Original Article

Prediction of Low Birth Weight by Quadruple Parameters in High-Risk Pregnancies

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Abstract

Context: Aneuploidy screening is done in the early second trimester of pregnancy among all pregnant women as compulsory, with a special focus on those who had abnormal ultrasound parameters, higher dual marker risk, or other comorbidities. Recently, all individual quad markers of conventional trisomy screening have been suggested as useful in predicting adverse pregnancy outcomes (APO) such as preeclampsia, preterm labor, small for gestational age, and placental abruptions. However, similar studies on Indian pregnant women are limited. Hence, this study was intended to find the relation of quadruple markers with any other APO than aneuploidy. **Materials and Methods:** A retrospective study was conducted in a Tertiary Care multi-specialty hospital in North India. Data from 252 pregnant women's quadruple test was analyzed. The association of abnormal value of quadruple markers (human chorionic gonadotropin [HCG]/alpha-fetoprotein/uE3/Inhibin A) with adverse outcomes was evaluated. Multiple logistic regression analysis and classification and regression tree were used to predict the significant risk factor in high-risk pregnancies. **Results:** In the study, a total (n = 252) of pregnant women, 190 were screened as high-risk pregnancies, whereas the remaining 62 were reported as low-risk using trisomy screening in the quadruple test. Baby birth weight was observed to be significantly associated with Inhibin-A, and HCG ($P < 0.001$), whereas Corrected (Corr)-multiple of median (MoM)-HCG (>1.415) and Inhibin-A Corr-MoM (>364.175) were the suitable predictor for the LBW. Both parameters were significantly higher in the high-risk group as compared to the low-risk group (each $P < 0.05$). **Conclusion:** Abnormal deviation of biochemical markers from aneuploidy screening assessment could help predict other perinatal adverse outcomes such as low birth weight babies.

Keywords: *Adverse pregnancy outcome, low birth weight, prenatal screening, quadruple screening test*

Introduction

Double marker combined screen (blood test along with ultrasound) in the late first trimester (11–13th week), and Triple marker and Quadruple marker tests in the second trimester (16–20th week) are conventionally performed for assessing trisomy risk in pregnancy in routine, but in last three decades, this aneuploidy screening has continued to grow and evolved enormously as an alternative tool to detect other associated high risks in pregnancies such as Preeclampsia (PE), Intra uterine growth restriction (IUGR), preterm labor (PTL), small for gestational age (SGA) and Placental abruptions. This became feasible by observing abnormal levels in serum biomarkers in pregnancy multimarker screening tests in few of the previous studies.[1-4]

The routine Quadruple test is used for the detection of the risk of chromosomal abnormality, mainly Trisomy 21 and combined risk of tri 18 and 13 in pregnancy, from a combination of four different serum markers (human chorionic gonadotropin [HCG] hormone, alpha-fetoprotein [AFP], Estriol; uE3, Inhibin A), and Ultrasound markers (nuchal translucency, crown rump length) along with maternal characteristics (age, weight, gestational age, smoking or diabetic history). These parameters had individually proven significant relation in depicting certain adverse pregnancy outcomes (APOs) in the antenatal and perinatal period in many studies.[5-8] However, in our population, no study has reported such an effect so far, to the best of our knowledge; hence, this study was designed to check the effectiveness of this perspective retrospectively.

Mothers with anemia, gestational diabetes mellitus (GDM), gestational

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hypertension (GHTN), PE, PTL, intrauterine growth restriction (IUGR), poly and oligohydramnios, premature rupture of membrane (PROM), and placental abruption were included in the study. Patients with a viral infection, thalassemia, multiple pregnancy, and congenital disorders, severe heart, hepatic or renal illnesses were excluded from the study to avoid potential cause of higher risks.

Materials and Methods

A retrospective unicentric study was conducted in a Tertiary Care Hospital in North India between the period of 2016 and 2019. Patients' data was collected and compiled from the hospital information system (HIS) in Department of Molecular Medicine and Biotechnology, SGPGIMS, Lucknow UP after approval from the Institutional Ethical Committee and in accordance with the Helsinki declaration. Maternal serum marker screening results, were taken from the database for the participants who had visited the lab for secondary level quadruple marker testing after clinicians' referral.

Biochemical testing on patients' serum was performed by the IMMULITE 1000, Siemens Inc. and risks were determined with the Prisca 5.0.0.1.0 software. In addition, clinical data and postpartum data were collected from HIS to check any reported APOs at the time of patients' discharge. Results were compared to see the correlation of biochemical markers with the APOs in screened patients.

