

# Prediction of Preterm Birth Using the Second-Trimester Quadruple Test

**Dr.Geethanjali Gonimadata**

Vardhman Mahavir Medical College & Safdarjung Hospital

**Dr.Upma Saxena**

[drupmasaxena@gmail.com](mailto:drupmasaxena@gmail.com)

Vardhman Mahavir Medical College & Safdarjung Hospital

**Dr.Abhishek Lachyan**

Vardhman Mahavir Medical College & Safdarjung Hospital

**Dr.Neha Kawatra Madan**

Vardhman Mahavir Medical College & Safdarjung Hospital

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## Research Article

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# Abstract

## Background

Preterm birth is a major contributor to neonatal morbidity and mortality. Maternal serum biomarkers measured during the second trimester may help identify pregnancies at increased risk of preterm delivery. This study evaluated the predictive performance of the second-trimester quadruple test and its individual components for preterm birth.

## Methods

This prospective observational study included 200 pregnant women attending the antenatal and fetal medicine outpatient departments of Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi. Women with singleton pregnancies between 15 + 0 and 20 + 6 weeks of gestation were enrolled. Maternal serum alpha-fetoprotein (AFP), free beta-human chorionic gonadotropin (free  $\beta$ -hCG), unconjugated estriol (uE3), and inhibin A were measured using validated laboratory assays. The predictive performance of individual biomarkers and combinations of the four markers was evaluated using receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy were assessed at the selected cut-off values.

## Results

The four-marker model demonstrated the highest area under the ROC curve (AUC) of 0.747, followed by inhibin A (0.721), AFP (0.680), free  $\beta$ -hCG (0.659), and uE3 (0.644). At the selected cut-offs, AFP demonstrated a sensitivity of 74.5% and specificity of 48.4%, whereas inhibin A showed a sensitivity of 53.2% and specificity of 79.1%. The four-marker model demonstrated a sensitivity of 72.3%, specificity of 72.5%, positive predictive value of 44.8%, and negative predictive value of 89.5%.

## Conclusion

The second-trimester quadruple test demonstrated moderate discriminatory ability for predicting preterm birth, with better overall discrimination than individual biomarkers. These findings support further evaluation of the quadruple test as a potential component of preterm birth risk assessment in larger prospective cohorts.

## Introduction

Preterm birth, defined as delivery before 37 completed weeks of gestation, remains a major global public health concern and an important contributor to neonatal morbidity and mortality. Infants born preterm are at increased risk of respiratory complications, infections, neurodevelopmental impairment, and long-

term health consequences. Early identification of pregnancies at increased risk may facilitate appropriate surveillance, timely preventive interventions, and improved perinatal outcomes.

Several maternal and obstetric factors are associated with preterm birth, including a previous history of preterm delivery, maternal medical conditions, cervical abnormalities, and pregnancy-related complications. However, clinical risk factors alone may not adequately identify all women who will subsequently deliver preterm. Maternal serum biomarkers offer a potential complementary approach to risk assessment because they may reflect placental function, fetal development, and the maternal response to pregnancy. The second-trimester quadruple test, traditionally used for aneuploidy screening, measures alpha-fetoprotein (AFP), free beta-human chorionic gonadotropin (free  $\beta$ -hCG), unconjugated estriol (uE3), and inhibin A [1,2].

Abnormal concentrations of these biomarkers have been investigated in relation to adverse pregnancy outcomes, including preterm birth. Elevated maternal serum AFP has been associated with an increased risk of preterm delivery, potentially reflecting placental dysfunction or disruption of the fetomaternal interface [3]. Inhibin A has also been investigated as a marker of placental abnormalities and adverse pregnancy outcomes [4]. Moreover, evidence from population-based research suggests that the second-trimester quadruple test may have a role in identifying pregnancies at increased risk of spontaneous preterm birth, although its predictive performance may vary according to the population studied and the outcome definition used [5].

Despite these observations, the predictive value of individual biomarkers and their combined performance may differ across clinical settings. Evidence from Indian populations is particularly relevant because the burden of preterm birth is substantial and accessible methods of risk stratification are needed. Evaluating the quadruple test in a hospital-based population may help determine whether routinely available second-trimester biomarkers provide useful information beyond conventional clinical risk factors.

