"AN ANALYSIS OF CERVICOVAGINAL β-hCG AND PROLACTIN LEVELS AS A PREDICTIVE BIOMARKER OF PRETERM BIRTH IN SYMPTOMATIC WOMEN: A PROSPECTIVE COHORT STUDY"

By

Dr. AKSHITHA SAI RAGAM MBBS

DISSERTATION SUBMITTED TO SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SURGERY

IN

OBSTETRICS AND GYNAECOLOGY

Under the Guidance of

DR. SHEELA S R

PROFESSOR

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

Co-Guide

DR. SHASHIDHAR K N

PROFESSOR & HOD

DEPARTMENT OF BIOCHEMISTRY

SRI DEVARAJ URS MEDICAL COLLEGE

TAMAKA, KOLAR 563101

JUNE 2023

ALMA MATER

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R L JALAPPA HOSPITAL AND RESEARCH CENTRE

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TAMAKA, KOLAR - 563101

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Date:

Place: Kolar

SIGNATURE OF THE GUIDE

Dr. SHEELA S R

Professor
Department of OBG Sri
Devaraj Urs Medical College,
Tamaka, Kolar- 563101

SRI DEVARAJ URS MEDICAL COLLEGE

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Date:

Place: Kolar

SIGNATURE OF THE CO-GUIDE

Dr. SHASHIDHAR K N

Professor and Head of Department

Department of Biochemistry

Sri Devaraj Urs Medical College,

Tamaka, Kolar - 563101

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Dr. RATHNAMMA P
Associate Professor & Head of Department
Department Of Obstetrics & Gynaecology
Sri Devaraj Urs Medical College,
Tamaka, Kolar

Dr. SREERAMULU P N
Principal
Sri Devaraj Urs Medical College,
Tamaka, Kolar

SRI DEVARAJ URS MEDICAL COLLEGE

TAMAKA, KOLAR - 563101

ETHICS COMMITTEE CERTIFICATE

This is to certify that the Ethics committee of Sri Devaraj Urs Medical College, Tamaka, Kolar, has unanimously approved **Dr. AKSHITHA SAI RAGAM ,** a post-graduate student in the subject of **OBSTETRICS AND GYNAECOLOGY** at Sri Devaraj Urs Medical College, Kolar to take up the dissertation work entitled **"AN ANALYSIS OF CERVICOVAGINAL β-hCG AND PROLACTIN LEVELS AS A PREDICTIVE BIOMARKER OF PRETERM BIRTH IN SYMPTOMATIC WOMEN: A PROSPECTIVE COHORT STUDY"** to be submitted to **SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH CENTRE, TAMAKA, KOLAR.**

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ABSTRACT

BACKGROUND: Preterm birth is a major cause of neonatal and infant illness and mortality in developing countries. In Northern India, there is an increase in preterm delivery and the prevalence is about 5-9%. It is associated with severe suffering for both the mother and neonate as well as long-term disabilities such as developmental delays, cerebral palsy and learning disabilities. Hence, interventions to prevent preterm birth are critical. Hence, accurate markers for determining whether a pregnant woman is at high risk for preterm delivery could lead to better surveillance and a more timely intervention to improve outcomes.

AIMS: To determine and compare the predictive value of cervicovaginal β-hCG and Prolactin levels for preterm delivery in symptomatic women.

MATERIALS & METHODS: All the consenting eligible pregnant women who were hospitalized in the labour room were recruited for the study. Cervicovaginal fluid samples from the women who were for the measurement of β-hCG and Prolactin levels. They were followed up till their delivery and divided into two groups depending on the outcome i.e. whether they had a term delivery or preterm delivery.

RESULTS: A total of 40 women were included in the study of which 28 (70%) progressed to have a preterm delivery and the rest 12 (30%) continued till term. There was a statistically significant difference found between delivery outcome and serum cervicovaginal β-hCG and Prolactin levels with p-value < 0.001. The study reported that at a cut-off of > 15.24 mIU/mL the sensitivity, specificity, PPV and NPV of cervicovaginal β-hCG in predicting preterm delivery was found to be 89.7%, 100%, 100% and 92.3% respectively. Whereas, the sensitivity, specificity, PPV and NPV of cervicovaginal prolactin at cut-off > 8.24 ng/ml in predicting preterm delivery was found to be 89.29%, 83.3%, 92.6% and 76.9% respectively.

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Dr. AKSHITHA SAI RAGAM

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LIST OF ABBREVIATIONS

β-hCG	Beta - Human Chorionic Gonadotropin
PPROM	Preterm Prelabour Rupture of Membranes
PROM	Prelabour Rupture of Membranes
CTG	Cardiotocography
GBS	Group B Streptococcus
RDS	Respiratory Distress Syndrome
NEC	Necrotizing Enterocolitis
IVH	Intraventricular Haemorrhage
PVL	Periventricular Leukomalacia
BPD	Bronchopulmonary dysplasia
ROP	Retinopathy of Prematurity
IV	Intravenous
fFN	fetal Fibronectin
phIGFBP-1	phosphorylated Insulin-like Growth Factor Binding Protein-1
kDa	kilo Daltons

IU	International Units
LH	Luteinizing Hormone
ELISA	Enzyme Linked Immuno Sorbent Assay
PRL	Prolactin
3D	3-Dimensional
mIU	Milli International Units
CI	Confidence Interval

ABSTRACT

BACKGROUND: Preterm birth is a major cause of neonatal and infant illness and mortality in developing countries. In Southern India, there is an increase in preterm delivery and the prevalence is about 5-8%.[3] It is associated with severe suffering for both the mother and neonate as well as long-term disabilities such as developmental delays, cerebral palsy and learning disabilities; hence interventions to prevent preterm birth are critical. Hence, accurate markers for determining whether a pregnant woman is at high risk for preterm delivery could lead to better surveillance and a more timely intervention to improve outcomes.

AIMS: To determine and compare the predictive value of cervicovaginal β-HCG and Prolactin levels for preterm delivery in symptomatic women.

MATERIALS & METHODS: All the consenting eligible pregnant women who were hospitalized to the labour room were recruited for the study. Cervicovaginal fluid samples from the women was sent for the quantitative estimation of β-hCG and Prolactin with commercially available chemiluminescent enzyme immunometric assay kit. They were followed up till their delivery and divided into two groups depending on the outcome i.e, whether they had a term delivery or preterm delivery.

RESULTS: A total of 40 women were included in the study of which 28 (70%) progressed to have a preterm delivery and the rest 12 (30%) continued till term. There was a statistically significant difference found between delivery outcome and mean cervicovaginal β-hCG and Prolactin levels with p-value < 0.001. This study reported that at a cut-off of > 15.54 mIU/ml, the sensitivity, specificity, PPV and NPV of cervicovaginal β-hCG in predicting preterm delivery was found to be 60.7%, 100%, 100% and 52.2% respectively. Whereas, the sensitivity, specificity, PPV and NPV

of cervicovaginal prolactin at cut-off > 6.24 ng/ml in predicting preterm delivery was found to be 89.29%, 83.3%, 92.6% and 76.9% respectively.

CONCLUSION: The optimal cut-off value for cervicovaginal β-hCG and Prolactin in predicting preterm delivery was reported to be 15.54 mIu/ml and 6.24 ng/ml respectively. Cervicovaginal Prolactin level was found to be a better predictor of preterm delivery in symptomatic women when compared to cervicovaginal β-hCG level.

INTRODUCTION

Preterm birth, as defined by The World Health Organisation, is "Any birth before 37 completed weeks of gestation or fewer than 259 days of gestation since the first day of the last menstrual period." [1] Approximately 15 million premature babies are born every year with around 1 million deaths being attributed to the complications arising from prematurity.[2] Two-thirds of preterm births are spontaneous, while the rest comprise of induced preterm births for medical reasons such as fetal growth restriction, pre-eclampsia, placental abruption among many others. Preterm birth is responsible for approximately 75% of neonatal deaths, with the majority of them occurring in babies born before 34 weeks of gestation. [3-4]

The causes of spontaneous preterm labour are complex and the pathophysiology is largely unexplored. However, predisposing factors include maternal, fetal and placental factors, as well as mechanical factors such as uterine overdistention and cervical incompetence, bacterial infection, inflammation, and hormonal changes. [5]

Accurate prediction of preterm birth has been a continuing and vexing challenge as clinical symptoms alone lack adequate predictive accuracy. Although research on various biomarkers in maternal serum, cervicovaginal fluid and amniotic fluid is currently underway, no single biomarker with the sensitivity and reliability to detect spontaneous preterm birth has emerged to date. A single biomarker or even a combination, if found, can help expedite timely intervention, improve the outcome and reduce the associated perinatal morbidity and mortality.

During pregnancy, prolactin, a protein, is secreted by the decidua, amnion, cytotrophoblast, and syncytiotrophoblast. Its concentration in amniotic fluid peaks during the second trimester and then plateaus thereafter.[6-8]

β - Human Chorionic Gonadotropin (β-hCG) is a glycoprotein secreted by the syncytiotrophoblast during pregnancy, with fluctuating concentrations in maternal serum, amniotic fluid, and urine. It is found in high concentrations until 20 weeks of pregnancy but then falls to a steady low level in the second and third trimesters. [9]

The rationale behind the presence of both these markers i.e, β-hCG and prolactin in the cervicovaginal fluid has been hypothesized to be due to subclinical membrane rupture or injury or decidual-membrane separation during the process of labour which could serve as effective predictors of preterm birth. [10]

NEED FOR STUDY

The distribution of the burden of preterm birth across the world is disproportionate, with the highest rates being reported from Asia and Africa i.e, 54% and 31% respectively. India shares the highest burden of preterm births and contributes to two-thirds of the total with the incidence being reported to be 14.5%.[11-12]

Although our knowledge of human parturition has advanced, the ability to precisely predict preterm labour which precedes preterm delivery has remained elusive. Preterm labour has traditionally been diagnosed using clinical indicators such as a previous history of preterm birth, symptoms, and a clinical examination. However, they have been shown to be of limited use in predicting spontaneous preterm labour.[13] A number of biochemical markers in various body fluids such as fetal Fibronectin, Phosphorylated insulin-like growth factor binding protein-1, Plasma urocortin, Human chorionic gonadotropin are being studied and have shown promise to be potential

predictors.[14-16] However, the statistical determinants have varied markedly in different studies and are far from optimal in some.

Accurate markers for determining whether a pregnant woman is at high risk for preterm delivery could lead to better surveillance and a more timely intervention to improve outcomes.

Various studies have shown the presence of β-hCG and prolactin in cervicovaginal fluid and have established the importance of these biomarkers in preterm labour. However, there is a dearth in studies comparing the diagnostic value of these markers in predicting preterm birth. Hence, this study has been planned to further the knowledge about potential biomarkers that could possibly help in predicting preterm births.

AIMS & OBJECTIVES

1. To determine the predictive value of cervicovaginal β-HCG and Prolactin levels for preterm delivery in symptomatic women
2. To compare the predictive value of cervicovaginal β-HCG and Prolactin levels for preterm delivery in symptomatic women

REVIEW OF LITERATURE

1) Preterm labour:

Definition,

Preterm labour is defined as "onset of labour associated with regular and painful uterine contractions that occur with increasing frequency and intensity with progressive cervical changes of effacement and dilatation after 28 weeks of gestation and before 37 completed weeks of gestation." [17]

Based on the gestational age, preterm is subdivided into the following: [18]

1. Extremely preterm: < 28 weeks
2. Very preterm: 28 $^{0/7}$ weeks to 31 $^{6/7}$ weeks
3. Moderate preterm: 32 $^{0/7}$ weeks to 33 $^{6/7}$ weeks
4. Late preterm: 34 $^{0/7}$ weeks to 36 $^{6/7}$ weeks

Epidemiology,

There is no recent accurate data on the global prevalence of preterm labour, but estimates range from 5% in developed countries to 25% in developing countries. [19] Preterm birth accounts for 70% of fetal deaths and around 50% of neural deficits in neonates. [20-21]

Risk factors associated with Preterm Labour, [22]

A. Black race
B. Advanced maternal age
C. Cocaine, tobacco or heroin use
D. History of preterm labour/ delivery

E. Cervical insufficiency - Idiopathic, Iatrogenic (Cervical surgeries or forced dilatation)

F. Infections of the maternal urogenital tracts - Gardnerella vaginalis, trichomoniasis, bacterial vaginosis etc.

G. Low pre-pregnancy body mass index ($\leq$ 19.8 kg per m^2)

H. Medical disorders such as diabetes mellitus, thyroid disease, hypertension, autoimmune diseases

I. Hormonal changes - maternal or fetal stress

J. Mechanical factors such as uterine overdistension due to multi-fetal pregnancy and polyhydramnios

K. Fetal causes - Growth restriction, anomalies, abnormal lie or presentation

L. Uterine distortion due to fibroid uterus or mullerian duct abnormalities

M. Placental abruption or placenta previa

N. Environmental factors

O. Genetic factors

Pathophysiology of Preterm Labour, [23-24]

Each pathway to preterm labour has its own set of initiators, with the majority of these pathways eventually sharing common effectors like cytokines, matrix metalloproteinases, and prostaglandins. Ultimately, uterine contractions and cervical changes are initiated, resulting in premature delivery and premature preterm membrane rupture (PPROM).