Patient data were categorized into high- and low-risk groups vertically and in adverse outcomes horizontally. Normality distribution was checked, and cross-tabulation was performed on SPSS software between blood serum markers, their multiple of median (MoM's) and selected adverse outcomes (dichotomous variable), Other clinical signs helped in understanding the severity of diseases. Statistical analysis was performed on SPSS-23, IBM, Chicago, IL, USA.

MoM is a measure of deviation in test results. It gets calculated for each marker with inbuilt PRISCA software (used with IMMULITE 1000 Siemens' healthineers inc) by using different types of regressions on every week's pregnancy test data from all the participating labs to find suitable median with standard deviation.

Adverse obstetric outcomes were examined by spontaneous fetal loss after 24 weeks, preterm delivery starting at 32 weeks of gestation or less, Baby weight was considered low if found <2.5 kg and marked as low birth weight (LBW), and between 2.5 and 3.5 kg as standard baby weight, whereas >3.5 kg as overly weighed.

GDM was declared by the level of blood sugar of more than 140 in PP and more than 100 units at fasting level during the first trimester of pregnancy. PE was defined as GHTN (if blood pressure was more than or equal to 140/90 in second trimester) in the setting of significant proteinuria (a minimum of 300 mg/24 h or 0.1 g/L (more than 2_on a dipstick) in at least in two random samples collected in 6 or more hours apart). Hypothyroidism was defined by the level of thyroid stimulating hormone in blood by <4.5 SI units. Moreover, finally, delivery was considered as early or PTL on <37 weeks of delivery, whereas medical termination of pregnancy was done at <24 weeks of gestation, if mandatory, as per ICMR norms.

Statistical analysis

Continuous variables are presented in median (Q1, Q3, or interquartile range) as variables data were skewed. Categorical variables are presented in number and %. Patient's data were categorized into high- and low-risk groups. To compare the continuous variables between low- and high-risk groups, Mann–Whitney *U*-test was used. To test the association of the risk groups with categorical variables, Chi-square test/Fisher's exact test was used. Binary logistic regression analysis was used to assess the factors associated with high-risk group. Classification and regression tree (CART), which is a method of predicting analysis, was used to assess the most significant predictors of high-risk pregnancy. It is one of the most used, practical approaches for supervised learning to solve both Regression and Classification tasks. It breaks down a dataset into smaller and smaller subsets while, at the same time, an associated decision tree is incrementally developed. A $P < 0.05$ was considered statistically significant. The Statistical Package for the Social Sciences, version-23 (SPSS-23, IBM, Chicago, IL, USA) Software was used for data analysis.

Results

In the study of expectant mothers ($n = 252$), 190 were screened as high-risk pregnancy, whereas the remaining 62 were reported as low risk of pregnancies using trisomy screening in the quadruple test. Mean ± standard deviation, age, and weight of the patient were 30.75 ± 5.05 years and 60.8 ± 10.7 kg, respectively. All patient's ethnicity was Asian and were nonsmoker. Only 4 (1.6%) and 5 (2.0%) patients had diabetes history, and *In vitro* fertilization conceived, respectively. The descriptive statistics of demographic and maternal variables were compared between high- and low-risk pregnancies [Table 1]. There was no significant difference in mothers' age and weight at delivery, AFP and AFP Corrected (Corr) MoM were also nonsignificantly increased in both the high- and low-risk group (each $P > 0.05$). Gestational age, Trisomy Biochemical risk, uE3, uE3 Corr MoM were significantly lower, whereas Baby birth weight, HCG, Corr MoM-HCG, Inhibin-A, Inhibin-A Corr MoM were significantly higher as compared to low-risk group [each $P < 0.05$, Table 1].

The distribution of pregnancy and other adverse outcome variables between high- and low-risk groups are presented

Table 1: Distribution of demographic and maternal variables between high- and low-risk pregnancy (*n*=252)

