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Maternal Serum Markers as Second Trimester Screening for Chromosomal Aneuploidies and Neural Tube Defects Followed by their Diagnosis and Impact on the Outcome of the Pregnancy

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Abstract: ***Background: As we are aware serum markers estimation is currently in use to screen aneuploidies and neural tube defects as prenatal care should be offered to all if possible, or at least high-risk patients. Down syndrome (DS) has major socio-economic and psychological implications being the most frequent cause of mental handicap in developing world countries. Objective: The present study was aimed at detecting the aneuploidies and other neural tube defects by second-trimester quadruple biochemical marker screening along with sonographic soft markers to compute risk assessment in current pregnancy followed by their impact on detection and deciding medical termination of pregnancy if patient desires. Methods: This study was carried out in pregnant women undergoing quadruple screening tests between June 2020 and June 2021. Biomarkers alpha-fetoprotein, human chorionic gonadotropin, unconjugated-estriol and inhibin A were tested, and risk of a pregnancy being affected with Down Syndrome, Edward's syndrome, Patau syndrome or Neural Tube Defects were calculated. Screen-positive patients were referred for confirmatory amniocentesis/NIPT. A follow-up of screen-positive patients were carried out by telephonically whether they continued or terminated the pregnancy in covid era. Results: Out of 200 pregnant women who underwent quadruple marker screening, 8 (4%) were screen-positive, including all 8 positives for Down syndrome. Out of eight, three cases were confirmed as DS by amniocentesis. No case of trisomy 13/18 was detected. All confirmed patients terminated the pregnancy (100% termination rate), rest continued the pregnancy. One fetus was detected with hydrocephalous and cleft palate in anomaly scan who was screened negative forthe quadruple marker. Conclusions: Quadruple screening in the second trimester is reasonably effective for the detection of major chromosomal defects and NTDs who come for their first antenatal visit in 2nd trimester.***

Keywords: Down syndrome, Prenatal screening, combined screening test, Quadruple screening test, Amniocentesis

1. Introduction

Over the past 20 years, there have been major advances in the field of prenatal screening for Down's syndrome and the efficacy of ultrasound scanning for the detection of fetal anomalies. Previously, older pregnant women were offered diagnostic tests like chorionic villus sampling [CVS] or amniocentesis to detect Down's syndrome, both associated with a risk for causing miscarriage. Offering an amniocentesis to the oldest 5% of women identified about 30% of pregnancies with Down's syndrome.1 Today, several of different non invasive screening tests, which can be offered to women of any age, are available. These tests have different detection and false-positive rates.[2–4]

Screening for chromosomal anomalies and open neural tube defects is part of prenatal care which should be offered to all if possible or at least high risk patients.

Currently, there are several screening tests available involving maternal serum and/or ultrasound, each with different test characteristics and differing in the gestational range at which they can be carried out. The maternal serum screening (MSS) quadruple test is offered between 14 and 20 weeks gestation. The maternal serum screening (MSS) quadruple test is offered between 14 and 20 weeks gestation.[5] Despite the high detection rate of quadruple screening, studies have shown that this test has a false-positive rate of 5–7.5%.[6, 7]

Regardless of maternal age, all women should be counseled regarding an option to pursue aneuploidy screening and follow-up diagnostic testing. Women should discuss these options with their providers to decide the best test for them. Factors affecting the choice of screening include the mother's desire for prenatal information, previous pregnancies, family history, gestational age at the first visit, cost, and desire to pursue follow-up pregnancy care or termination in the case of an abnormal diagnostic test.[8]

In this study, we retrospectively collected the data of patients who underwent quadruple marker screening and took follow up of those patients who came screen positive to find out the outcome of pregnancy.

2. Method

This is a retrospective study done in Umaid Hospital, Jodhpur Rajasthan. This study was conducted by