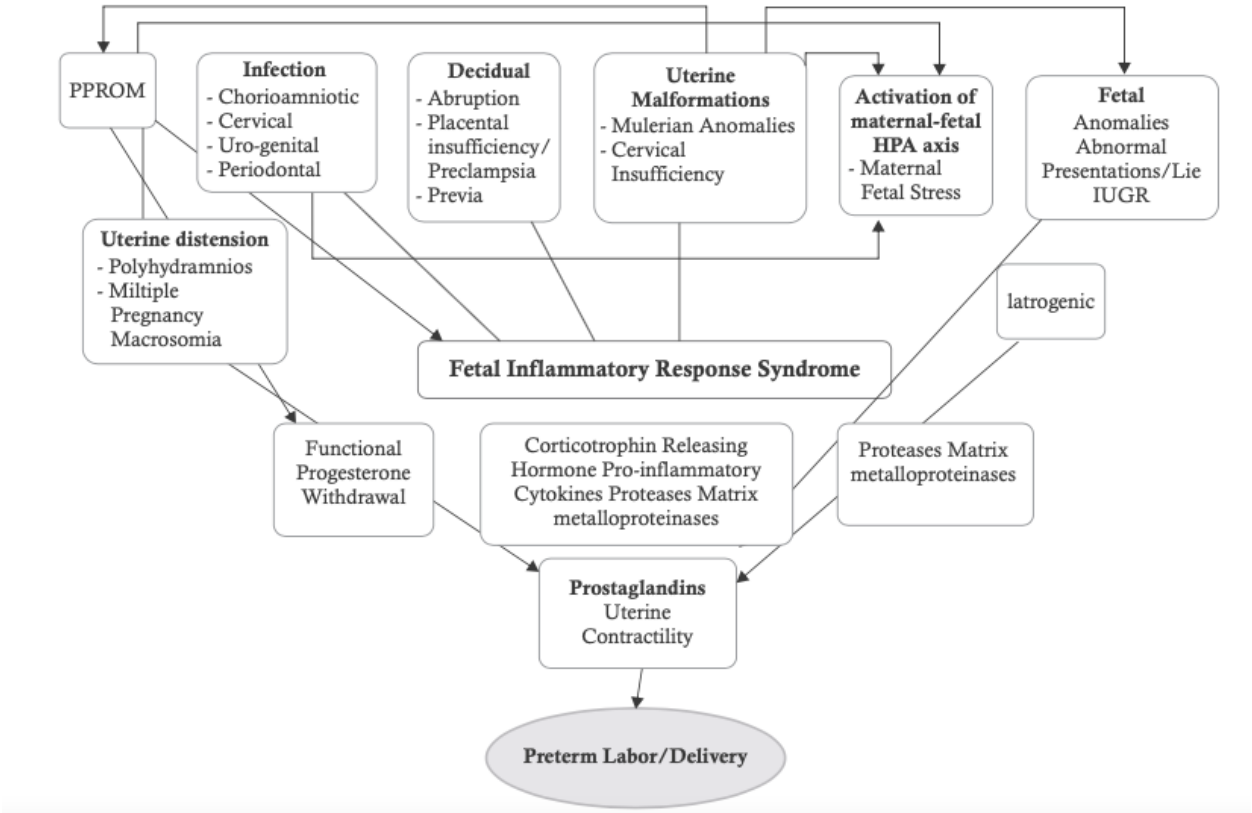


Figure 1: Pathophysiology of Preterm Labour

Clinical Assessment of Preterm Labour, [25]

- History taking: Sociodemographic, Medical, Surgical and Obstetrical history - antenatal notes, estimated date of delivery and ultrasound scans
- Signs and Symptoms: The most common order preceding preterm birth is cervical ripening, followed by decidual membrane activation and contractions which are characterized by:
    - Cervical effacement and dilatation
    - Lower abdominal cramping
    - Lower back pain
    - Regular uterine activity
    - Vaginal discharge (mucous, blood or fluid)

- Physical examination:
    - Vital signs: Pulse rate, Blood pressure, Temperature, Respiratory rate
    - Abdominal palpation to assess: Uterine tone, contractions, fetal size and presentation
- Sterile speculum examination to:
    - Confirm or exclude rupture of membranes
    - Assess liquor (Clear/ meconium stained/ bloody)
    - Visualize cervix and membranes
- Sterile digital vaginal examination is done to assess cervical changes
- Fetal surveillance:
    - Ultrasound examination: Fetal number, presentation, liquor volume and placental localization
    - Monitoring of fetal well-being by Cardiotocography (CTG) and intermittent auscultation
- Laboratory Investigations:
    - High vaginal swab for Bacterial vaginosis (microscopy culture and sensitivity)
    - Genital swabs for Group B Streptococcus (GBS)
    - Midstream specimen of urine for bacteriology

Complications, [26]

Early Neonatal Complications,

Respiratory Distress Syndrome (RDS): It was one of the first recognised complications of prematurity and is still the major cause of neonatal morbidity and mortality. It results from inadequate production of surfactant by the premature lung resulting in decreased lung compliance and gaseous exchange. Signs and symptoms include grunting, retractions, tachypnea, hypoxia, hypercarbia and acidosis.

Sepsis: It is a systemic inflammatory response, resulting from bacterial infections such as Staphylococcus, Streptococcus or Gram negative bacteria. In severe cases, it advances to multiorgan dysfunction and sometimes death, regardless of appropriate antimicrobial therapy. It has been linked to poor neurodevelopmental and growth outcomes in infants, particularly those with recurrent infections.

Necrotizing Enterocolitis (NEC): The most serious neonatal gastrointestinal complication, it's pathogenesis is complex and is poorly understood. The immaturity of the gastrointestinal mucosa along with abnormal bacterial colonization and ischemic insult are believed to lead to Necrotizing Enterocolitis. It has a varied presentation. The disease onset can be insidious with abdominal distension, feed intolerance or lethargy, or it could have a precipitous onset with intestinal perforation, metabolic acidosis or hypotension. In a majority of the cases, conservative management consisting of antibiotic therapy and bowel rest is sufficient. In 20 - 40 % of cases, intervention would be needed. Long term complications include intestinal strictures, feed

intolerance, short bowel syndrome and neurodevelopmental affliction. Mortality rates range from 15 - 30 %. [27-28]

Intraventricular Haemorrhage (IVH): Intraventricular haemorrhage refers to bleeding into the ventricles of the brain with probable extension into the parenchyma in severe cases which could lead to post haemorrhagic hydrocephalus. Mortality rates range from 28 - 37 %, while long-term disabilities such as recurrent seizures, cerebral palsy, and cognitive impairment are a risk for surviving infants.[29]

Periventricular Leukomalacia (PVL): Periventricular leukomalacia is a kind of white matter injury associated with the development of cerebral palsy. The key factors involved are cerebral ischemia and systemic inflammation following infections which lead to the activation of microglia causing the release of a variety of toxic inflammatory mediators such as cytokines and reactive oxygen species that damage the pre-myelinating oligodendrocytes. [30] In such cases, there is a delayed manifestation of associated neurocognitive and motor deficits, much after the discharge from hospital.

Bronchopulmonary dysplasia (BPD): Bronchopulmonary dysplasia is a chronic disease that affects nearly 30% of infants born with extremely low birth weight. Factors implicated in the pathogenesis are barotrauma, production of reactive oxygen species following inflammation, which injure the small airways and interfere with alveolarization and development of pulmonary microvasculature. Preterm infants requiring prolonged ventilatory support are more likely to develop Bronchopulmonary dysplasia. Sequelae include recurrent pulmonary infections, increased airway reactivity and failure to thrive. [31-32]

Retinopathy of prematurity (ROP): Each year, nearly 50,000 infants are affected by this major cause of severe visual impairment or blindness in preterm infants. It is marked by abnormal vascular proliferation in the immature retina caused by increased angiogenic growth factors and local reactive oxygen species. It is most commonly associated with fetal growth restriction, male gender, extreme prematurity, hyperoxia and septicemia. [33]

Preterm infants who suffer one or more of the complications listed above are at risk for neurodevelopmental disabilities such as developmental delay, mental retardation, and cerebral palsy. Recent research has also shown that these infants may have more subtle impairments such as impaired social skills, cognitive impairment, and psychiatric issues, particularly anxiety, depression and autism spectrum disorders. [34]

Management, [35]

The gestational age at which the pregnant woman presents to the hospital determines management. If she presents after 34 weeks of gestation, she is admitted and observed for about four to six hours for any progressive cervical changes and fetal well-being. If it is an uncomplicated pregnancy with no progressive cervical changes with a reactive non-stress test, she can be sent home with instructions for weekly follow-up and to return in case of persisting signs and symptoms or other pregnancy concerns.

Pregnant women who present with signs and symptoms of preterm labour before 34 weeks of gestation are hospitalized. In preterm labour with intact membranes with no contraindications, tocolytic drugs are used to inhibit the progression of labour for up to 48 hours until the course of corticosteroids is completed.

The only preferred intervention is a single course of corticosteroids administered between 24 and 34 weeks of gestation once preterm labour is confirmed in order to improve the neonatal outcomes. Either betamethasone (two 12 mg doses given intramuscularly 24 hours apart) or dexamethasone (four 6 mg doses given intramuscularly every 12 hours) is given.

Magnesium Sulphate has been shown to offer neuroprotection for the fetus, reducing fetal cerebral palsy and motor dysfunction.[36-37] The administration of a bolus of Magnesium Sulphate in women at imminent risk of preterm birth at less than 30 weeks gestation has been shown to reduce neonatal deaths and cerebral palsy by as much as 15%. [38] An intravenous (IV) bolus of 4 gram of Magnesium Sulphate is administered over 15 minutes which is followed by IV infusion of 1 gram per hour until the delivery or for 24 hours, whichever is sooner. Maternal monitoring of pulse, blood pressure, respiratory rate and deep tendon reflexes every 4th hourly for signs of toxicity is necessary during the administration. [36]

Antibiotics have no place in the treatment of preterm labour as they have been linked to an increased risk of cerebral palsy. However, if a woman is in active preterm labour, IV antibiotics for Group B Streptococcus (GBS) prophylaxis are advised to reduce the risk of neonatal sepsis. [39]

2) Biochemical markers of preterm birth :

Biomarkers have been defined as "parameters which can be measured in a biological sample, and which provide information on an exposure, or on the actual or potential effects of that exposure in an individual or in a group." In recent times, biological fluids such as amniotic fluid, cervicovaginal fluid, plasma, serum, urine, saliva and cord blood have been analyzed to assess the predictive value of biomarkers for preterm birth. The two widely used biochemical predictors of preterm birth are fetal Fibronectin (fFN), and phosphorylated Insulin-like Growth Factor Binding Protein-1 (phIGFBP-1) which are suggestive of disruption of the choriodecidual membrane when present in cervicovaginal fluid.

The fetal Fibronectin (fFN) test is most reliable in the prediction of spontaneous preterm birth within 7-10 days of a woman presenting with threatened preterm labour. The absence of fetal Fibronectin (fFN) is typically utilized as a negative diagnostic test, meaning that the pregnant woman has a low probability of giving birth prematurely. [40] A meta-analysis of six trials on 546 symptomatic singleton pregnancies conducted in 2016 found that fetal Fibronectin (fFN) testing was costly and had no relation to perinatal outcomes or the prevention of preterm delivery in singleton pregnancies. [41]

Various studies have shown that cervical detection of phosphorylated Insulin-like Growth Factor Binding Protein-1 (phIGFBP-1) is a rapid and easily applicable test that could help in predicting preterm delivery in patients at risk. [42]

Alternative biomarkers have been explored for their ability to predict preterm birth due to the relatively low sensitivity and positive predictive value of the fFN and phIGFBP-1 tests. They include activin–A [43], albumin/vitamin D-binding protein (VDBP)[44], alkaline phosphatase (AP)[45],

alpha-fetoprotein (AFP)[46], angiogenin[47], Corticotropin releasing hormone (CRH)[48], C-reactive protein (CRP)[49], defensins[50], elastase[51], endoglin[52], ferritin[53], Granulocyte- Colony Stimulating Factor (G-CSF)[54,57], IGFBP-4[55], Interleukin-1, 2, 6, 8, IL-2 receptors (IL2R)[56], interferon-gamma (IFN-γ)[57], lactate[58], matrix metalloproteinases [59], placental alpha macroglobulin-1 (PAMG-1)[60], pregnancy associated plasma protein-A (PAPP-A)[61], relaxin[62], salivary estriol and progesterone [63], sex hormone-binding globulin (SHBG)[64], sialidase[65], thioredoxin[66], Tumour Necrosis Factor - Alpha (TNF-α)[57], thrombin-antithrombin III complex[67], vascular endothelial growth factor[52] and proteomic profile.[68] In clinical settings, however, none of these biomarkers have consistently predicted spontaneous preterm birth.

Menon R et al., [69] in 2011, reviewed articles from the last 40 years on preterm birth biomarkers to trace the existing knowledge and to understand the lacunae. 217 studies between 1965 and 2008 were analyzed and a total of 116 different biomarkers were reported. They concluded that due to heterogeneities in research design, sampling difficulties, assay methods and analysis, finding comparable studies on biomarkers for preterm birth prediction was a difficult task. Inadequate phenotypic definition, non-ideal study designs, population stratification problems, and inadequate rationalization for biomarker selection are some of the main areas of concern found in this analysis.