Variable's	Total (*n*=252)	High (*n*=190)	Low (*n*=62)	*P*
Women age at delivery (years)	30 (26.5–34)	31 (27–34)	30 (26–34)	0.074
	30.62	30.97	29.53	
Women's weight at delivery (kg)	60 (54–67)	60 (54–67)	60 (53–69)	0.698
	60.70	60.57	61.10	
Gestational age (weeks)	37 (35–38)	37 (34–38)	38 (36–39)	0.011
	35.4	35.02	36.75	
Baby birth weight (kg)	2.61 (2.1–3.0)	2.6 (2.1–2.9)	2.5 (2.0–3.0)	0.003
	2.4	2.4	2.5	
Trisomy biochemical risk	138.5 (59.3–247.3)	99 (50–176)	324 (275–1640)	<0.001
	473.1	117.6	1550.9	
AFP	46.7 (35.1–64.3)	46.8 (34.2–65.1)	44.5 (35.3–61.3)	0.669
	57.3	57.9	55.1	
AFP corrected MoM	0.9 (0.7–1.2)	31 (27–34)	0.91 (0.7–1.1)	0.807
	1.1	30.9	1.01	
HCG (×1000)	35.4 (24.4–49.4)	37.1 (25.7–52.0)	26.9 (14.7–41.9)	<0.001
	40.7	43.9	29.2	
Corrected MoM-HCG	1.73 (1.2–2.5)	1.8 (1.4–2.6)	1.18 (0.9–1.8)	<0.001
	2.0	2.2	1.3	
uE3	0.72 (0.4–1.2)	0.7 (0.4–1.1)	0.9 (0.5–1.2)	0.03
	1.1	1.44	1.00	
uE3 corrected MoM	2.34 (1.77–3.37)	0.60 (0.4–0.9)	0.8 (0.6–1.1)	<0.001
	2.98	0.70	0.89	
Inhibin-A	373.8 (280.8–526.7)	394 (299.8–563.4)	317.7 (252.4–408.7)	0.001
	551.7	607.6	353.4	
Inhibin-A corrected MoM	2.35 (1.77–3.375)	2.51 (1.85–3.57)	2.1 (1.5–2.5)	0.001
	2.99	3.2	2.1	

P<0.05 significant. Data are presented in median (Q1–Q3); compared by Mann–Whitney *U*-test. AFP: Alpha-fetoprotein; HCG: Human chorionic gonadotropin; MoM: Multiple of median

in Table 2. The result showed that GDM, PTL, IUGR, PE, hypothyroid, oligohydramnios, low birth weight, and fetal distress were higher in the high-risk group. Although except for fetal distress, the rest APOs were statistically insignificant. Quadruple test biomarkers, which were found to be significantly associated with quadruple test severity (high/low), were further included in multivariate analysis. Adjusted odds ratio (AOR) with a 95% confidence interval was calculated for the independent predictors after mutual adjustment. Results showed that patients with the high-risk group had 2.13 times the chances of low birth weight (AOR: 2.13, $P > 0.05$), 3.89 times of had chances of Corr MoM for HCG, 1.77 times the chances of Corr MoM for Inhibin-A (AOR: 1.77, $P < 0.05$) whereas had a lower risk of Corr MoM for uE3 [AOR: 0.13, $P < 0.05$, Table 3].

Similarly, CARTs analysis showed that Corr MoM for Inhibin-A and Corr MoM for HCG were the most significant predictors in high-risk patients. Whereas AFP could not have a significant association [Figure 1].

Discussion

The importance of quadruple screening as an alternative tool is being tried to be established in the past three decades as an alarming tool to predict adverse outcomes other than trisomy in pregnancies.[9-11] Studies quoted high-risk prevalence of sufferings with GDM, Hypothyroid, PTL, IUGR, PE, intrauterine death, and oligohydramnios during pregnancy globally with morbidity of 5%–10%[12] but were not in significant association with any of the trisomy marker.[10,13] In our study, we found fetal distress was observed to have a significant correlation ($P < 0.031$), and low birth weight was also close to the significance value ($P < 0.051$). While comparing the odds ratio of HCG MoM, Inhibin-A MoM and uE3 MoM, low birth weight (LBW) was mostly in accordance with biochemical markers, and fetal distress did not show a significant effect later, which might be due to small group size.

The odds of having a low birthweight baby in the high-risk group were observed to be almost four times increased in comparison to the low-risk group with an increase in HCG MoM, whereas it was almost two times higher with Inhibin A MoM value. In contrast, a study by Androutsopoulos *et al*., in 2016, reported an inverse relation between HCG with pregnancy complications, although they noticed a significant correlation of high-risk screen with LBW and oligohydramnios alike our findings,[10] they could

Table 2: Distribution of pregnancy and other variables between high- and low-risk pregnancy (*n*=252)