Therefore, this study aimed to evaluate the predictive performance of AFP, free  $\beta$ -hCG, uE3, and inhibin A, individually and in combination, for preterm birth among pregnant women attending Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi. The study also compared the discriminatory performance and diagnostic accuracy of individual biomarkers with those of the four-marker model.

## Methods

### Study Design and Setting

This prospective observational study was conducted over 18 months in the Department of Obstetrics and Gynaecology at Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India. The study evaluated the association between second-trimester maternal serum biomarkers and subsequent preterm birth.

## Study Population and Sample Size

A total of 200 pregnant women attending the antenatal care and fetal medicine outpatient departments were included. Eligible participants had singleton pregnancies confirmed by ultrasonography and presented between 15 + 0 and 20 + 6 weeks of gestation.

## Eligibility Criteria

Women with a confirmed singleton pregnancy within the specified gestational age range were eligible for inclusion. Exclusion criteria comprised fetal structural anomalies or suspected aneuploidy, pregnancy termination, progesterone use beyond 20 weeks of gestation, a previous history of preterm birth, current infection, and smoking or alcohol abuse, as specified in the study protocol.

## Maternal Serum Biomarker Assessment

Maternal serum concentrations of alpha-fetoprotein (AFP), free beta-human chorionic gonadotropin (free  $\beta$ -hCG), unconjugated estriol (uE3), and inhibin A were assessed during the second trimester. AFP, free  $\beta$ -hCG, and uE3 were measured using the DELFIA platform on the PerkinElmer Victor 2D system. Inhibin A was measured using an enzyme-linked immunosorbent assay (ELISA). Biomarker concentrations were evaluated using the cut-off values determined in the study. Where applicable, concentrations were expressed as multiples of the median (MoM).

## Study Outcome

The primary outcome was preterm birth, defined as delivery before 37 completed weeks of gestation. The predictive performance of individual biomarkers and combinations of the four markers was assessed in relation to this outcome.

## Statistical Analysis

The predictive performance of AFP, free  $\beta$ -hCG, uE3, and inhibin A was assessed individually and in combination. Receiver operating characteristic (ROC) curve analysis was used to evaluate discriminatory ability, expressed as the area under the curve (AUC). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated at the selected cut-off values. The performance of the four-marker model was compared with that of individual biomarkers and selected combinations. Statistical results were interpreted using the study's prespecified analytical approach.

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Ethical Considerations Ethical approval was obtained from the Institutional Ethics Committee of Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, before study initiation (approval number: IEC/VMCC/SIH/THESIS/2023/CC-163; approval date: 27 March 2023). Written informed consent was obtained from participants before enrolment. Study procedures were conducted in accordance with applicable institutional ethical requirements.

## Results

### Study Population and Biomarker Assessment

A total of 200 pregnant women with singleton pregnancies between 15+0 and 20+6 weeks of gestation were included in this prospective observational study. Maternal serum alpha-fetoprotein (AFP), free beta-human chorionic gonadotropin (free  $\beta$ -hCG), unconjugated estriol (uE3), and inhibin A were evaluated individually and in combination to assess their predictive performance for preterm birth.

### Receiver Operating Characteristic Analysis

ROC curve analysis showed that the combined four-marker model had the highest area under the curve (AUC) at 0.747. Among the individual biomarkers, inhibin A demonstrated the highest AUC (0.721), followed by AFP (0.680), free  $\beta$ -hCG (0.659), and uE3 (0.644) (Table 1).

**Table 1. Area under the receiver operating characteristic curve for individual biomarkers and the four-marker model**

| Biomarker/model                                               | AUC   |
|---------------------------------------------------------------|-------|
| AFP                                                           | 0.680 |
| Free $\beta$ -hCG                                             | 0.659 |
| uE3                                                           | 0.644 |
| Inhibin A                                                     | 0.721 |
| Four-marker model (AFP + free $\beta$ -hCG + uE3 + inhibin A) | 0.747 |

*Abbreviations:* AFP, alpha-fetoprotein;  $\beta$ -hCG, beta-human chorionic gonadotropin; uE3, unconjugated estriol; AUC, area under the curve.

### Diagnostic Performance of Individual Biomarkers

The selected cut-off values were 1.07 MoM for AFP, 0.77 MoM for free  $\beta$ -hCG, 1.38 MoM for uE3, and 1.15 MoM for inhibin A. At the selected AFP cut-off, sensitivity was 74.5%, specificity was 48.4%, PPV was 32.6%, and NPV was 86.0%. For inhibin A, sensitivity was 53.2%, specificity was 79.1%, PPV was 43.9%, and NPV was 84.6% (Table 2).