3) Human chorionic gonadotropin (hCG):

Human chorionic gonadotropin is a glycoprotein, i.e, it has a peptide framework with carbohydrate side chains. It comprises of 237 amino acids with a molecular weight of 25.7 kDa and a half life of 24 hours. hCG is made up of two subunits called alpha (α) and beta (β), which are non-covalently linked by disulphide bonds. The α-subunit consisting of 92 amino acids is identical to FSH, LH and TSH. The β-subunit has a larger carbohydrate moiety and 145 amino acid residues, including a unique 23-amino acid carboxy terminal tail piece. This distinct feature of the hCG structure enables the production of highly specific antibodies and the use of highly specific immunological assays. [70]

The α-subunit is encoded by one single gene on chromosome 6, while the β-subunit is encoded by six highly homologous genes on chromosome 19. [71]

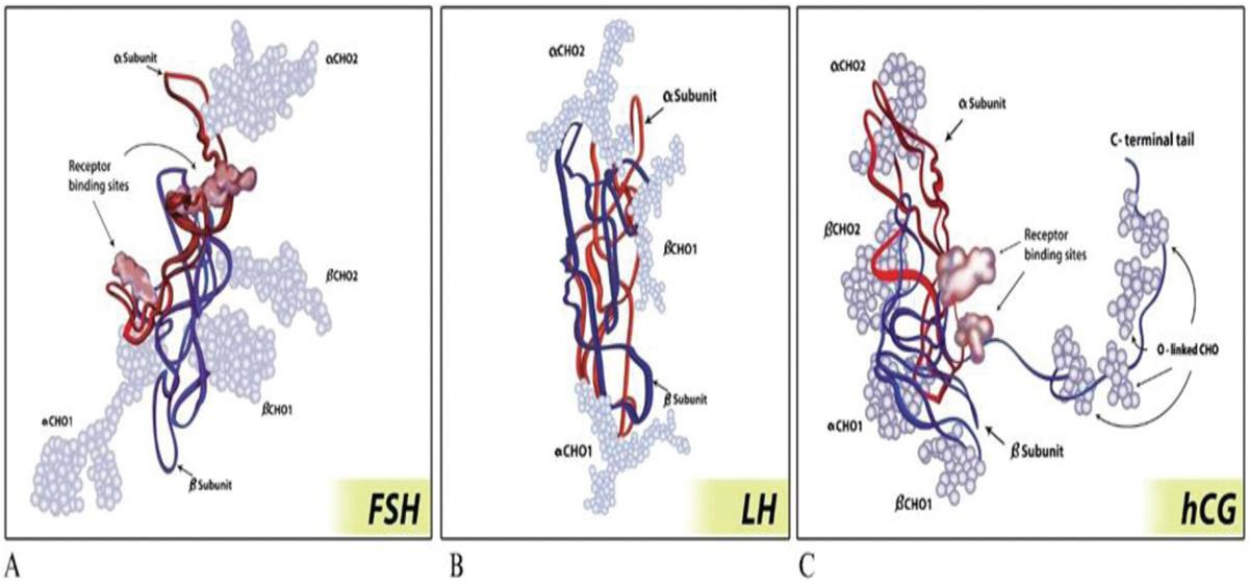


Figure 2: Molecular structures of (A) Follicle Stimulating Hormone (B) Luteinizing Hormone (C) Human Chorionic Gonadotropin

hCG secretion is regulated by a number of factors such as: Placental Gonadotropin releasing hormone (GnRH) [72] and Corticotropin releasing hormone (CRH) [72], Butyrated cyclic AMP, Interleukin-1, 6 [73-74], Colony Stimulating Factor - 1 (CSF-1) [75], Epidermal Growth Factor (EGF)[76], retinoic acid[77] either increasing the gene transcription of hCG subunit or by enhancing the stability of the hCG subunit mRNAs or by enhancing secretory mechanisms.

β-hCG is mainly synthesized by syncytiotrophoblasts. Although β-hCG appears to be produced in trace amounts by all human tissues, the placenta is unique in that it can glycosylate the protein, slowing its metabolism thus increasing the half life and biological activity. On the other hand, β-hCG produced in other sites has little or no carbohydrate side chain and thus is rapidly cleared from the circulation through the kidneys. [78]

At the time of missed menses, the maternal circulating β-hCG concentration is approximately 100 IU/L. In a normally progressing early intrauterine pregnancy, β-hCG levels should rise by at least 66% every 48 hours at concentrations less than 10,000 IU/L. The maximum level in maternal circulation is approximately 100,000 IU/L at 8 - 10 weeks gestation, which decreases to approximately 10,000 - 20,000 IU/L by 18-20 weeks gestation and remains at that level until delivery. β-hCG levels in cervicovaginal secretions mirror those in maternal serum and fluids, rising until the beginning of the second trimester and then falling to a plateau level for the reminder of pregnancy by 18 weeks gestation.[79] This is due to the fact that during the process of labour, the choriodecidual interface is disrupted leading to the release of β-hCG into the cervical secretions.

Functions of hCG [80],

1. Both hCG subunits have a binding propensity to the LH / hCG receptor, which is most pronounced in corpus luteum tissues.
2. The fertilised ovum requires support for survival shortly after embedding, for which continued Progesterone is required. Progesterone is only available from the corpus luteum at this stage of pregnancy. As a result, the life of the corpus luteum must be extended which is provided by hCG.
3. hCG stimulates the corpus luteum to secrete relaxin. Furthermore, It is thought that hCG causes vascular dilatation and myometrial relaxation, thereby supporting the fertilized ovum.

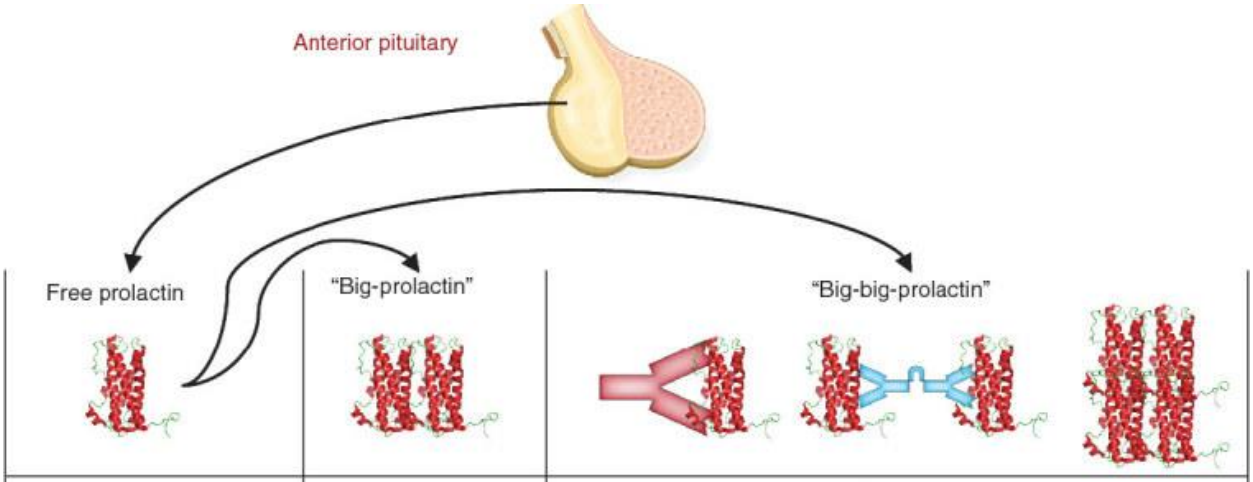
β-hCG levels can be qualitatively measured by ELISA using a sandwich technique with monoclonal antibodies specific to the β - subunit and quantitative estimation is done with the help of immunochromatographic methods.

4) Prolactin:

Prolactin (PRL), otherwise known as lactotropin, is a polypeptide protein, encoded by PRL gene on Chromosome 6 [81], and is synthesized from the lactotrophs of the anterior pituitary gland and secreted in a pulsatile manner. It has a biological half-life of 15-20 minutes.

It belongs to the cytokine family of proteins and has strong structural homology with growth hormone and placental lactogen. It has a 3D structure composed of four antiparallel α-helices which are folded due to the activity of three disulfide bonds. Several prolactin protein variants have been identified, many of which are the result of post-translational modifications of the mature protein such as phosphorylation, glycosylation, sulfation, and deamidation. The different variants of prolactin are: [82 - 85]

1. Little prolactin: The predominant form, it is a 199 amino acid single-chain polypeptide with a molecular weight of approximately 23 kDa.
2. Big prolactin: A polypeptide chain with a molecular weight of about 48 kDa. It appears to have very little biological activity and may be the result of the interaction of several prolactin molecules.
3. Big big prolactin: It has a molecular weight of approximately 150 kDa and has low biological activity.



Monomeric	Dimeric	IgG-prolactin	IgA-prolactin	Prolactin aggregates
23 kDa	40–60 kDa	>100–150 kDa		
Active	Inactive	Inactive		
60%–90%	15%–30%	0%–8%	0%–2%	0%–3%

Figure 3: Different variants of Prolactin

The secretion of prolactin is mainly under the inhibitory control of the neurotransmitter dopamine which is produced by the arcuate nucleus by acting through the D2 receptors.

1. Factors that inhibit actions of the dopamine neurons, thereby stimulating prolactin release: Serotonin, noradrenaline, histamine, opioids, galanin, somatostatin, cholecystokinin, γ amino butyric acid (GABA), nitric oxide and oestrogen. [86]
2. Factors that stimulate dopamine neurons, thereby inhibiting prolactin release: Acetylcholine, thyrotropin releasing hormone (TRH), oxytocin, vasopressin, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide, angiotensin II, neurotensin, neuropeptide Y, calcitonin, bombesin-like peptides, atrial natriuretic peptide and prolactin itself. [87]
3. Oestradiol also regulates prolactin gene expression to promote prolactin secretion. [88]

The hormone acts both in an autocrine and paracrine manner through its prolactin and cytokine receptors. [89] It has diurnal and ovulatory cycles. The levels are found to rise during activities such as exercise, sexual intercourse, breast examination, minor surgical procedures, epileptic seizures, physical or emotional stress, or the consumption of a high protein meal.

Prolactin is synthesized during pregnancy by the decidua, the maternal adenohypophysis, and the fetal pituitary where the decidual secretion is stimulated by the α-subunit of hCG, whose regulation differs remarkably from that present within the pituitary. [90] It is transported across the fetal membranes to the amniotic cavity, where it aids in the suppression of prostaglandin synthesis and the enhancement of pulmonary maturation.

Prolactin levels increase by 10 to 20 fold during pregnancy due to high levels of oestrogen and progesterone, and it reaches its peak in amniotic fluid during the second trimester and remains elevated throughout the pregnancy. [91-96] It was considered to be a potential marker for preterm birth for two reasons: it is actively secreted by the decidua throughout pregnancy, and high concentrations have been found in amniotic fluid. Its concentration in cervicovaginal fluid may indicate decidua-membrane disruption or subclinical injury to the membranes.

Functions of Prolactin [97],

1. The primary function of prolactin is to promote milk synthesis and to maintain postpartum lactation. It promotes the growth of the mammary gland and along with oestradiol, progesterone, placental lactogen, insulin, and cortisol, prepares the breast for postpartum lactation. High estrogen concentrations, on the other hand, inhibit the lactotropic effect of prolactin in the mammary gland, and the return of oestrogen levels to pre-pregnancy levels after delivery results in the onset of lactation.

2. Prolactin influences the functioning of gonads by inhibiting gonadotropin releasing hormone (GnRH) secretion leading to hypogonadotropic hypogonadism in extreme cases and also decreases the sensitivity of the Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) receptors in the gonads.
3. It improves glucose homeostasis by increasing β-cell mass under certain conditions, such as pregnancy.[98-99]
4. It also enhances dihydroepiandrosterone (DHEA), cortisol and aldosterone secretion by the adrenal cortex cells. [100]
5. It is also produced by lymphocytes and other immune cells and acts as a cytokine playing an important role in human immune responses ranging from immunostimulation at modest concentrations and inhibition at high levels. [101-102]
6. It is involved in osmoregulation - it increases absorption of absorption in all segments of the bowel and reduces renal excretion of Sodium and Potassium. [103]
7. It has been shown to promote the proliferation of glial and oligodendrocyte precursor cells, resulting in myelination of the central nervous system. [103]
8. It affects the fetal lung surfactant synthesis and helps in strengthening the immunity of the fetus.

1. O'Brien JM et al., [104] conducted a study in a cohort of 80 women in 1994 to examine whether prolactin levels in cervicovaginal washings was associated with premature birth. Cervicovaginal prolactin was identified in significantly more symptomatic patients than asymptomatic controls (50% vs 5%, $p < 0.0001$) and exhibited an 80% positive predictive value and a 65% negative predictive value for delivery at or before 34 weeks of gestation in symptomatic patients. They concluded that cervicovaginal prolactin levels could serve as a useful predictor for preterm birth in asymptomatic women since they were linked to a shorter latency period to delivery in symptomatic patients.