collecting the quadruple test data from SRL laboratory between June 2020 and June 2021. All patients visiting Out Patient's Department and antenatal clinic of the department of obstetrics and gynecology with the first visit in the second trimester of pregnancy (15–19weeks) were counseled about the prenatal quadruple marker screening tests and advised to get it done. A sample of 5mLvenous blood was collected in a plain vial under the aseptic condition from the patients, and serum was separated and stored at – 60C until further processing. Clinical details including maternal age, weight, gravida, parity, past and family history of congenital and chromosomal disorders, smoking status and history of insulin dependent diabetes mellitus with h/o IVF pregnancy were recorded in a structured proforma. Gestational age was calculated ultrasonographically using either crown rump length or biparietal diameter. Two patient's pregnancies were IVF and none of the pregnant women had a positive smoking status. Biomarkers AFP, bhCG and uE3 were quantitatively measured using DELFIA AFP/free b hCG dual kit and DELFIA unconjugated estriol kit of PerkinElmer (Turku, Finland). The Quadruple biomarker results were adjusted for maternal weight, age and Asian ethnicity, and the likelihood ratio of the fetus being affected with trisomy 21, trisomy 18 and NTD was calculated using Wallac Life Cycle software with Elipse Screening Engine of PerkinElmer. The patients were categorized as high-risk (screen-positive) using 1: 250 as the cut-off for DS and low-risk (screen-negative) with 1: 100 for trisomy 18, and an AFP multiple of median (MoM) of greater than 2.5 for open NTDs. All screen-positive patients were provided detailed counseling for further confirmatory testing in the form of level II ultrasonography, amniocentesis or NIPT (non-invasive prenatal test). In case of confirmed diagnosis for chromosomal aneuploidies, the couple was counseled for informed decision making. A follow-up of screen positive patients were carried out by telephonically whether they continued or terminated the pregnancy.

3. Results

A total of 200 patients were analyzed who underwent second trimester quadruple marker screening in the last year from June 2020 and June 2021. The age distribution of women is given in table no 1. The mean maternal age is 27.2 and 8 women older than 35 or more.

Table 1: Enrolment of pregnant women in the study with respect to age and weight

AGE	NO	%	MATERNAL WEIGHT	NO	%
<20.0	2	1	<50.0	30	15
20.0–25	80	40	50.0–59.9	90	45
26.0–30	82	41	60.0–69.9	45	22.5
31.0–35	28	14	70.0–79.9	18	9
36.0–40	7	3.5	80.0–89.9	10	5
>40.0	1	.5	>90.0	7	3.5
TOTAL	200			200	

Of all the women tested, 3.5% (07) women screened positive; the screen positive rate was higher among women of 30 years or older than among those younger than 35. Screen-positive cases included those positive for trisomy 21 (n=7 3.5% of total), for trisomy 18 (n=0), and for NTDs (n=0). Follow-up (either at delivery or by telephone interview) was obtained.

Table 2: Biochemical parameters and ultrasonographic findings in screen positive Down syndrome (DS) cases

S. No.	Risk for DS in Quadruple test	Age (years)	Weight	GA	AFP MoM	uE3 MoM	Bhcg MoM	Inhibin A MoM	Ultrasonographic fetal markers
1	1: 95	27	54	16 wks	0.48	0.71	1.34	2.28	
2	1: 79	35	53	15 wks	1.23	0.18	0.86	2.56	
3	1: 171	24	60	16.3 wks	1.27	1.01	1.87	5.00	
4	1: 216	36	80	15.1	0.85	1.50	1.56	2.15	
5	1: 2	22	72	18.1	0.36	0.10	2.05	2.79	Coarctation of aorta, pericardial effusion, lt pylectasis
6	1: 45	24	68	16.5	0.83	1.18	3.85	2.43	
7	1: 16	30	58	15	0.69	1.36	3.06	5.12	
8	1: 169	36	69	14.5	1.03	0.84	1.63	3.13	

4 (50%) of women were of age 30 or more, out of total 8 patients who screened positive.

Among screen positives, the confirmation was done by amniocentesis and NIPT.3 cases were confirmed Down syndrome out of 8. Ultrasonography followed by amniocentesis revealed DS in 37.5 % of these screen-positive cases. Following counseling, all parents opted for the termination of pregnancy. One additional case of hydrocephalous was detected at 20 weeks with negative quadruple markers (sensitivity of the DS screen=7/8 or 87.5%). Table 2 displays the serum results in patients with screened positive DS fetuses (Table 2). Ultrasonography in these patients revealed ventriculomegaly, pericardial effusion, bilateral pyelectasis and coarctation of the aorta.

