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Original Article

Triple marker study in midtrimester of pregnancy and risk of chromosomal abnormality

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Abstract

Objectives : Every year about 18,000 babies are born in India with trisomy 21. With the availability of well established, documented and widely used maternal serum triplemarker screening during midtrimester of pregnancy, every pregnancy can be monitored for the most common aneuploidy like trisomy 21, trisomy 13, and trisomy 18 in addition to open neural tube defects. *Methods :* MoM values were derived from 1738 normal pregnant women between 14-20 weeks of gestation who later had full term normal delivery. Two thousand one hundred and eleven women were investigated by triple marker screening between 14-20 weeks of gestation. *Results* : Two hundred twenty four women were considered as screen positive for trisomy 21, of which, 105 were further investigated for karyotyping and eight of these had trisomy 21, one each had mosaic trisomy 21, der (14:15) and del (X) (p11). Twentythree women with low hCG MoM were considered as screen negative for trisomy 21 and trisomy 18 but positive for other chromosomal abnormalities like iso (X) (q10) and der (13:14) one each, and two with polyploidy. *Conclusion :* The results suggest that triple marker screening is an effective screening program for noninvasive diagnosis of pregnancies with suspected Down syndrome fetus and also detects other chromosomal anomalies.

Key words : triple marker study, AFP, uE3, hCG, Down syndrome, structural chromosomal abnormality

Introduction

Current census of India shows that, 1768 babies are born every hour in the country [1]. Considering the birth incidence of 1:920 for Down syndrome (DS) child, every hour two new DS babies are born with an annual incidence of about 18,000 [2]. The average lifetime cost of one DS baby would be approximately 5 million rupees considering their average life span of 45-50 years. This is a tremendous cost to the society. Though the molecular-cellular events responsible for DS birth have shown the promising future for prevention of their birth [3,4], the only preventable and most acceptable noninvasive methods available today are the maternal serum screening (MSSI) and fetal ultrasound markers for the prenatal identification of fetal chromosomal anomalies [5,6]. The first trimester markers like pregnancy associated plasma protein-A (PAPP-A) and the free-beta form of human chorionic gonadotropin (free β-

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hCG) and the second trimester serological markers α feto-proteins, uE3, hCG and inhibin-A have been used in different comibinations [7-8] whereas the inclusion of proform of eosinophilic major basic protein (ProMBP) looks promising but is still being investigated [9]. The use of different serological markers in combination with maternal age and ultrasound markers such as nuchal translucency (NT) in the first trimester [10] has been shown to increase the detection rate markedly and reduce screen positive rate (SPR).

With the many possible marker combinations available today, it becomes imperative to assess the performance of different screening strategies in order to be able to offer the most oppropriate screening test with a minimum false positive rate (FPR) and enhanced specificity.

The aim of present study was to investigate the distribution of second trimester markers (AFP, uE3 and hCG) in Indian Gujarati women together with maternal age and weight in normal pregnancies and use of these findings in the midtrimester screening protocol for other chromosomal abnormalities in addition to trisomy 21 (T21). Mean maternal weight in midtrimester was 62 kg. Since screening performance is affected in the very obese all the values were weight corrected.

Methods

To establish the indigenous MoM we screened 1738 pregnancies between 14-20 weeks of gestation for triple marker screen (TMS) all of which ended in normal delivery. Further 2111 women were screened between 14-20 weeks of gestation. Of these, 1555 were in the age range of 19 to 34 years and 556 were above 35 years of age. Blood samples were processed for AFP, uE3 and hCG. All pregnancies were spontaneous conception or IVF singletons.

Gestational age dependant MoMs were calculated for all markers using logarithmic regression of marker values based on gestational ages determined by the crownrump length. The distribution of MoM values of different markers in relevant gestational age intervals were established by followup of all pregnancies.

Glenn E. Paulomaki (Foundation for Blood Research, Portland Maine, USA) software was used for risk calculation of DS pregnancy using the likelihood ratio derived from the trivariate Gaussian distribution of the analytes and the prior risk (maternal age). Cases with DS risk >1:270 at birth were considered as high risk and genetic counseling was offered to them for amniocentesis.

Results

The calculated median and MoM values in normal pregnancies for three markers (AFP, uE3, hCG) are shown in Table 1. As can be seen from the results, the distribution of MoM is identical in all periods of gestation irrespective of the median values. For AFP, uE3 and hCG the MoM value remains 1,13,1.1 and 1.24 respectively at 14 to 20 weeks of gestation and these are independent of analyte concentration at different

Table 1. AFP, uE3 and hCG concentrations in normal pregnancies (n=1738).