2. Leylek O.A et al., [105] in 1997, conducted a study in 66 women in Turkey, to ascertain the utility of cervicovaginal washing prolactin assay in prediction of preterm birth. It was found that prolactin levels greater than 50 ng/ml in the 12 days preceding preterm delivery were found to have sensitivity, specificity, positive and negative predictive values of 65%, 95%, 86%, and 81%, respectively. They concluded that the good feasibility, easy application, and cost effectiveness of the test makes it an able biochemical marker of preterm delivery.

3. Bernstein S et al., [106] in 1997, conducted a study that demonstrated that β-hCG levels in the cervicovaginal fluid mirror those in the maternal serum and amniotic fluid, and that elevated levels of β-hCG in the cervicovaginal fluid may be due to the inflammatory process that precedes the onset of labour. Disruption of the chorion and decidua, a possible precursor of active labor, may also be related to the same. They noted that a single β-hCG value of > 50 mIU/ml between 24 weeks and 28 weeks' of gestation was associated with a significant increase in the incidence of delivery between 34 weeks' gestation with a sensitivity, specificity, PPV and NPV of 50%, 87%, 33% and 93%, respectively.

4. Anai T et al., [107] in 1997, conducted a study to determine if the estimation of hCG levels in vaginal fluid is useful for the diagnosis of premature rupture of membranes (PROM). It was reported that hCG level in vaginal washings of women with PROM was higher than those of controls during the first, second, and third trimesters. The sensitivity, specificity, positive and negative predictive values in the third trimester, with the threshold value of 50 mIU/ml was reported to be 100%, 96.5%, 88.9% and 100% respectively and concluded that hCG level in vaginal fluid is a useful marker of PROM during the second and third trimesters.

5. Jotterand AD et al., [108] in 1997, conducted a study in 64 pregnant women between 21 and 34 weeks of gestation to determine whether the presence of cervicovaginal prolactin during pregnancy was significantly associated with preterm delivery. The study reported a positive cervicovaginal prolactin level in preterm delivery before 34 weeks with sensitivity, specificity, positive and negative predictive value of 57%, 88%, 36%, and 94%, respectively, while it was reported to be 31%, 87%, 45%, and 79% in preterm delivery before 37 weeks. Women who tested positive for prolactin had a significantly shorter time between testing and delivery.

6. Guvenal T et al., [109] in 2001 compared the predictive value of cervicovaginal β-hCG and prolactin levels in spontaneous preterm delivery in 60 women. The preterm labour and normal pregnancy groups each had 17 and 43 patients, respectively, and both groups had a single cervicovaginal β-hCG and prolactin measurement. It was reported that cervicovaginal β-hCG and prolactin levels were found to be significantly higher in the preterm group when compared to the term delivery group. They concluded that cervicovaginal β-hCG measurement in preterm labour patients can be

used as a predictive test and that cervicovaginal prolactin is not a sensitive test when compared to β-hCG.

7. Sanchez-Ramos L et al., [110] conducted a study in 2003, to determine whether human chorionic gonadotropin (hCG) detected in cervicovaginal secretions of patients with symptoms suggestive of preterm labor is a predictor of preterm birth. The study reported that a positive rapid qualitative assay from cervicovaginal secretions between 24 and 34 weeks' gestation was associated with a significant increase in the incidence of preterm delivery. The likelihood ratios for a positive and negative test were 2.19 and 0.51 respectively. Using a cut-off of 19 mIU/ml, a similar diagnostic accuracy for predicting preterm birth was obtained with the quantitative test with the likelihood ratios for a positive and negative test being 1.93 and 0.62 respectively. It concluded that qualitative and quantitative hCG measurements from cervicovaginal secretions may be useful predictors of preterm birth in symptomatic patients.

8. Gurbuz A et al., [111] in 2003, conducted a study in 102 pregnant women with aim to determine the cut-off values for hCG in predicting delivery within 7 and 14 days, as well as before 35 and 37 weeks of gestation in a group of women at high risk for preterm delivery. The study reported the cut-off value for cervical hCG concentration and its sensitivity, specificity, positive and negative predictive values, accuracy, relative risk and likelihood ratio for accurate determination of delivery within 7 days was $\geq$ 32 mIU/ml, 97%, 84%, 89%, 95%, 92%, 17.37 and 6.06, respectively, and $\geq$ 30 mIU/ml, 97%, 79%, 87%, 94%, 89%, 15.15 and 4.62, respectively, for prediction of delivery within 14 days; It was reported to be $\geq$ 33 mIU/ml, 89%, 92%, 94%, 83%, 90%, 5.83 and, 11.55,

respectively, for prediction of delivery before 35 weeks; and ≥ 27 mIU/ml, 76%, 50%, 85%, 37%, 71%, 1.34 and 1.52, respectively, for prediction of delivery before 37 weeks.

9. Garshasbi A et al., [112] in 2004 undertook a cohort study to determine whether concentrations of β-HCG in cervicovaginal secretions could predict spontaneous preterm birth in asymptomatic high risk pregnancies. This study was undertaken with cervicovaginal samples collected from 540 pregnant women between 20 to 28 weeks of gestation. A 3.2-fold increase in cervicovaginal β-HCG concentrations among patients with SPB vs. term delivery was reported while A single cervicovaginal β-HCG >77.8mIU/ml, between 20 and 28 weeks' gestation, identified patients with subsequent SPB vs. term delivery with sensitivity of 87.5% (95% CI: 47.4–97.9) and a specificity of 97% (95% CI: 86.5–99.4) with positive and negative predictive values of 88.5% and 98%, respectively. They concluded that cervicovaginal β-HCG was a sensitive and specific predictor of subsequent preterm delivery.

10. Buyukbayrak EE et al., [113] conducted a study in 70 women in 2004 to determine the reliability and diagnostic cut-off value of the vaginal washing-fluid prolactin assay for the diagnosis of premature rupture of membranes (PROM). The optimal diagnostic cut-off value was reported to be 30 μIU/ml with 95% sensitivity, 78% specificity, 84% positive predictive value and 93% negative predictive value with 87% accuracy and 11.30 relative risk. They concluded that the vaginal washing-fluid prolactin test can be used for the diagnosis of PROM as it is simple, cheap, rapid and reliable.

11. Adhikari K et al., [114] in 2008, conducted a study in 75 pregnant women with the objective of predicting the risk of preterm birth (< 37 weeks) or early preterm birth (< 34 weeks) by

cervicovaginal β-hCG and cervical length measured between 24-28 weeks of gestation in asymptomatic women at high risk for preterm birth. To predict delivery before 37 weeks, cervical length < 2.95 cm had a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 75%, 80.1%, 71.4% and 90.7% respectively, while cervicovaginal β-hCG > 4.75 mIU/ml was reported to be 70%, 61.81%, 40% and 85% respectively. To predict delivery before 34 weeks, cervical length < 2.65 cm had a sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 50%, 85.50%, 23.08% and 95.16% respectively whereas, cervicovaginal β-hCG >14 mIU/ml was reported to be 83.3%, 85.5%, 33.3% and 98.3%, respectively. They concluded that cervical length was superior to predict delivery at 37 weeks, whereas β-hCG was superior to predict delivery at 34 weeks. Their combination outperformed either parameter alone in predicting preterm birth at 37 or 34 weeks.

12. Sak ME et al., [115] in 2010, conducted a study in 55 women with the aim to investigate the β-hCG levels in cervicovaginal secretions as an early marker for preterm delivery. They observed that the cervicovaginal β-hCG levels in women with preterm delivery was three times more than the women who had a term delivery while the cut-off value of cervicovaginal β-hCG was reported to be 77.8 mIU/ml between 24 and 36 weeks of gestation with the sensitivity, specificity, positive and negative predictive values being reported as 76%, 91.6%, 95% and 79.9%, respectively.

13. Bagga R et al., [116] conducted a study in 2010 in 100 pregnant women with the aim to evaluate the role of cervicovaginal hCG along with measurement of cervical length by transvaginal ultrasound in women with signs and symptoms of preterm labor in order to predict which of these women are likely to deliver preterm. It was reported that an hCG value of ≥ 45 mIU/ml was the optimal cut-off, with sensitivity, specificity, positive predictive value (PPV), and negative predictive value

(NPV) of 95.8%, 73.7%, 53.5%, 98.2%, and 85.7%, 80%, 69.8%, 91.2%, respectively, for predicting delivery within 48 h and 7 days. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cervical length ≤ 2.5 cm to predict delivery within 48 h and 7 days of admission were 62.5%, 89.5%, 65.2%, 88.3%, and 60.0%, 96.9%, 91.3%, and 81.8%, respectively. They concluded that combining hCG assay with cervical length significantly increased sensitivity and negative predictive value (NPV) for predicting preterm delivery within 48 h and 7 days when compared to cervical length alone.

14. Ranjbar M et al., [117] in 2012, conducted a cross-sectional study with the purpose of evaluating the relationship between the levels of β-hCG cervicovaginal secretions and preterm delivery. They reported that elevated cervicovaginal levels of β-hCG (22.5 mIU/ml) was associated with preterm delivery with a 97% sensitivity, 76% specificity, 81% positive predictive value and 96% negative predictive value and concluded that cervicovaginal β-hCG levels could be used as a useful parameter for preterm delivery prediction.

15. Ibrahim MI et al., [118] in 2013, conducted a prospective study in 390 pregnant women to assess the diagnostic accuracy of qualitative and quantitative assay of human chorionic gonadotropin (hCG) in cervicovaginal secretion as a biochemical predictor of preterm birth. The study reported that for the qualitative test, the cut-off value was 25 mIU/mL with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy of 68.3%, 96.1%, 76.9%, 94.3% and 91.8%, respectively; and that for quantitative test, with a cut-off level of 34.5 mIU/mL, was 100%, 98.5%, 92.3%, 100% and 98.7%, respectively. They concluded that both the qualitative and quantitative assessment of cervicovaginal fluid hCG at 26–36 weeks of

gestation was valuable in the prediction of preterm birth in symptomatic and asymptomatic population.

16. Bahasadri S et al., [119] in 2013, conducted a cross-sectional study in 123 pregnant women with the main aim of evaluating vaginal fluid β-human chorionic gonadotropin (β-hCG) for the diagnosis of preterm premature rupture of membranes (PPROM). β-hCG concentration was reported to be 7.71 ± 15.7 mIU/mL in the intact membrane control group, 468.06 ± 366.34 mIU/mL in the PPROM group and 176.43 ± 316.37 mIU/mL in the suspected PPROM group, indicating a significant difference between the three groups. The optimal cut-off value was found to be 79.5 mIU/mL with a sensitivity of 95% and specificity of 84%. The study concluded that β-hCG levels were higher in the PPROM and suspected PPROM groups, and that it could be used as a suitable, fast, and reliable test for detecting membrane rupture.

17. Alakananda et al., [120] in 2016, conducted a prospective study in India to determine the association of β-hCG levels in cervicovaginal secretion with preterm labour and preterm delivery. They reported the predictive value of β-hCG to be > 14mIU/ml with a sensitivity of 85.3% and specificity of 84.7% and concluded that it can be used to predict preterm delivery in patients who present with preterm labour.

18. Mishra N et al., [121] in 2017, conducted a prospective study to evaluate cervicovaginal β-hCG and cervical length as a diagnostic marker of preterm delivery. The study included 100 women with singleton pregnancy with gestational age between 24-28 weeks. The study reported that β-hCG value of > 13.5 mlU/ml should be taken as a cut-off point to predict preterm delivery as sensitivity (71.11%) and specificity (81.82%) were both high for that value while the cut-off point for cervical

length should be taken as < 2.5 cms with sensitivity of 66.67% and specificity of 89.09%. The findings of this study confirm that raised β-hCG levels and decreased cervical length can be used as a diagnostic marker for preterm delivery.

19. El-Sayed MLM et al., [122] in 2018, conducted a prospective observational study in 220 women with the aim to evaluate the diagnostic accuracy of qualitative cervicovaginal β-hCG versus qualitative fetal Fibronectin (fFN) for prediction of preterm labour in asymptomatic high risk women during antenatal care. The study showed that for the prediction of preterm labour, β-hCG had a sensitivity, specificity, positive and negative predictive value of 72%, 85%, 41%, and 95.5%, respectively, while fetal Fibronectin (fFN) had a sensitivity, specificity, positive and negative predictive value of 73%, 87%, 38%, and 96%, respectively. They concluded that qualitative β-hCG assessment in cervicovaginal fluid can be used as an alternative method to qualitative fFN assessment as it is a valid and less expensive test.

20. Radwan AM et al., [123] in 2019, conducted a study in 120 women with the aim to assess the correlation between cervicovaginal levels of β-hCG and preterm labor. They concluded that β-hCG level in cervicovaginal secretion can be used as a predictor of preterm labor in women presenting with preterm labor pain, with cut off value > 5.8 mIu/ml.