**Table 2. Diagnostic performance of selected individual biomarkers**

| Biomarker | Cut-off (MoM) | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-----------|---------------|-----------------|-----------------|---------|---------|
| AFP       | 1.07          | 74.5            | 48.4            | 32.6    | 86.0    |
| Inhibin A | 1.15          | 53.2            | 79.1            | 43.9    | 84.6    |

*Abbreviations:* MoM, multiples of the median; PPV, positive predictive value; NPV, negative predictive value.

**Diagnostic Performance of Combined Biomarkers**

The four-marker model, comprising AFP, free  $\beta$ -hCG, uE3, and inhibin A, demonstrated a sensitivity of 72.3%, specificity of 72.5%, PPV of 44.8%, and NPV of 89.5% at the selected model cut-off of 0.279. The combination of free  $\beta$ -hCG, uE3, and inhibin A showed a sensitivity of 78.7% and specificity of 65.2%. The combination of free  $\beta$ -hCG and AFP showed a sensitivity of 67.4% and specificity of 84.3% (Table 3).

**Table 3. Diagnostic performance of selected biomarker combinations**

| Biomarker combination               | Sensitivity (%) | Specificity (%) |
|-------------------------------------|-----------------|-----------------|
| Free $\beta$ -hCG + uE3 + inhibin A | 78.7            | 65.2            |
| Free $\beta$ -hCG + AFP             | 67.4            | 84.3            |
| Four-marker model                   | 72.3            | 72.5            |

**Comparison of Diagnostic Accuracy**

The reported diagnostic accuracy was 73.0% for inhibin A, 68.2% for AFP, and 72.5% for the four-marker model (Table 4). Although the four-marker model achieved the highest AUC, inhibin A demonstrated the highest reported diagnostic accuracy among these three approaches.

**Table 4. Comparison of reported diagnostic accuracy**

| Biomarker/model   | Diagnostic accuracy (%) |
|-------------------|-------------------------|
| AFP               | 68.2                    |
| Inhibin A         | 73.0                    |
| Four-marker model | 72.5                    |

Overall, the four-marker model demonstrated the highest discriminatory ability based on AUC, while inhibin A showed the highest reported diagnostic accuracy among the individual-marker and combined-model comparisons presented.

## Discussion

This prospective observational study evaluated the predictive performance of second-trimester maternal serum alpha-fetoprotein (AFP), free beta-human chorionic gonadotropin (free  $\beta$ -hCG), unconjugated estriol (uE3), and inhibin A for preterm birth among 200 pregnant women. The principal finding was that the combined four-marker model demonstrated the highest area under the receiver operating characteristic curve (AUC = 0.747), indicating moderate discriminatory ability. Among the individual biomarkers, inhibin A demonstrated the highest AUC (0.721), followed by AFP (0.680), free  $\beta$ -hCG (0.659), and uE3 (0.644). These findings suggest that a combination of maternal serum biomarkers may provide more useful discrimination than individual markers alone.

The second-trimester quadruple test is routinely used for fetal aneuploidy screening and measures biomarkers associated with fetal development and placental function. Previous research has examined the relationship between these markers and adverse pregnancy outcomes, including preterm birth [1,2]. Dugoff et al. investigated the association between second-trimester serum screening markers and subsequent pregnancy outcomes, highlighting the potential of these routinely collected biomarkers to identify pregnancies at increased risk of complications [2]. However, their value for predicting preterm birth should be considered separately from their established role in aneuploidy screening.

In the present study, AFP demonstrated a sensitivity of 74.5% and specificity of 48.4% at the selected cut-off of 1.07 multiples of the median (MoM). Although this sensitivity indicates that AFP may identify a proportion of pregnancies subsequently resulting in preterm delivery, its relatively low specificity suggests a considerable risk of false-positive classification. A systematic review and meta-analysis by Yuan et al. reported an association between elevated maternal serum AFP in the second trimester and increased preterm birth risk [3]. This association may reflect placental dysfunction or disruption of the fetomaternal interface. Nevertheless, AFP alone may have limited clinical utility as a screening marker because its performance depends on the threshold applied and the characteristics of the population being assessed.