		AFP		uE3		hCG	
Gestation (weeks)	**No. of women**	**IU/mL Median**	**MoM**	**ng/mL Median**	**MoM**	**mIU/mL Median**	**MoM**
14	51	23.14	1.10	0.70	1.10	50000.00	1.10
15	210	26.44	1.00	0.82	1.10	48000.00	1.20
16	335	32.90	1.10	1.05	1.10	45000.00	1.30
17	372	35.53	1.10	1.20	1.10	34000.00	1.20
18	368	41.27	1.10	1.50	1.10	32000.00	1.30
19	255	47.93	1.10	1.70	1.00	28000.00	1.10
20	147	57.85	1.20	2.00	1.00	30000.00	1.10

Table 2. AFP, uE2, hCG concentration in pregnancies at risk for Down syndrome [a] (n=224).

Gestation (weeks)	No. of women	AFP		uE3		hCG	
		Median	IU/mL MoM	ng/mL Median	MoM	mlU/mL Median	MoM
14	13	16.94	0.73	0.42	0.60	88000.00	1.76
15	22	20.66	0.78	0.58	0.71	80000.00	1.68
16	35	22.75	0.69	0.78	0.74	67000.00	1.49
17	51	25.00	0.90	0.90	0.75	60869.00	1.79
18	53	33.05	0.80	1.10	0.73	57000.00	1.78
20	25	33.88	0.82	1.20	0.71	50000.00	1..79
19	25	47.75	0.83	1.70	0.85	54000.00	1.80

[a] cut off risk 1:270

Table 3. Screening performance of α fetoprotein.

Age (Years)	No. of women	Triple marker screening			Pregnancies at risk neural tube defect based on Increased α feto-proteins		Screen negative Down syndrom + neural tube defects
		Normal at risk for	No.of pregnancies rate Down syndrome	False positive rate (%)	Number	%	
19-34	1555	1448	78	5.02	29	1.86	93.12
(35-45)	556	390	146	26.26	20	3.60	70.14
Total	2111	1838	224	10.61	49	2.32	87.07

Table 4. Chromosomal study on amniotic fluid of triple marker screen positive cases.

Findings	No. of cases	
Normal findings	90	
47.X*, +_21	8	
92, XXYY	1	
46,X*/47, X*,+21	1	(Mosaic)
45,X*,der (13:14) (q10;q10)	1	
Total	101	

gestational age. The age, weight and markers with corrected MoM are shown in Table 2 for pregnancies at risk for DS birth using cut off risk at 1:270. The FPR was 5.02% in women less than 35 years of age and 26.26 % in those greater than 35 years of age. The combined FPR obtained was 10.61% (Table 3).

Forty five percent women (101/220) with positive screen had opted for genetic confirmation by amniocentesis and 10.89% of these (11/101) showed chromosomal anomalies (Table 4). The most common was free trisomy 21 in 8 (72.72%) followed by mosaic trisomy, Robertsonian translocation and Turner variant in one each. Of 23 women with low hCG MoM, seven (30.43%) opted for genetic study by amniocentesis and three were normal while four (17.39%) showed confirmed chromosomal abnormality (Table 5). Two women with AFP MoM of 3.3 and 3.4 were found to have open NTD's in the fetus and opted for termination of pregnancy. Rest of the pregnancies were followed till term and resulted in normal child. No followup was available in those who refused genetic testing.

Table 5. Triple marker screen positive and amniocentesis.

Gestation weeks	Women Down Syndrome risk > 1:270	AFP		uE3		hCG		Amniocentesis
		IU/mL	MoM	µg/mL	MoM	mIU/mL	MoM	Report
15	1	82.60	3.30	1.00	1.30	33000.00	0.70	Fetus with NTD (n=1)
16	2	53.71	1.950.91	1.10	16500.00	0.45	No follow-up	
17	5	98.21	3.40	1.39	0.92	14102.00	0.48	Normal (N=1), NTD Fetus (N=1)
18	5	37.19	1.10	1.50	1.00	13800.00	0.50	92, X *** (N=1)
19	5	44.62	1.00	1.55	0.95	12000.00	0.60	46,X,iso(Xq)(q10) (N=1), 92,X*** (N=1)
20	5	121.15	2.40	2.12	1.05	13000.00	0.52	Normal (N=21),, 45,X*, der (13;14) (q10;q10) (N=1)

Discussion

Present study carried out in Indian Gujarati women clearly demonstrates that the unaffected normal pregnancies have an identical MoM value for AFP, uE3 and hCG to those reported in various ethnic populations [11].