21. Mehrotra S et al., [124] in 2020, conducted a prospective study on 229 women between 24 and 36 weeks of gestation to evaluate the diagnostic value of cervicovaginal prolactin levels for predicting preterm delivery in women with preterm labor. They observed that mean prolactin levels was three times higher in women with preterm labor than in women who were not and reported a cut off value of 7ng/ml with a sensitivity, specificity, positive and negative predictive values of 80%, 80%,

88.64% and 64.52% respectively. The study suggests that patients with preterm labor having a cut-off value of prolactin between 2.5 and 7 ng/mL belong to the high- risk group and need close monitoring and early intervention in the form of prophylactic tocolysis.

22. Gupta R et al., [125] in 2020, conducted a prospective observational study in 134 asymptomatic pregnant women at 24 - 34 weeks of gestation with at least one risk factor for preterm delivery, with the main goal of determining whether cervicovaginal β-hCG levels can be used as a predictor. They observed that the cervicovaginal β-hCG level was significantly higher in the preterm group as compared to the term group, and the optimal cut-off value for predicting preterm delivery was 36.45 mIU/ml, with a sensitivity of 71.9%, specificity of 81.8%, positive predictive value of 74.5%, negative predictive value of 79.7%, and diagnostic accuracy of 77.6%.

MATERIALS AND METHODS

Study site: Department of Obstetrics & Gynaecology, R.L Jalappa Hospital & Research Centre, attached to Sri Devaraj Urs Medical College under the aegis of SDUAHER, Tamaka, Kolar-563103

Study population: All women with singleton pregnancies with gestational age between 28 weeks to 36 weeks admitted to the labour room with labour pains associated with uterine contractions of 4 or more in 20 minutes, cervical dilatation < 3 cms, effacement of < 80%, with intact membranes with absence of any other maternal or fetal complications were considered as the study population.

Study design: Prospective Cohort study

Sample size: The sample size was estimated based on the sensitivity of prolactin and β -hCG in cervicovaginal secretion which was 80% for cut-off value 7ng/ml and 27.1 mIU/ml in predicting preterm delivery as per studies done by Seema Mehrotra et al and Guvenal T et al using the below formula:

$n=Z_{\alpha 2)}{}^{2}\, P\,\hat{}\,(1 - P\,\hat{}\,)/ d^2$

Where P ^ is the pre-determined value of sensitivity (or specificity) that is ascertained by previous published data or clinician experience/judgment and for $\alpha = 0.05$, $Z\alpha 2$ is inserted by 1.96.

P ^ = 80% or 0.80

d = 12.5% or 0.125.

Using the above values at 95% Confidence level, a sample size of 40 subjects will be included

in the study.

Sampling method: All the eligible subjects were recruited into the study consecutively by convenient sampling till the sample size was reached.

Study duration: The data collection was done between January 2021 to August 2022.

Inclusion Criteria:

- Singleton pregnancies
- Gestational age of 28-36 weeks
- Uterine contractions of 4 or more in 20 minutes or 8 in 60 minutes
- Cervical dilatation of < 3 cms
- Cervical effacement of < 80%
- With intact membranes

Exclusion criteria:

- Pregnancy induced Hypertension
- Diabetes in Pregnancy
- Multiple gestation
- Antepartum hemorrhage (Placenta previa, Abruptio placenta)
- Preterm premature rupture of membranes (PPROM)
- Premature rupture of membranes (PROM)
- Chorioamnionitis
- Fetal growth disorders
- Fetal congenital anomalies

Ethical considerations: The approval was obtained by the Institutional ethics committee. . Informed written consent was taken from the study participants and only those participants who have given consent were included in the study. The risks and benefits involved in the study and the voluntary nature of participation were explained to the participants before obtaining consent. The confidentiality of the study participants was maintained.

Data collection tools: All the relevant parameters were collected and documented in a structured study proforma.

Methodology:

All the consenting eligible pregnant women who were hospitalized to the labour room were recruited for the study. Cervicovaginal fluid samples were sent for all the women for the measurement of β-hCG and Prolactin levels. Irrespective of tocolysis, they were followed up till their delivery and divided into two groups depending on the outcome i.e,

Group I: Women who came with preterm labour and had a preterm delivery

Group II: Women who came with preterm labour and had a term delivery

Cervicovaginal fluid collection and laboratory study: Cervicovaginal fluid samples were obtained before per vaginal examination. For obtaining samples, a sterile cotton tipped swab was placed first, into the endocervical canal and then into the posterior fornix for 20-30 seconds which was then placed in a sterile tube containing 1 ml of saline solution. This solution was shaken for 1 minute followed by centrifuging for 5 minutes at the rate of 1500 rpm. Refrigeration of this supernatant was done at 20 degree celsius for upto 30 days which was quantitatively tested for the presence of β-hCG and Prolactin and was assayed with commercially available chemiluminescent enzyme immunometric assay kit.

STATISTICAL ANALYSIS

Data was entered into Microsoft excel data sheet and analyzed using SPSS 22 version software. Categorical data was represented in the form of frequencies and proportions. Chi-square test or Fischer's exact test (for 2 x 2 tables only) was used as test of significance for qualitative data.

Continuous data was represented as mean and standard deviation. Independent t test was used as test of significance to identify the mean difference between two quantitative variables. Receiver operating characteristic curves (ROCs) were constructed for both β-hCG and Prolactin in predicting preterm delivery. Comparison of β-hCG with prolactin was done. Receiver operating characteristic (ROC) curve and optimal cut-off points was chosen for the calculation of sensitivity, specificity, positive and negative predictive values. A test that predicts an outcome no better than chance has an area under the ROC curve of 0.5. An area under the ROC curve above 0.8 indicates a fairly good prediction.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram, Pie diagram.

P value (Probability that the result is true) of < 0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

RESULTS

The final analysis consisted of 40 subjects.

Table 1:- Distribution of subjects according to age group (N=40)

Age group (in years)	Frequency (n)	Percentage (%)
18 - 21	11	27.5
22 - 25	19	47.5
26 - 29	6	15.0
30 - 33	4	10.0

Figure 4:- Bar chart showing distribution of subjects according to age group (N=40)

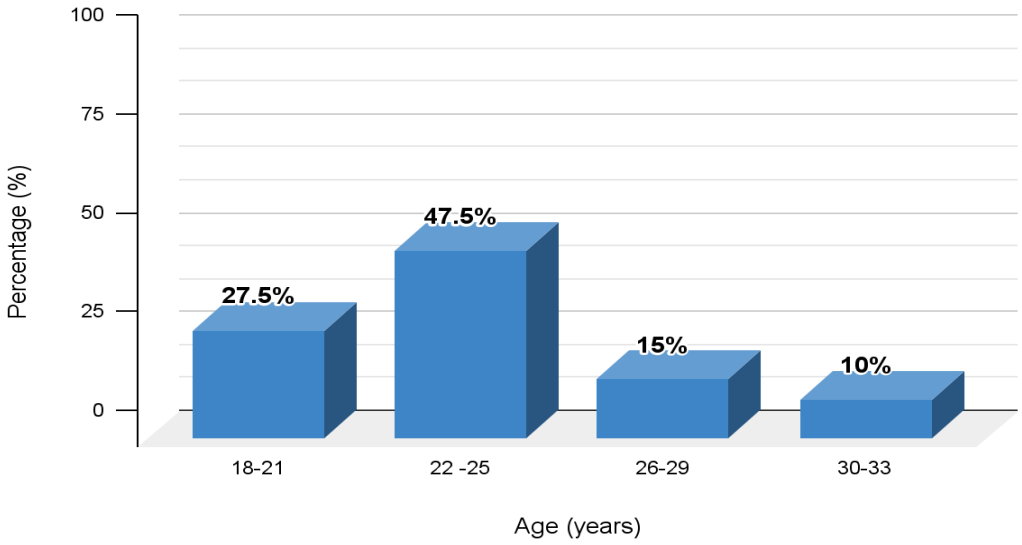


Table 1 and Figure 4: There were a total of 40 subjects with ages ranging from 18 to 33 years, out of which 11 (27.5%) subjects were between 18 to 21 years old, 19 (47.5%) subjects were between 22 to 25 years old, 6 (15%) subjects were between 26 to 29 years old and 4 (10%) subjects were between 30 to 33 years old.

Table 2 :- Association between age and cervicovaginal β-hCG level (N=40)

	Age group (in years)	n	Mean	Standard Deviation	P-value
Cervicovaginal β-hCG level	18 - 21	11	15.94	7.10	0.267
	22 - 25	19	15.32	9.88	
	26 - 29	6	14.33	6.28	
	30 - 33	4	24.60	10.41	

Figure 5:- Bar chart showing comparison of age and mean cervicovaginal β-hCG level (N=40)

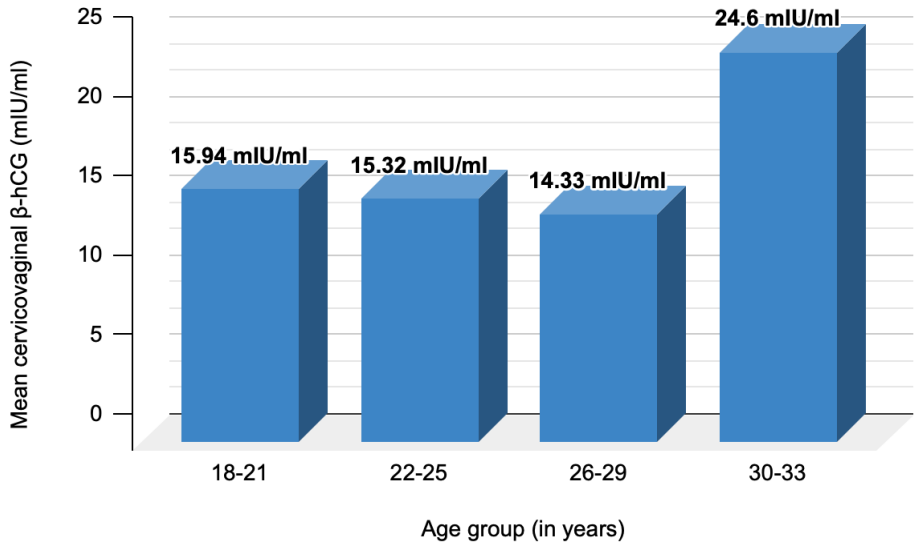


Table 2 and Figure 5: The mean cervicovaginal β-hCG level was highest (24.6 mIU/ml) among the 30 to 33 years age group. No significant statistical difference was seen between cervicovaginal β-hCG levels among the different age groups with $p > 0.05$.

Table 3:- Association between age and cervicovaginal Prolactin level (N=40)

	Age group (in years)	n	Mean	Standard Deviation	Standard Error	P-value
Cervicovaginal Prolactin level	18 - 21	11	11.03	3.61	1.08	0.018
	22 - 25	19	8.94	3.94	0.90	
	26 - 29	6	4.78	2.59	1.05	
	30 - 33	4	11.09	5.36	2.68	

Figure 6:- Bar chart showing comparison of age and mean cervicovaginal Prolactin level (N=40)

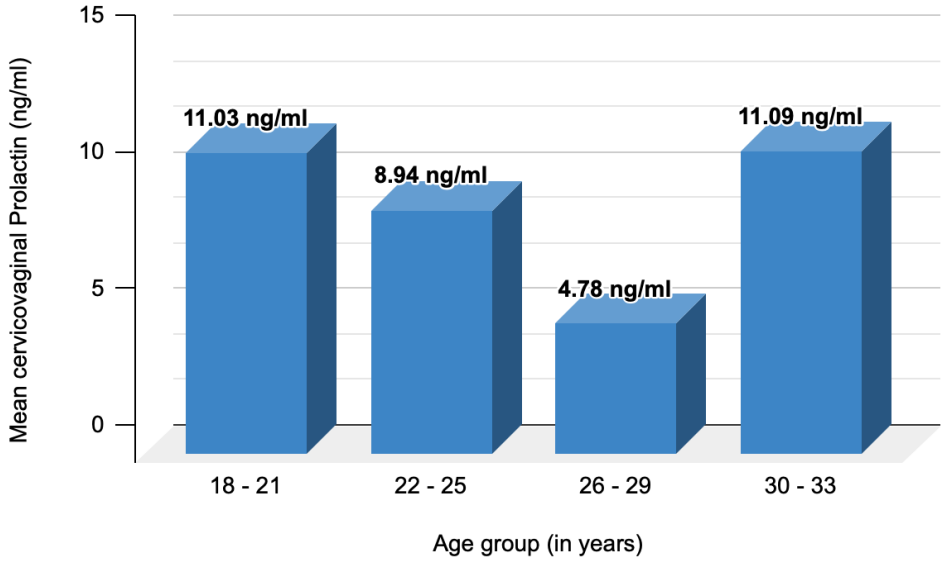


Table 3 and Figure 6: The mean cervicovaginal Prolactin level was highest (11.09 ng/ml) among the 30 to 33 years age group followed by the 18 to 21 years age group (11.033ng/ml). The association between cervicovaginal Prolactin level and age was statistically significant with $p < 0.05$.