Table 3: Biochemical parameters and pregnancy outcome in screen positive Down syndrome (DS) cases

S. No.	Risk for DS in Quadruple test	Age (years)	Weight	GA	Confirmation for aneuploidy	Pregnancy outcome
1	1: 95	27	54	16 wks	Amniocentesis+ve	Terminated
2	1: 79	34	53	15 wks	Amniocentesis+ve	Terminated DM2
3	1: 171	24	60	16.3 wks	NIPT-ve	Continued
4	1: 216	36	80	15.1	Amniocentesis-ve	Continued
5	1: 2	22	72	18.1	Amniocentesis +ve	Terminated
6	1: 45	24	68	16.5	Amniocentesis-ve	Continued
7	1: 16	30	58	15	NIPT-ve	Continued
8	1: 169	32	69	14.5	NIPT-ve	Continued

S. No.	Risk for DS in Quadruple test	Age (years)	Weight	GA	AFP MoM	uE3 MoM	Bhcg MoM	Inhibin A MoM	Ultrasonographic fetal markers
1	1: 6097	27	70	16 wks	1.03	0.34	0.98	1.14	Severe hydrocephalous and cleft palate

In our study, we did not detect any case of trisomy 18/1. All confirmed cases opted for termination. And rest continued their pregnancy. In the screen negative group, one baby was detected with severe hydrocephalous and cleft palate in anomaly scan. The mother decided to terminate the pregnancy.

4. Discussion

The quadruple marker test is the most commonly used method for second trimester screening. The detection rate is between 81% and 85.8% for false positive rate between 7%-8.3% respectively.[9] The quadruple test for Down's syndrome estimates the risk of a Down's syndrome term pregnancy from maternal age at term and the concentration of four markers in the maternal serum-alphafetoprotein, unconjugated oestriol, human chorionic gonadotropin (total hCG or, more usually, the free subunit), and inhibin-A-at 14–22 weeks of period of gestation.[9] In developed countries, down syndrome screening in first and second trimesters is well established. For the first and second trimesters, different tests are available accordingly. The worldwide criteria for screening Down syndrome has been shifted towards ist trimester, early screening as soon as possible. But here in India/developing countries, the scenario is different because of many reasons-limited resources, lack of education, financial problems/can't afford, late antenatal check-ups and many others.

In India and many other Asian countries, prenatal screening in ist and second trimesters is still an incipient stage.1[0] At present in India, there is no nationwide consensus regarding the nature and timing of the prenatal-screening protocols. Due to the lack of any proper guidelines and the present lacunae of awareness regarding the appropriate prenatal screening in the country, the optimum benefits of these screening protocols are not reaching the population. The disorders with a significant prevalence in India for which we highly need population-based prevention programs include Down syndrome and neural tube defects (NTDs) [11, 12, 13]. Canada like many countries has national guidelines for Down syndrome screening[14]. We don't have a population-based government program for prenatal screening despite being India's second most populated country.

The prevalence of genetic screening tests among obstetricians has been increased in last several years. But these tests are only available in most private Hospitals and laboratories in large cities. The methods of prenatal diagnosis and fetal tissue sampling have been well established in dedicated centers for genetic studies in metropolitan towns in India. Among those obstetricians who offer and counsel patients regarding screening, limitation comes at the patient level either they don't understand the importance of the test or financially they can't afford the test. So a very small junk of population left who avail the tests.

In our study period total of 200 pregnant women were able to get screened themselves in the second trimester by quadruple marker test, as their first visit was in the second trimester only.8 (4%) were screen-positive, including all 8 positive for Down syndrome. Out of eight, three cases were confirmed as Down syndrome by amniocentesis. The earlier triple test was being used as second-trimester screening. The DR of detection of the triple test is approximately 69% for a FPR of 5%. It has become less popular as the accuracy of first trimester screening has improved. But those patients who come in their 2nd trimester, the quadruple marker test is the most commonly used and appropriate method for second trimester screening. Its DR is between 81% and 85.8% for FPRs of 7%-8.3%, respectively. The DR of trisomy 21 in a woman over 35, using maternal age only, is 30% for a FPR of approx 5%. By combining maternal age and NT, the DR increases to 75%-80%.

Screening Method	DR (%)	FPR (%)
Maternal Age	30	5
Double Test (AFP + bHCG)	58	5
Triple Test (AFP + bHCG + uE3)	69	5
Quadruple Test (AFP + bHCG + uE3 + inhibin A)	85	5
Combined Test (maternal age + NT + PAPP-A + b HCG)	85-90	5
Integrated Test (NT + PAPPA-A + T2 QUAD)	85	1.2
NT alone	77	4.7