Variables	Total (*n*=252), *n* (%)	High (*n*=190), *n* (%)	Low (*n*=62), *n* (%)	*P*
Aneuploidy diagnosis				
No	245 (97.2)	183 (74.7)	62 (25.3)	0.199
Yes	7 (2.8)	7 (100)	0	
GDM				
No	186 (74.4)	136 (74.8)	50 (25.3)	0.322
Yes	64 (25.6)	51 (79.7)	13 (20.3)	
Hypothyroid				
No	174 (75.1)	131 (68.9)	43 (31.2)	1
Yes	79 (24.9)	59 (74.7)	20 (25.3)	
PTL				
No	202 (75.2)	149 (74.7)	53 (24.8)	0.369
Yes	52 (24.8)	42 (80.8)	10 (19.2)	
IUGR				
No	207 (75.6)	157 (75.8)	50 (24.2)	0.833
Yes	35 (24.4)	26 (74.3)	9 (25.7)	
PE				
No	228 (90.5)	172 (75.4)	56 (24.6)	1
Yes	24 (9.5)	18 (75.0)	6 (25.0)	
Fetal distress				
No	241 (75)	184 (76.3)	57 (23.7)	0.031
Yes	11 (25)	5 (45.5)	6 (54.5)	
IUD				
No	105 (78.8)	81 (74.7)	24 (25.3)	0.199
Yes	8 (21.2)	8 (100)	0	
Oligohydramnios				
No	213 (75.2)	159 (74.6)	54 (25.4)	0.698
Yes	41 (24.8)	32 (78.0)	9 (22.0)	
LBW				
No	145 (74.3)	104 (69.7)	44 (3.03)	0.051
Yes	96 (25.7)	78 (81.2)	18 (185.8)	

P<0.05 significant. Data are presented in frequency (%); compared by Chi-square test/Fisher's exact test. IUD: Intrauterine death; GDM: Gestational diabetes mellitus; PE: Preeclampsia; PTL: Preterm labor; LBW: Low birth weight; IUGR: Intra uterine growth restriction

Table 3: Independent outcomes of the quadruple test (*n*=252)

Variable's	OR	Lower	Upper	*P*
LBW	2.13	0.97	4.68	0.060
Corrected MoM for HCG	3.89	2.10	7.18	<0.001
Corrected MoM for uE3	0.13	0.05	0.35	<0.001
Corrected MoM for Inhibin-A	1.77	1.16	2.72	0.009

P<0.05 significant. Multivariate binary logistic regression analysis was used. OR: Odds ratio; HCG: Human chorionic gonadotropin; MoM: Multiple of median; LBW: Low birth weight

not predict birth weight with the help of any of the second-trimester markers.

Another study on Asian women in Finland addressed elevations of serum AFP, and free beta-hCG level in case of adverse perinatal outcomes in false-positive screens for Down syndrome were shown higher risk. Careful fetal ultrasound examination and thoughtful strategy for perinatal management were also warranted for those patients in their study[14] but that study lacked in correlating Corr MoM of the marker with adverse outcomes, which could help make it precise. Hypothyroidism was also found to be a major cause of miscarriage in some studies, but in our study, we did not observe much variations in adversity among the group comparatively.[15,16] Whereas a prospective study on elderly pregnant (>35 years) women belonging to the western part of India has found a significantly higher proportion of maternal and fetal mortality and morbidity among them. Antenatal and intranatal complications were increased in their study group along with an increased incidence of LBW babies compared to the comparison group (40.20% vs. 24%). High AFP, low hCG, and high inhibin A were risk factors for low birth weight,[17-19] which is similar to our results. In another study from Thailand in 2020, over 578 women with SGA among 10115 total pregnant women were in agreement up to an extent with our study and found a higher level of AFP (area under the curve [AUC] 0.724) and b-HCG (AUC 0.655) and lower level of uE3 (0.597) was significantly associated to the SGA in the fetal aneuploidy testing results.[20]