Table 4:- Distribution of subjects according to parity (N=40)

Parity	Frequency (n)	Percentage (%)
Primigravida	20	50.0
Multigravida	20	50.0

Figure 7:- Pie chart showing distribution of subjects according to parity (N=40)

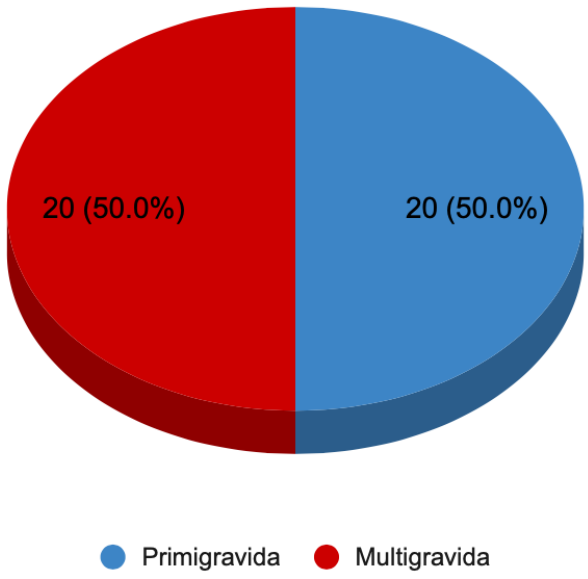


Table 4 and Figure 7: Out of 40 subjects, 20 (50%) subjects were Primigravida and the rest 20 (50%) subjects were Multigravida.

Table 5:- Association between parity and cervicovaginal β-hCG and prolactin levels (N=40)

	Parity	n	Mean	Standard Deviation	P-value
β-hCG (mIU/ml)	Primigravida	20	13.66	7.52	0.063
	Multigravida	20	18.89	9.60	
Prolactin (ng/ml)	Primigravida	20	8.88	4.11	0.741
	Multigravida	20	9.33	4.45	

Figure 8:- Bar chart showing comparison of parity and mean cervicovaginal β-hCG and Prolactin levels (N=40)

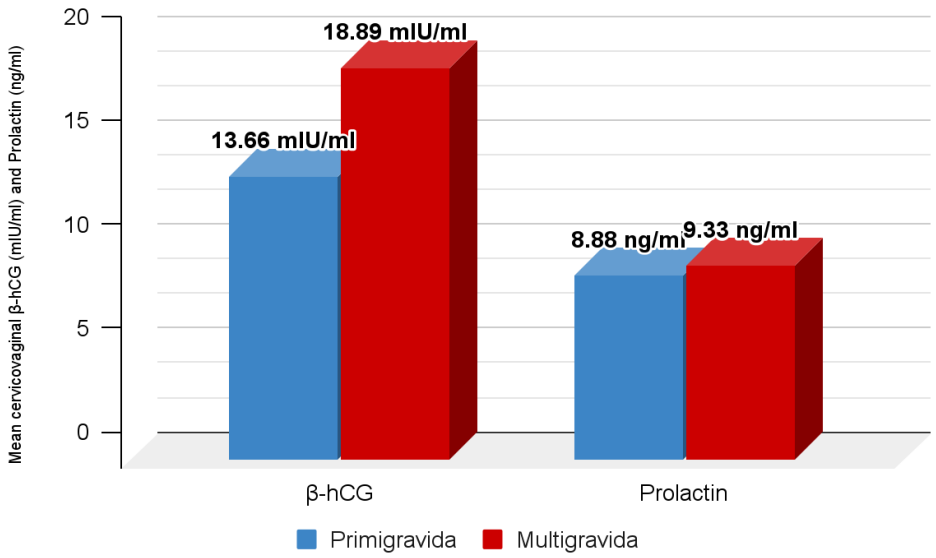


Table 5 and Figure 8: Shows no significant statistical difference between parity and cervicovaginal β-hCG and Prolactin levels with $p > 0.05$ for both.

Table 6:- Distribution of subjects according to gestational age* at sample collection (N=40)

Gestational age (in weeks)	Frequency (n)	Percentage (%)
Very Preterm (28 - 31 completed weeks)	3	7.5
Moderate Preterm (32 - 33 completed weeks)	13	32.5
Late Preterm (34 - 36 completed weeks)	24	60.0

*According to World Health Organization (WHO) classification

Figure 9:- Bar chart showing distribution of subjects according to gestational age at sample collection (N=40)

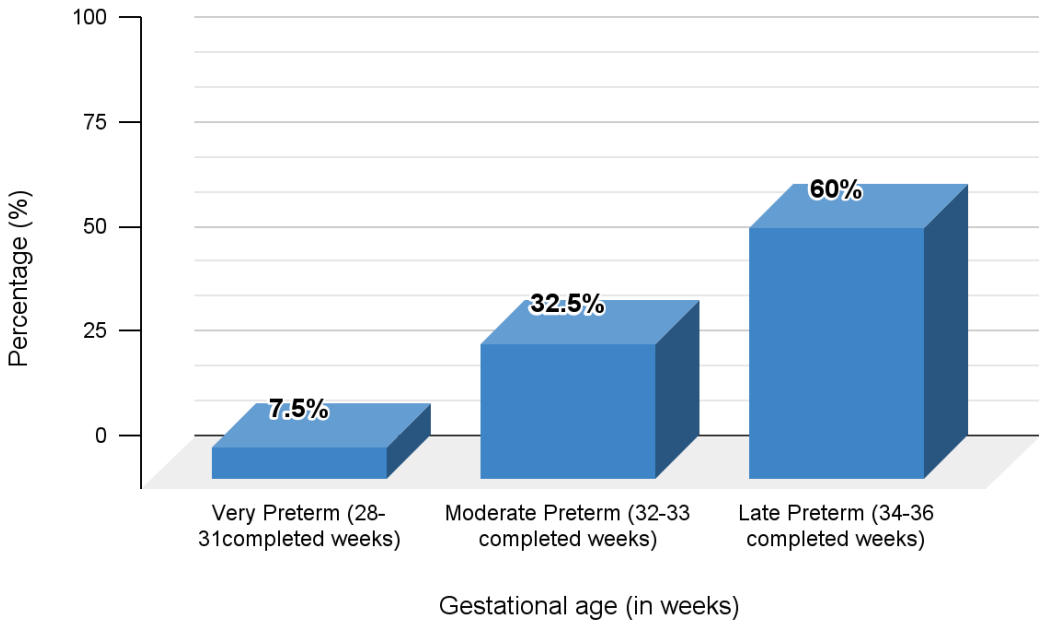


Table 6 and Figure 9: Among the 40 subjects, 3 (7.5%) subjects belonged to very preterm, 13 (32.5%) subjects belonged to moderate preterm and the maximum subjects i.e, 24 (60%) belonged to late preterm.

Table 7:- Comparison of mean cervicovaginal β-hCG level according to gestational age at sample collection (N=40)

Gestational age	Mean cervicovaginal β-hCG level (mIU/ml)	Standard Deviation	P - value
Very Preterm (28 - 31completed weeks)	34.39	1.51	< 0.001
Moderate Preterm (32 - 33 completed weeks)	17.76	7.74	
Late Preterm (34 - 36 completed weeks)	13.07	7.65	

Figure 10:- Bar chart showing comparison of mean cervicovaginal β-hCG level and gestational age at sample collection (N=40)

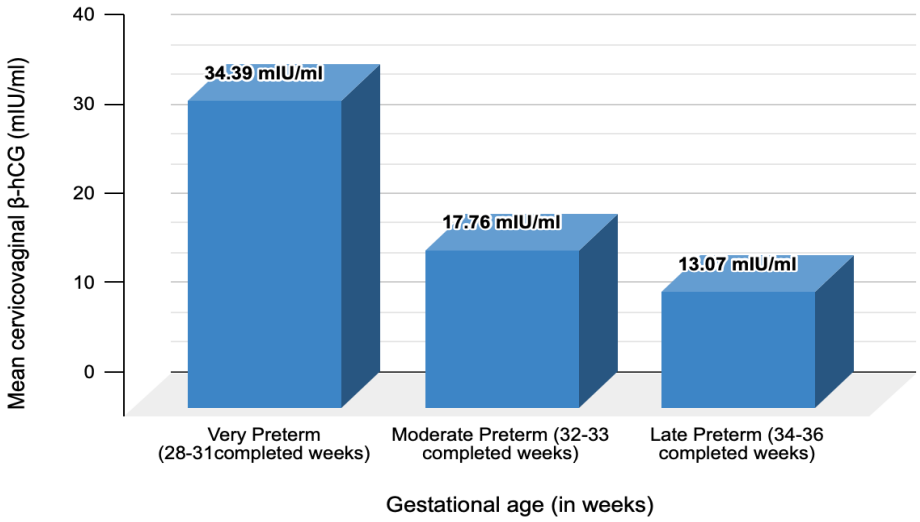


Table 7 and Figure 10: Mean cervicovaginal β-hCG level was highest i.e, 34.39 ± 1.5 mIU/ml among subjects with very preterm gestation followed by subjects with moderate preterm gestation (17.76 ± 7.7 mIU/ml) and late preterm gestation (13.07 ± 7.6 mIU/ml). The association between cervicovaginal β-hCG level and gestational age at sample collection was found to be statistically significant with p-value < 0.001.

Table 8:- Comparison of mean cervicovaginal Prolactin level according to gestational age at sample collection (N=40)

Gestational age	Mean cervicovaginal Prolactin level (ng/ml)	Standard Deviation	P - value
Very Preterm (28 - 31 completed weeks)	12.61	0.79	0.342
Moderate Preterm (32 - 33 completed weeks)	8.68	4.73	
Late Preterm (34 - 36 completed weeks)	9.9	4.2	

Figure 11:- Bar chart showing comparison of mean cervicovaginal Prolactin level and gestational age at sample collection (N=40)

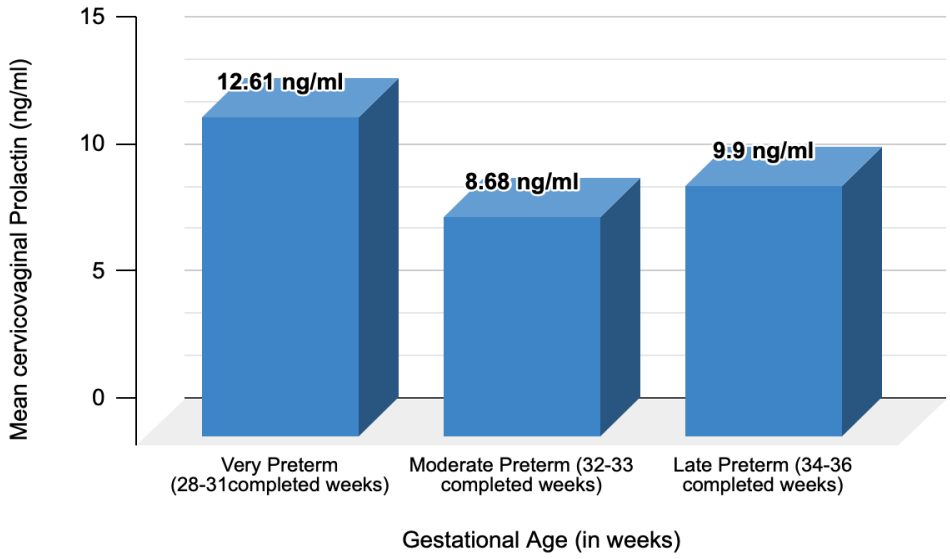


Table 8 and Figure 11: Mean cervicovaginal Prolactin level was found to be highest i.e, 12.61 ± 0.79 ng/ml among subjects with very preterm gestation followed by subjects with late preterm gestation (9.9 ± 4.2 ng/ml) and moderate preterm gestation (8.68 ± 4.7 ng/ml). There was no statistically significant difference found between gestational age at sample collection and mean cervicovaginal prolactin level with p-value of 0.342.

Table 9:- Distribution of subjects according to delivery outcome (N=40)

Delivery Outcome	Frequency (n)	Percentage (%)
Group 1: Preterm delivery	28	70.0
Group 2: Term delivery	12	30.0

Figure 12:- Pie chart showing distribution of subjects according to delivery outcome (N=40)

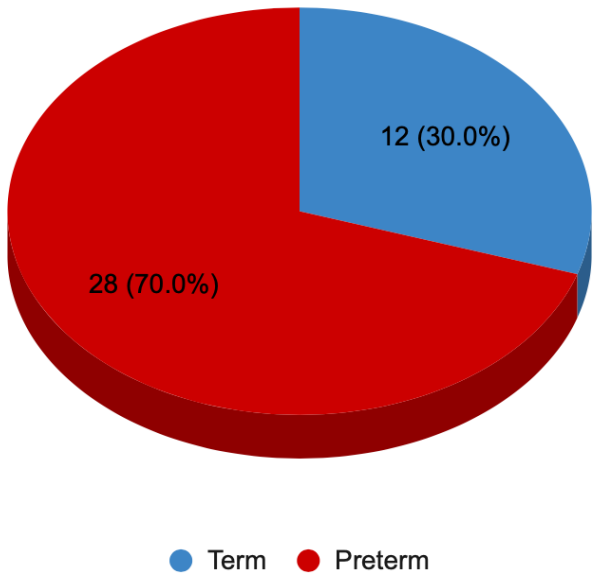


Table 9 and Figure 12: Out of the 40 subjects, majority of them i.e, 28 (70%) progressed to have a preterm delivery while, 12 (30%) of them continued till term and had a term delivery.

