamp C, Barth CK. Prenatal screening using maternal serum alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol: two-year experience in a healthmaintenance organization. J Matern Fetal Med. 1996 Mar-Apr;5(2):70-3.
12. Šimundić A-M. Measures of Diagnostic Accuracy: Basic Definitions. EJIFCC. 2009;19(4):203-211.
13. Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. J Clin Epidemiol. 2003 Nov;56(11):1129-35.
14. Littenberg B, Moses LE. Estimating diagnostic accuracy from multiple conflicting reports: a new meta-analytic method. Med Decis Making. 1993 Oct-Dec;13(4):313-21.
15. Moses LE, Shapiro D Littenberg B. Combining independent studies of a diagnostic test into a summary ROC curve: Data-analytic approaches and some additional considerations. Statistics Medicine. 1993;12 14):1293-316.

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