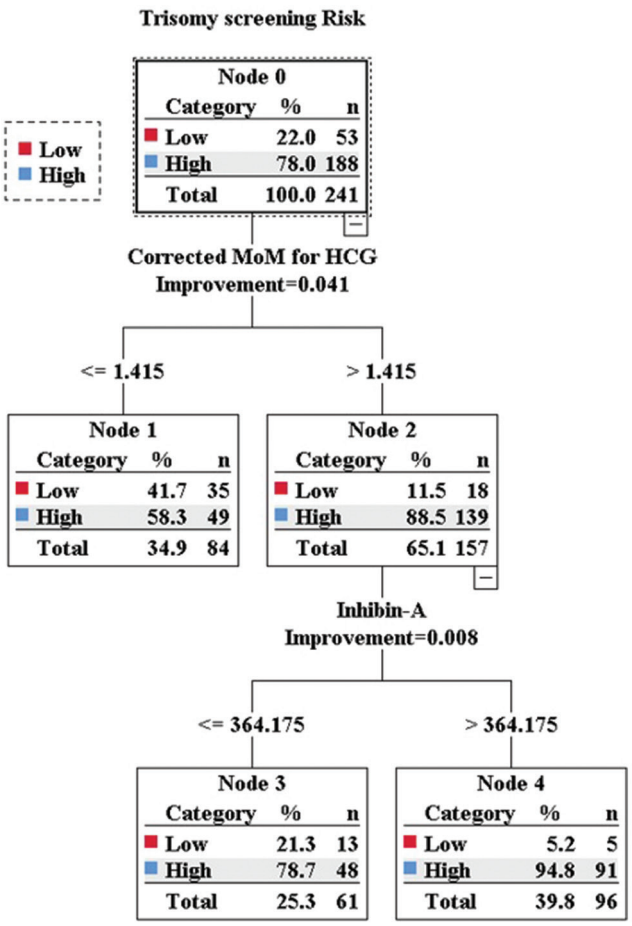

Figure 1: Classification and regression tree analysis showing the most significant predictors of the high-risk patients. Classification tree Plot shows 2 terminal Node, blue is for higher events, whereas red is for lower event. Node 1 is primary parameter and predictive power for Corr MOM-HCG > 1.415, and secondary Node is for Corr-MoM Inhibin A > 364.175 as critical value. HCG: Human chorionic gonadotropin, MoM: Multiple of median, Corr: Corrected

HCG is an important hormone for embryo implantation and growth. HCG synthesises in trophoblastic cells and helps in the development of the placenta. Its concentration increases in the first trimester and decreases in the second trimester in healthy pregnancy but deranged (greater or lesser) values projects abnormalities in placental function.[21,22] In this study, we found its increased value may predict low birth weight babies ($P < 0.005$)

When examining the statistical relationships of each marker without accounting for the impact of other markers that may be abnormal, each of the three markers was significantly associated with a low-birth weight pregnancy; For LBW outcomes, combinations of at least two markers were more strongly associated than any single marker [Table 3]. As the number of abnormal markers increased, the association with adverse outcomes became stronger.

Inhibin A is a glycoprotein hormone that has a fetoplacental origin during pregnancy and is known to have a negative feedback effect on pituitary follicle-stimulating hormone secretion. It is important in the growth of the placenta's angiogenesis and impacts direct/indirect mothers' blood pressure levels. A study from Iran in 2018 by the Broumand *et al*.[23,24] group established Inhibin A MoM = 1.25 in the second trimester had significantly associated with PE (sensitivity = 83.83%) in 300 pregnant women. However, they have considered only inhibin as one biomarker and PE in one adverse outcome in account for association establishment in limit. Whereas we found other markers among the same second-trimester screening test were also affecting many complications.

Another study for the same reason in 2015 by Yazdani *et al*.[25] on 80 quadruple positive samples among 231 total pregnancies found adverse outcomes were increased in the quadruple positive group by a significant amount. They observed PE ($P = 0.008$) was associated with inhibin A ($P < 0.001$), IUGR was ($P = 0.028$) was associated with inhibin ($P = 0.020$) and AFP ($P = 0.015$) with PROM was also ($P = 0.040$) present in trisomy high-risk group, but they could not find the association with the low birth weight with any marker in their study and the age difference between the study groups were significant in contrast of our analysis.

One more study by Moghadam *et al*. group in Iran on 240 pregnant women find uE3 (attribute = 0.265) with fetal death and AFP (attribute 1.765) with abortion with a sensitivity of 100% and 86%, respectively.[26] In our study, we did not find a significant connection with the increased level of AFP, but a decreased level of estradiol is associated with APO.[27]

A study in the nearby area in Meerut on ethnically similar people was conducted in 2018 on 360 pregnant women for finding the clinical significance of the elevated level of AFP in the 15th–20th week of pregnancy from maternal serum, but their study group had a significant difference in age of women, and they find moderate sensitivity and specificity in using this marker for diagnostic purposes.[28]

An increased level of AFP has been observed to be linked to the APOs in the absence of other abnormalities.[28,29] In Tellapragada *et al*.'s study, on the Canadian population, it was found to be associated with lower birth weight centile and gestational age at delivery with histological evidence of advanced placental maturation.[15,30] In contrast, our study AFP could not show any significant association with any of the adverse outcomes. Further studying socioeconomic causes of the rise in the marker may also address the other aspect of adversity increment.

Conclusion

Deranged values of biomarkers in quadruple screening tests may help predict APOs other than trisomy, and

these deranged values may help in monitoring them well in advance, which can reduce the level of bad obstetric outcomes concerning low-weight baby births.

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Ethical statement

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Conflicts of interest

There are no conflicts of interest.

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