Table 10:- Comparison of mean cervicovaginal β-hCG level according to delivery outcome (N=40)

	Term		Preterm		P-value
	Mean	Standard Deviation	Mean	Standard Deviation	
β-hCG (mIU/ml)	9.43	2.67	19.20	9.07	< 0.001

Figure 13:- Bar chart showing comparison of mean cervicovaginal β-hCG level according to delivery outcome (N=40)

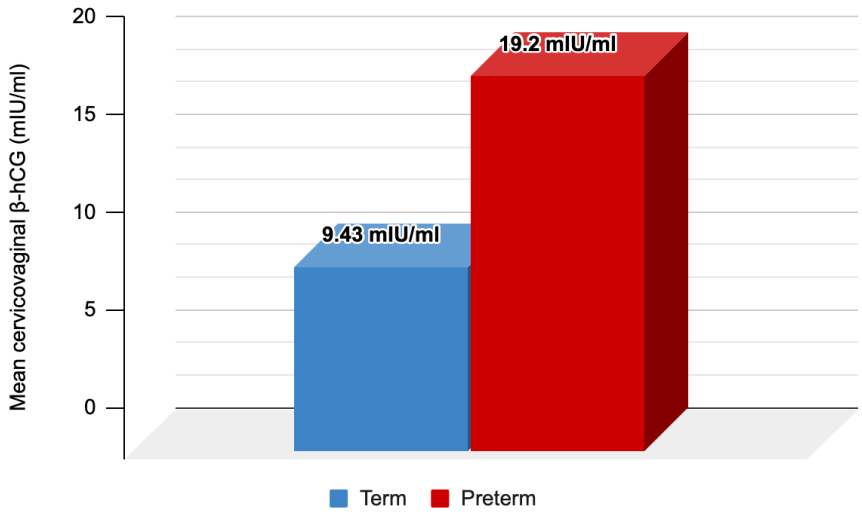


Table 10 and Figure 13: Mean cervicovaginal β-hCG level was found to be more among subjects with preterm delivery (19.20 mIU/ml) as compared to term delivery (9.43 mIU/ml). There was a statistically significant difference found between delivery outcome and mean cervicovaginal β-hCG level with p-value < 0.001.

Table 11:- Comparison of mean cervicovaginal Prolactin level according to delivery outcome (N=40)

	Term		Preterm		P-value
	Mean	Standard Deviation	Mean	Standard Deviation	
Prolactin (ng/ml)	4.95	2.49	10.88	3.52	< 0.001

Figure 14:- Bar chart showing comparison of mean cervicovaginal Prolactin level according to delivery outcome (N=40)

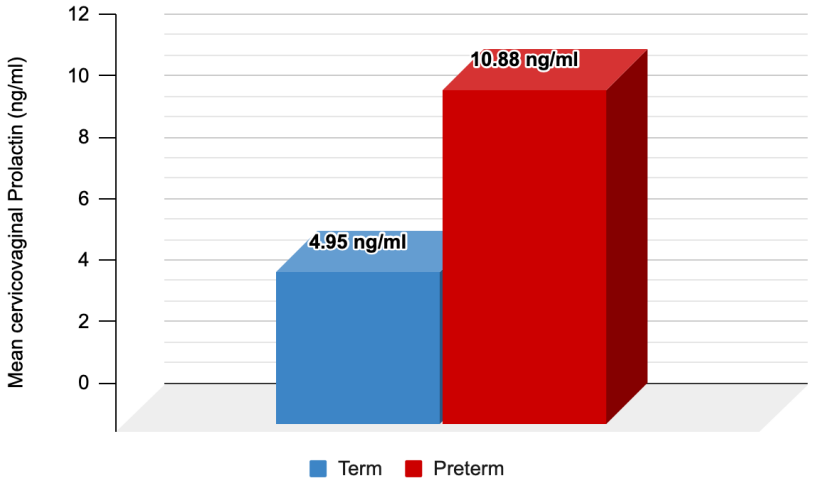


Table 11 and Figure 14: Mean cervicovaginal Prolactin level was found to be more among subjects with preterm delivery (10.88 ng/ml) as compared to term delivery (4.95 ng/ml). There was a statistically significant difference found between delivery outcome and mean cervicovaginal Prolactin level with p-value < 0.001.

Figure 15:- Receiver Operating Characteristic (ROC) curve for cervicovaginal β-hCG level in predicting preterm delivery

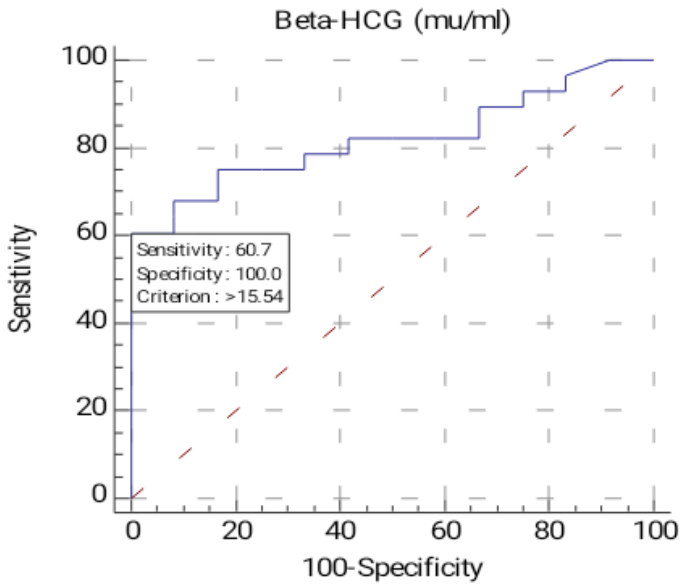


Table 12:- Sensitivity, Specificity, PPV, NPV of cervicovaginal β-hCG at cut-off >15.54 mIU/ml in predicting preterm delivery

	Value	**95% CI**
Sensitivity	60.7%	40.6 – 78.5
Specificity	100.00%	73.5 - 100.0
PPV	100.0%	80.5 - 100.0
NPV	52.2%	30.6 – 73.2

Figure 15 and Table 12: The optimal cut-off value for cervicovaginal β-hCG in prediction g preterm delivery was taken as 15.54 mIU/ml. Area under the curve was 0.820 which was

statistically significant with P value < 0.001. Sensitivity, Specificity, PPV, NPV of cervicovaginal β-hCG at cut-off >15.54 mIU/ml in predicting preterm delivery was 60.7%, 100%, 100% and 52.2% respectively. Hence cervicovaginal β-hCG level was found to be the good diagnostic marker in predicting preterm delivery.

Figure 16:- Receiver Operating Characteristic (ROC) curve for cervicovaginal Prolactin level in predicting preterm delivery

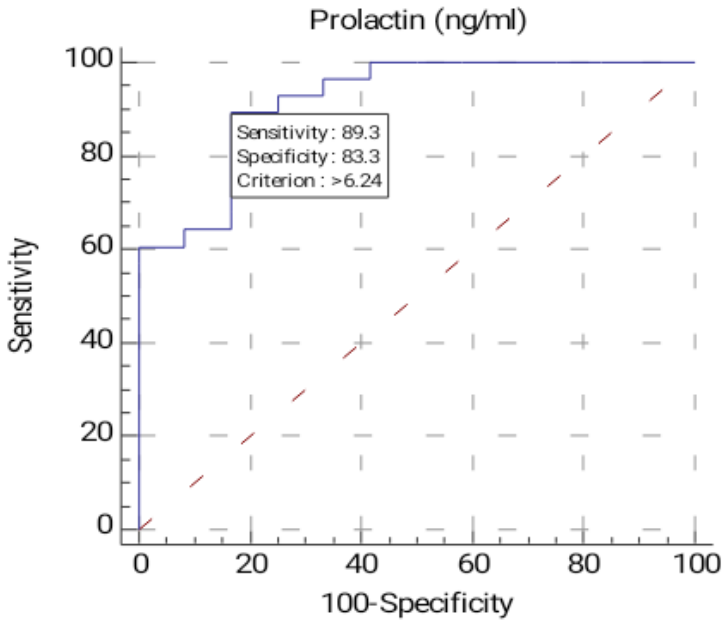


Table 13:- Sensitivity, Specificity, PPV, NPV of cervicovaginal Prolactin at cut-off > 6.24 ng/ml in predicting preterm delivery

	Value	95% CI
Sensitivity	89.29%	71.8 – 97.7
Specificity	83.3%	51.6 - 97.9
PPV	92.6%	75.7 - 99.1
NPV	76.9%	46.2 - 95

Figure 16 and Table 13: The optimal cut-off value for cervicovaginal Prolactin in predicting preterm delivery was taken as 6.24 ng/ml. Area under the curve was 0.920 which was statistically significant with P value < 0.001. Sensitivity, Specificity, PPV, NPV of cervicovaginal Prolactin at cut-off > 6.24 ng/ml in predicting preterm delivery was 89.29%, 83.3%, 92.6% and 76.9%. Hence cervicovaginal Prolactin level can be used as a diagnostic marker in predicting preterm delivery.

Figure 17:- Comparison of Receiver Operating Characteristic (ROC) curves for cervicovaginal β-hCG and Prolactin level in predicting preterm delivery

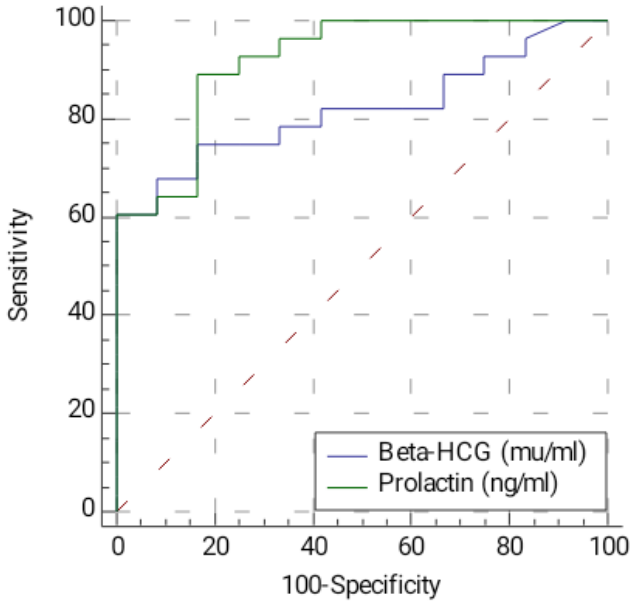


Table 14:- Comparison of Sensitivity, Specificity, PPV, NPV of cervicovaginal β-hCG and Prolactin in predicting preterm delivery

	β-hCG	**Prolactin**
Sensitivity	60.7%	89.29%
Specificity	100.00%	83.3%
PPV	100.0%	92.6%
NPV	52.2%	76.9%

Figure 17 and Table 14: Area under the curve for cervicovaginal β-hCG was 0.820 whereas for cervicovaginal Prolactin, it was 0.920. Therefore, cervicovaginal Prolactin is slightly better than cervicovaginal β-hCG in predicting preterm delivery. The specificity and PPV (100%, 100%) of cervicovaginal β-hCG in predicting preterm delivery was comparatively higher than that of cervicovaginal Prolactin (83.3%, 92.6%) whereas, the sensitivity and NPV of cervicovaginal prolactin (89.29%, 76.9%) was comparatively higher than that of cervicovaginal β-hCG (60.7%, 52.2%).

DISCUSSION

Preterm birth is a complicated obstetric condition that causes significant newborn death and morbidity throughout the world. In the developing world, India alone is responsible for 40% of all cases of low birth weight (LBW), and more than half of all cases in Asia.[126] Recently, it has been suggested to employ biological markers to improve clinical methods for predicting preterm birth. These tests have a great chance of identifying these asymptomatic and symptomatic people who are more likely to have preterm births and who may therefore need more close monitoring and therapies, such as tocolysis and therapy for fetal lung maturation. On the other hand, intensive tocolysis, protracted hospitalization, and frequent follow-up visits might not be required if low risk of preterm birth is identified. Numerous biochemical indicators have been researched up to this point, but none have emerged as the industry standard.[124] The present study sought to compare and correlate cervicovaginal β-hCG and Prolactin levels in the prediction of preterm birth in symptomatic women.

Age:

In the present study, out of 40 subjects, majority (47.5%) belonged to the age group of 22 - 25 years followed by 27.5% belonging to 18 - 21 years group with a mean of 23.7 ± 3.53 years. Radwan AM et al. [123], performed a study in 120 women of which 80 of them presented with symptoms of preterm labour whose mean age was reported to be 25.57 ± 4.03 years. Similarly, the mean age was found to be in that of the comparable range in studies done by Gupta R et al. [125], Garshasbi et al. [112] and Gurbuz et al. [111] where the mean ages were identified to be 25.19 ± 4.1 years, 24.7 ± 6.3 years and 25.8 ± 4.7 years respectively. A study by Fuchs et al. [127], re-iterated that there was a tendency for women between the ages of 25 - 29 years to be at higher risk of preterm birth.

Table 15:- Comparison of mean of age in various studies

Study	Study Population	Mean of age (years)
Present study	40	23.70 ± 3.53
Radwan AM et al [123]	80	25.57 ± 4.03
Gurbuz et al [111]	102	25.80 ± 4.70

Parity:

In the present study, there was equal distribution between primigravida (50%) and multigravida (50%). However, contrasting results were obtained in a study by Gupta R et al. [125], where the majority (67.1%) belonged to the multigravida group, and in a study by Sanchez Ramos et al. [110], where 66.3% belonged to the multigravida group.