With India being one of the highest birthing nations, there is a greater incidence of Down Syndrome and it occurs in approximately 1 out of 830 live births, as per The Down Syndrome Federation of India (2018). The most common chromosomal aberration among all conceptions is trisomy 16-such fetuses almost always die in utero. Similarly, the other common chromosomal aneuploidies such as trisomy 18 (Edward syndrome) and trisomy 13 (Patau syndrome) have as high as 80% in utero mortality between the first trimester and term, while this figure is about 30% for trisomy 21[15]. Also, the vast majority of fetuses with Edward and Patau syndrome exhibit ultrasound detectable structural defects, whereas only about 50% of Down syndrome fetuses have any structural defects identifiable on prenatal ultrasound. Postnatally, infants with Patau syndrome rarely survive beyond the first week, and Edward syndrome is beyond the first year, whereas a significant proportion of those with Down syndrome reach adulthood. So above are the reasons for giving so much importance to the justification and principles of Down Syndrome Screening**[15].** In our study, the patients who were diagnosed with DS their age group was between 20-30 (2 patients) and one was above 30 yrs of age. Some authors have suggested a younger age distribution of the mothers with DS children in some Indian populations[16, 17].

In the present study, no cases of NTD were detected in screen-positive patients. The screen-negative group reported one case of closed NTDs, detected through ultrasound in the second trimester of pregnancy. The AFP assay in the diagnosis of NTDs is not specific for these defects, as it does not detect closed NTDs. About 90% of these defects are diagnosable prenatally early in the second trimester, the other 10% consisting of closed lesions that are not amenable to this approach. AFP levels are not elevated in most closed or skin/membrane-covered lesions.1[0] The one closed NTD has been reported in women in the age group of 20 to 30 years, which is the commonest childbearing age in India.

5. Conclusion

In India, as our population is increasing we urgently need a prenatal screening policy in the form of maternal serum screening. Although first trimester screening has a better detection rate than second trimester screen, in developing countries like India with limited resources and infrastructure, second trimester screening can be more easily introduced into the existing health care system, with the gradual shift to first trimester screening.

References

[1] Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. J Med Screen 1997; 4: 181–246.
[2] Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med 2005; 353: 2001–11.
[3] Wald NJ, Watt HC, Hackshaw AK. Integrated screening for Down's syndrome on the basis of tests performed during the first and second trimesters. N Engl J Med 1999; 341: 461–7.
[4] Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, et al. First-trimester screening for trisomies 21 and 18. N Engl J Med 2003; 349: 1405–13.
[5] Wald N, Huttly WJ, Hackshaw AK. Antenatal screening for Down's syndrome with the quadruple test. Lancet 2003; 361: 835–6
[6] Malone FD, Canick JA, Ball RH, Nyberg DA, Comstock CH, Bukowski R, et al: First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med 2005; 353: 2001–2011.
[7] Benn PA, Ying J, Beazoglou T, Egan JF: Estimates for the sensitivity and false-positive rates for second trimester serum screening for Down syndrome and trisomy 18 with adjustment for cross-identification and double-positive results. Prenat Diagn 2001; 21: 46–51.
[8] Practice Bulletin No.163: Screening for Fetal Aneuploidy. Obstet Gynecol.2016 May; 127 (5): e123-e137.
[9] Wald N, Huttly W, Hackshaw A. Antenatal screening for Down's syndrome with the quadruple test. Lancet.2003; 361; 835-836
[10] Kaur G, Srivastav J, Kaur A, et al. Maternal serum second trimester screening for chromosomal disorders and neural tube defects in a government Hospital of North India. Prenat Diagn.2012; 32 (12): 1192–6.
[11] Phadke S, Agarwal M. Neural tube defects: A need for population-based prevention program. Indian J Hum Genet.2012; 18: 145–7.
[12] Central Technical Co-ordinating Unit, ICMR Central Technical Co-ordinating Unit, ICMR. Multicentric study of efficacy of periconceptional folic acid containing vitamin supplementation in prevention of open neural tube defects from India. Indian J Med Res. 2000; 112: 206–11.
[13] Aggarwal S, Bogula VR, Mandal K, Kumar R, Phadke SR. Aetiologic spectrum of mental retardation & developmental delay in India. Indian J Med Res.2012; 136: 436–44.
[14] Chitayat D, Langlois S, Douglas Wilson R SOGC Genetics Committee, CCMG Prenatal Diagnosis Committee. Prenatal screening for fetal aneuploidy in singleton pregnancies. J Obstet Gynaecol Can.2011; 33: 736–50.
[15] K. Manikandan, Suresh Seshadri, Down Syndrome Screening in India: Are We There Yet? The Journal of Obstetrics and Gynecology of India.2017
[16] Rao VB. Mean maternal age of Down's syndrome in Hyderabad, India. J Indian Med Assoc.1999; 97: 25.
[17] Vundinti BR, Ghosh K. Incidence of Down syndrome: Hypotheses and reality. Indian J Hum Genet.2011; 17 (3): 117–9