Inhibin A demonstrated the highest AUC among the individual biomarkers (0.721), with a sensitivity of 53.2% and specificity of 79.1% at a cut-off of 1.15 MoM. Its comparatively higher specificity suggests potential value in identifying pregnancies with an increased likelihood of preterm delivery. However, the moderate sensitivity indicates that inhibin A alone would fail to identify a substantial proportion of pregnancies that subsequently deliver preterm. Previous research has investigated second-trimester inhibin A concentrations in relation to adverse pregnancy outcomes, supporting continued evaluation of this marker in obstetric risk assessment [4]. Differences in study populations, outcome definitions, and biomarker thresholds may account for variations in the reported predictive performance.

The four-marker model achieved an AUC of 0.747, compared with 0.721 for inhibin A alone. Its sensitivity was 72.3%, specificity was 72.5%, positive predictive value (PPV) was 44.8%, and negative predictive value (NPV) was 89.5% at the selected model threshold. These findings suggest that the combined model may offer a more balanced approach to identifying pregnancies at increased risk than some

individual biomarkers. However, the PPV indicates that a substantial proportion of women classified as high risk did not experience the outcome. Predictive values are influenced by outcome prevalence and should therefore be interpreted in the context of the study population. The relatively high NPV should likewise not be interpreted as proof that preterm birth can be reliably excluded in all women with a low-risk result [5-11]

The diagnostic accuracy results require a separate interpretation from the AUC findings. Inhibin A had a reported diagnostic accuracy of 73.0%, compared with 72.5% for the four-marker model and 68.2% for AFP. Thus, although the combined model demonstrated the highest AUC, inhibin A had the highest reported diagnostic accuracy among these comparisons. AUC reflects the ability of a marker or model to discriminate between individuals with and without the outcome across different thresholds, whereas diagnostic accuracy represents the proportion of correctly classified individuals at a particular threshold. Consequently, these measures may produce different rankings without being inherently contradictory.

Evidence from a population-based study in a developing-country setting has also evaluated the second-trimester quadruple test for predicting spontaneous preterm birth [5,12-16]. This research supports the importance of assessing the predictive performance of routinely available serum markers in different healthcare settings. Nevertheless, direct comparisons between studies should be made cautiously because predictive performance may vary according to the prevalence of preterm birth, the characteristics of the population, the definition of the outcome, and the analytical approach used. The findings of the present study provide additional hospital-based evidence from India but do not establish that the quadruple test can replace established clinical assessment or other validated approaches to preterm birth risk prediction.

The potential clinical relevance of the quadruple test lies in its use as an adjunct to routine antenatal assessment. Where the test is already performed for aneuploidy screening, its biomarkers may offer additional information without requiring a separate biomarker panel. However, the present findings do not establish the cost-effectiveness of this approach or demonstrate that biomarker-guided management improves maternal or neonatal outcomes. Before implementation, a prediction model should be evaluated for calibration, external validity, and incremental value beyond established clinical risk factors. The consequences of false-positive and false-negative classifications should also be considered when selecting thresholds for clinical use.

This study has several strengths, including its prospective observational design, assessment of four maternal serum biomarkers, and comparison of individual markers with a combined model. However, several limitations should be acknowledged. First, the sample size of 200 participants and the single-centre setting may limit the precision and generalisability of the findings. Second, the estimates of sensitivity, specificity, PPV, NPV, and diagnostic accuracy depend on the selected thresholds and the frequency of preterm birth in the study population. Third, the predictive performance of the model requires independent validation before it can be recommended for clinical use. In addition, distinguishing spontaneous preterm birth from medically indicated preterm delivery is important because these

outcomes may have different underlying mechanisms and clinical predictors. Future multicentre studies should report confidence intervals, evaluate model calibration, and compare the incremental predictive value of serum biomarkers with established maternal and obstetric risk factors.

## Conclusion

The second-trimester quadruple test may have potential as an adjunctive tool for preterm birth risk assessment. The combined evaluation of maternal serum biomarkers may provide additional information beyond individual markers. However, further validation in larger, multicentre studies is required before recommending its routine clinical use. Future research should assess its predictive performance across diverse populations and determine its incremental value alongside established maternal and obstetric risk factors.

## Declarations

### Ethics Approval and Consent to Participate

Ethical approval was obtained from the Institutional Ethics Committee of Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi, India (Approval No. IEC/VMCC/SIH/THESIS/2023/CC-163; dated 27 March 2023). Written informed consent was obtained from all participants before enrolment.