The marker values obtained in pregnancies at risk for DS were also identical to those reported in different populations [12,13]. The cumulative FPR for detection of trisomy 21 was 10.61% which is higher due to the inclusion of elder women where the age related risk for DS pregnancy is higher. Nonetheless the FPR is in acceptable range of 5 to 7% for women under 35 years of age [14]. Low uptake of amniocentesis (45%) in screen positive women indicates the need for pretest counseling. Eleven percent of these women showed confirmed chromosomal anomalies with the predominance of free trisomy 21 confirming DS as the most common aneuploidy encountered in screen positive pregnancies. Present study also shows higher detection rate of chromosomal anomalies in screen positive women as compared to the reported rate of 7 to 8 per cent [15]. This could be attributed to the population based mean values in risk assessment and small sample size.

It is well established that meiotic error is the cause of trisomy 21 that increases with age. However about 91% of Down babies are born to young mothers which implies that all women irrespective of their age should be screened for triple marker screening as almost equal number of women in younger and older age had confirmed trisomy 21 [16].

Pregnancies (1.3%) with screen negative and with low hCG MoM have shown Turner variant in one case and polyploidy in two cases. None of these pregnancies had fetal hydrops. This observation is in accordance with the earlier study reports [17].

Our results confirm the value of second trimester serum screening using maternal age, maternal weight and corrected gestational age. Study shows that maternal serum screening is an effective and practical method for large scale second trimester screening for DS and other chromosomal abnormalities. Those with screen negative along with low hCG MoM also need confirmative genetic study. Thus present data will provide an important tool to those involved in the noninvasive screening program for monitoring screen positive and screen negative pregnancies as well.

Conclusion

Triple marker serum screening should be routinely employed for pregnant women at risk of having Down syndrome fetus.

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References

1. Krivchenia E, Huther CA, Edmonds LD et al. Comparative epidemiology of Down syndrome in two United States populations, 1970-1989. Am J Epidemiol 1993;137:815-28.
2. Government of India Planning commission, National Population Policy 2001.
3. James SJ, Pogribna M, Pogribny IP et al Abnormal folate metabolism and mutation in the methylnete-trahydrofolate reductase gene may be maternal risk factors for Down syndrome. Am J Clin Nutr 1999;70:495-550.
4. Sheth JJ, Sheth FJ: Gene polymorphism and folate metabolism :A maternal risk factor for Down syndrome. Indian Pediatr 2003;40:115-23.
5. Wald NJ, Watt HC, Haddow JE et al. Screening for Down syndrome at 14 weeks of pregnancy. Prenatal Diagn 1998;18:291-3.
6. Canick JA, Saller DN Jr, - Messerlian Lambert, GM. Prenatal screening for Down syndrome: current and future methods. Clin Lab Med 2003;23:395-411.
7. Souter VL, Nyberg DA, Benn PA et al. Correlation of second - trimester sonographic and biochemical markers. J Ultrasound Med 2004;23:505-11.
8. Wald NJ, Huttly WJ, Hackshaw AK. Antenatal screening for Down's syndrome with the quadruple test. Lancet 2003;361:835-6.
9. Rode L, Wojdemann KR, Shalimi AC et al. Combined first -and second - trimester screening for Down syndrome: an evaluation of pro-MBP as a marker. Prenat Diagn 2003;23:593-8.
10. Snijders RJ, Noble P, Sebire N et al. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998;352;343-6.
11. Canick JA, Knight GJ. Multiple marker screening for fetal Down syndrome. In: Contemporary OB-Gyn, 1992. Medical Economic Publishing, at Montvale. NJ. 07645-1742.
12. Chard T, Lowings C, Kitau MJ. Alphafetoprotein and chorionic gonadotropin levels in relation to Down's syndrome. Lancet 1984;2:750.
13. Biggio JR, Morris TC, Owen J et al. An outcome analysis of five prenatal screening strategies for trisomy 21 in women younger than 35 years. Am J Obstet Gynecol 2004;190:721-9.
14. Haddow JE, Palomaki GE, Knight GJ et al. Prenatal screening for Down's syndrome with use of maternal serum markers. N Engl J Med 1992;327:588-93.
15. Summers AM, Farrell SA, Huang T et al. Maternal serum screening in Ontario using the triple marker test. J Med Screen 2003;10:107-11.
16. Sheth F, J, Rao S, Manisha D et al. Cytogenetic Analysis of Down syndrome in Gujarat. Indian Pediatrics. 2007;44:774-7. (Pending Publication).
17. Saller DN, Canick JA, Schwartz S et al. Multiple marker screening in pregnancies with hydropic and nonhydropic Turner syndrome. Am J Obstet Gynecol 1992;167:1021-4.