### Consent for Publication

Not applicable.

### Availability of Data and Materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to institutional policies and applicable ethical requirements.

### Competing Interests

The authors declare that they have no competing interests.

### Funding

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### Authors' Contributions

All authors contributed to the study conception and design, data collection, analysis and interpretation, manuscript drafting, and critical revision. All authors read and approved the final manuscript.

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## Figures

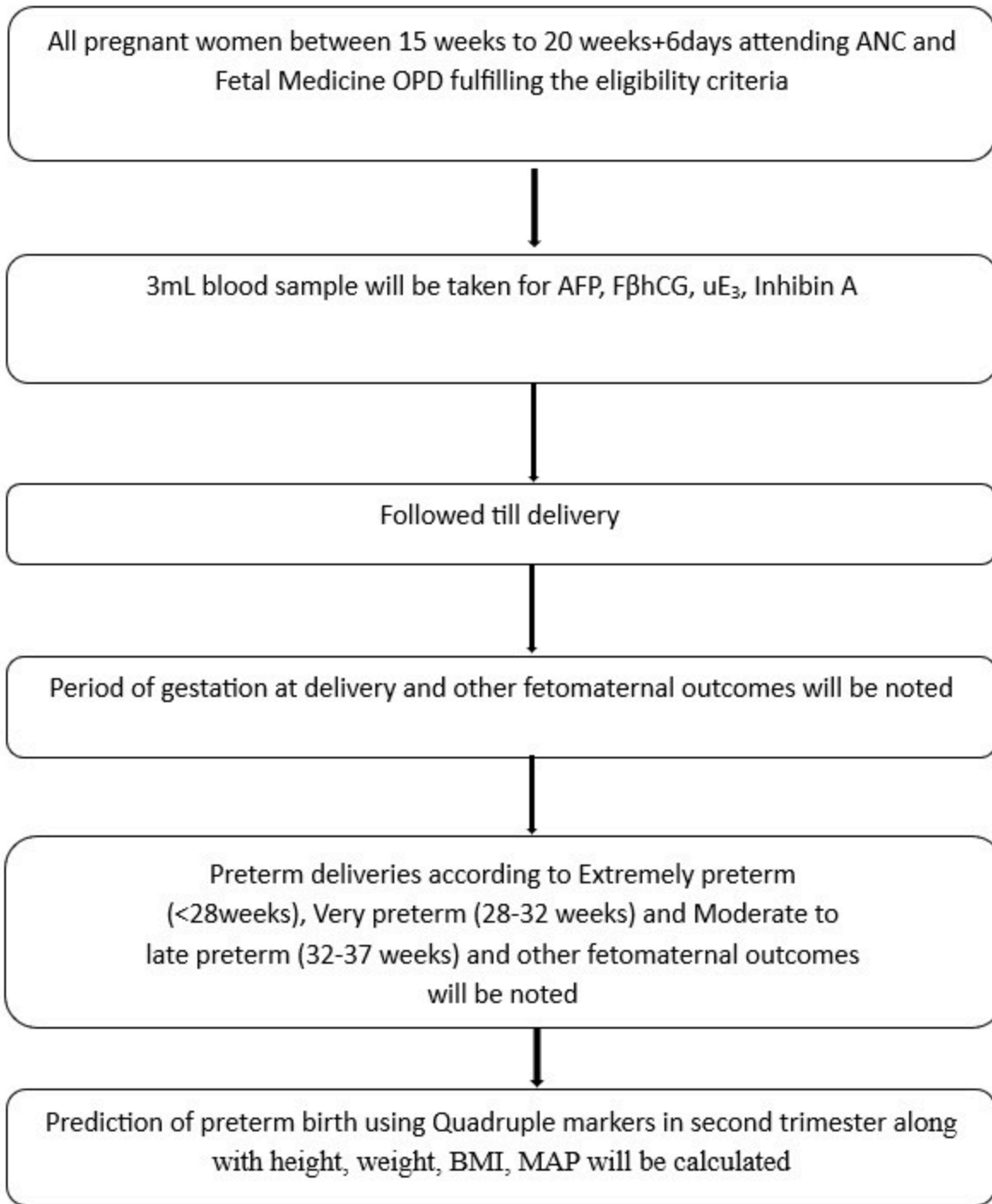


Figure 1

### Study Flow Chart