Table 16:- Comparison of parity in various studies

Study	Study Population	Parity
Present study	40	Primigravida: 50% Multigravida: 50%
Gupta R et al [125]	134	Primigravida: 32.8% Multigravida: 67.1%
Garshasbi et al [112]	153	Primigravida: 26.1% Multigravida: 73.8%
Sanchez Ramos et al [110]	86	Primigravida: 33.7% Multigravida: 66.3%

Gestational age:

Current study had a majority (60%) belonging to late preterm gestation i.e, between 34 to 36 completed weeks, followed by 32.5% who belonged to moderate preterm gestation i.e, between 32 to 33 completed weeks. Similar results were obtained in a study by Singh et al.[128] where it was observed that the gestational age range of 34 to 36 weeks saw the greatest percentage of women (48.5%) experiencing preterm labour. Similarly, Das et al. [129] noted that in their research, 65% women experienced preterm labour between 34 and 37 weeks of pregnancy.

Delivery outcome:

In the present study, 70% progressed to have a preterm delivery while 30% continued till term. Similar outcome was noted in a study done by Gurbuz et al. [111] where 78.4 % of the women had a preterm delivery. In a study done by Radwan et al., [123] 50% of them progressed to preterm delivery whereas, in a study done by Gupta R et al., [125] 42.5% of them had a preterm delivery. On the contrary, Ibrahim et al.[118] reported that only 15.4% had a preterm delivery.

Table 17:- Comparison of delivery outcomes in various studies

Study	Study Population	Delivery outcome
Present study	40	Term delivery: 30% Preterm delivery: 70%
Gupta R et al [125]	134	Term delivery: 57.5% Preterm delivery: 42.5%
Gurbuz et al [111]	102	Term delivery: 23.6% Preterm delivery: 78.4%

β-hCG:

The concept of using cervicovaginal β-hCG detection to foretell the potential occurrence of spontaneous preterm birth is based on the theory that a latent inflammatory process is the last common pathway that ends in spontaneous preterm birth, releasing maternal serum components, including β-hCG, into the cervicovaginal secretions.[106]Additionally, it is also attributed to the disruption of the chorion and decidua that often takes place at the beginning of labour.[109] In order to forecast preterm birth, Bernstein et al.[106] were the first to describe the detection of β-hCG in the cervicovaginal discharge.

The present study showed that the mean cervicovaginal β-hCG level was highest (24.6 mIU/ml) among the 30 to 33 years age group and no significant statistical difference was seen between cervicovaginal β-hCG levels among the different age groups.

In the present study, the association between gestational age at sample collection and cervicovaginal β-hCG level was statistically significant. The mean cervicovaginal β-hCG level was found to be highest in subjects between 28 - 31 completed weeks i.e, very preterm gestation (34.39 ± 1.5 mIU/ml) while it was least in those with gestational age between 34 - 36 completed weeks i.e, late preterm gestation (13.07 ± 7.6 mIU/ml).

Similarly, a statistically significant difference was found between delivery outcome and β-hCG where cervicovaginal β-hCG levels were found to be significantly more among subjects with preterm delivery (19.20 ± 9.07 mIU/ml) as opposed to term (9.43 ±. 2.67 mIU/ml). This was in concordance with the results obtained by Gupta R et al. [125] where the preterm group had considerably greater cervicovaginal β-hCG levels (39.38 ± 19.66 mIU/ml) compared to the term group (21.86 ± 11.18 mIU/ml). Similar results were obtained by Mishra et al. [121], who observed

the β-hCG level to be significantly higher in the study group i.e, preterm delivery (23.46 ± 8.86 mlU/ml) as compared to control group i.e, who had a term delivery (7.81 ± 16.19 mlU/ml). Similar observations were echoed in studies done by Radwan et al. [123], Guvenal et al. [109], Garshasbi et al. [112], Ranjbar et al. [117] among many others.

Table 18:- Comparison of association between mean cervicovaginal β-hCG level and delivery outcome in various studies

Study	Study Population	Mean cervicovaginal β-hCG level (mIU/ml)	
		Term delivery	Preterm delivery
Present study	40	9.43 ± 2.67	19.20 ± 9.07
Guvenal et al [109]	60	35.8 ± 43	87.4 ± 77
Gupta R et al [125]	134	21.86 ± 11.18	39.38 ± 19.66
Mishra et al [121]	100	7.81 ± 16.19	23.46 ± 8.86
Radwan et al [123]	80	7.6 ± 12.34	24.5 ± 9.23

The present study showed no significant association between the parity and mean cervicovaginal β-hCG level, although the levels in primigravida (13.66 ± 7.52 mIu/ml) were low as compared to multigravida (18.89 ± 9.6 mIU/ml). Radwan et al. [123] reported an insignificant and negative correlation between parity and mean β-hCG level.

Through the receiver operating characterisctic curve, the present study obtained the optimal cut-off of cervicovaginal β-hCG level in predicting preterm delivery as > 15.54 mIU/ml, with a sensitivity, specificity, PPV and NPV of 60.7%, 100%, 100% and 52.2% respectively. The current study's findings were comparable to those of other studies conducted by Bernstein et al. [106], who showed that a cervicovaginal β-hCG cut-off level of > 50 mIU/ml before 34 weeks of pregnancy has sensitivity, specificity, positive and negative predictive values of 50, 87%, 33, and 93%, respectively. With a cut-off value of 27.1 mIU/ml, Guvenal et al. [109] likewise discovered a substantial correlation between cervicovaginal β-hCG levels and preterm labour, with sensitivity, specificity, positive and negative predictive values of 87.5%, 65.4%, 28%, and 97%, respectively. Gupta R et al [(125)] in their research showed that a optimal cut-off of cervicovaginal β-hCG level of 36.45 mIU/ml might predict preterm delivery with a sensitivity of 71.9%, specificity of 81.8%, positive predictive value of 74.5%, negative predictive value of 79.7%, and diagnostic accuracy of 77.6%.

Table 19:- Comparison of various studies for optimal cut-off of cervicovaginal β-hCG with Sensitivity, Specificity, PPV, NPV

Study	Study Population	β-hCG Cut-off level (mIU/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Present study	40	15.54	60.7	100	100	52.2
Bernstein et al [106]	77	50	50	87	33	93
Guvenal et al [109]	60	27.1	87.5	65.4	28	97
Gupta R et al [125]	134	36.45	71.9	81.8	74.5	79.7
Sanchez Ramos et al [110]	86	19	55.6	71.2	46.9	77.8
Radwan et al [123]	80	11	92.5	67.5	74	90
Garshasbi et al [112]	540					
	20-24 weeks	77.8	66.7	86.5	33	94
	24-28 weeks	90.9	80	92.2	63	74
Adhikari K et al [114]	75					
	Delivery <37 weeks	4.75	70	61.81	40	85
	Delivery <34 weeks	22.12	83.3	85.50	33.3	98.3
Ibrahim IM et al [118]	390	34.5	100	98.79	93.75	100
Bagga R et al [116]	100	45	85.7	80	69.76	91.2

Prolactin:

It has been demonstrated that during pregnancy, chorionic cytotrophoblast, decidua, amnion, and placental syncytiotrophoblast all release prolactin. High levels of prolactin found in cervicovaginal irrigation fluids have been linked to premature birth in several studies, but the numbers vary. [105, 109, 130]This could be attributed to the fact that the cervicovaginal prolactin was measured at various gestational weeks.

In the present study, the highest level of prolactin was seen in women in the 30 - 33 years age group (11.09 ± 5.3 ng/ml) followed by 18 - 21 year group (11.03 ± 3.61 ng/ml). It was the lowest in the 26 - 29 years group (4.78 ± 2.59 ng/ml). The association between cervicovaginal prolactin and age was statistically significant. On the contrary, no statistical significance was observed in maternal age and prolactin levels in a study by Mazor et al. [130]

In the present study, the mean cervicovaginal Prolactin level was found to be highest among subjects with very preterm gestation i.e, 28 to 31 completed weeks (12.61 ± 0.79 ng/ml) and lowest in the late preterm group i.e, 34 to 36 completed weeks (9.9 ± 4.2 ng/ml). There was no statistically significant difference found between gestational age at sample collection and mean cervicovaginal Prolactin level.

The present study showed a statistically significant difference between mean cervicovaginal Prolactin level and delivery outcome. Mean cervicovaginal Prolactin levels were found to be significantly more among subjects with preterm delivery (10.88 ± 3.52 ng/ml) as opposed to term delivery (4.95 ± 2.49 ng/ml). Similar results were obtained in a study by Mehrotra S et al. [124] (11.81

± 9.3 ng/mL in women who had a preterm delivery vs 4.61 ± 6.2 ng/mL in women who had a term delivery). According to O' Brien et al., [104] cervicovaginal Prolactin level was found to be more in preterm labouring women than in asymptomatic controls (50% vs. 5%; p<0.0001). Similarly, the prolactin levels in the preterm group were found to be considerably higher than those in the term delivery group by Guvenal et al. [109] (2.2 ± 1.7 ng/mL vs 0.83 ± 0.66 ng/mL, respectively, p = 0.026).

Table 20:- Comparison of association between mean cervicovaginal Prolactin level and delivery outcome in various studies

Study	Study Population	Mean cervicovaginal Prolactin level (ng/ml)	
		Term delivery	Preterm delivery
Present study	40	4.95 ± 2.49	10.88 ± 3.52
Mehrotra et al [124]	75	11.81 ± 9.3	4.61 ± 6.2
Guvenal et al [109]	60	0.83 ± 0.66	2.2 ± 1.7

Current study showed no significant association between parity and cervicovaginal Prolactin level, although the levels in Primigravida (8.88 ± 4.11 ng/ml) were lower when compared to Multigravida (9.33 ± 4.45ng/ml).

Through the receiver operating characteristic curve, the present study obtained the optimal cut-off of cervicovaginal Prolactin level in predicting preterm delivery as > 6.24 ng/ml, with a sensitivity,

specificity, PPV and NPV of 89.29%, 83.3%, 92.6% and 76.9% respectively. In patients who were symptomatic and delivered before 37 weeks of gestation, O'Brien et al. [104] provided cut-off values > 2 ng/mL with sensitivity of 88%, specificity of 79%, PPV of 80% and NPV of 65%. In the research by Guvenal et al. [109], prolactin readings >1.8 ng/mL were considered positive. According to these numbers, the cervicovaginal prolactin had sensitivity, specificity, PPV and NPV of 50%, 96%, 67%, and 93%, respectively, for predicting premature birth.

Table 21:- Comparison of various studies for optimal cut-off of cervicovaginal Prolactin with Sensitivity, Specificity, Positive predictive value, Negative predictive value

Study	Study Population	Prolactin Cut-off level (ng/ml)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Present study	40	6.24	89.29	83.3	92.6	76.9
O' Brien et al [104]	40	2	88	79	80	65
Guvenal et al [109]	60	1.8	50	96	67	93
Mehrotra S et al [124]	75	7	80	80	88.64	64.52

Area under the curve in the present study for cervicovaginal β-hCG level was 0.820 while that for cervicovaginal Prolactin level was 0.920. This indicates that cervicovaginal Prolactin is slightly better than cervicovaginal β-hCG in predicting preterm delivery. A similar conclusion was drawn by Guvenal et al. [109] which reports that cervicovaginal β-hCG > 28 mIU/ml, when present between 24 and 36 weeks' gestation, can identify approximately 87% of women who will deliver preterm while, cervicovaginal prolactin > 1.8 ng/ml can identify 50% of women who will deliver

preterm. However, no significant difference in the prediction of preterm delivery was found between the two tests.

Both the tests had variable sensitivity, specificity, positive and negative predictive value across various studies. These discrepancies could have a variety of causes. Firstly, the variability in results may have arisen due to dissimilarities in case selection, as some studies selected both asymptomatic and symptomatic women, different timings of sample collection and diversities in the study population. Secondly, the different approaches used to obtain the sample could also alter the sensitivity of the tests. However, the cervicovaginal tests are useful in making predictions, despite the variations in cut-off values between studies.[109]

Current study also reports a high negative predictive value of cervicovaginal Prolactin as compared to other studies as well as with cervicovaginal β-hCG, because of which, its usage could lead to a reduction in unnecessary patient hospitalization and manipulative procedures or operations to some extent.

SUMMARY

The present study was undertaken to compare and correlate the cervicovaginal β-hCG and prolactin levels in women who were in preterm labour. A total of 40 subjects were taken where the majority (47.5%) of the subjects belonged to the 21 to 25 years age group. The mean cervicovaginal β-hCG and Prolactin level was highest among the 30 to 33 years age group which was 24.6 mIu/ml and 11.09 ng/ml. There was significant statistical difference between cervicovaginal Prolactin level and age with $p < 0.05$ whereas no significant statistical difference was seen between cervicovaginal β-hCG levels among the different age group.

The subjects were equally distributed according to parity - 50% were Primigravida and other 50% were multigravida. There was no significant statistical difference between parity and cervicovaginal β-hCG and Prolactin levels.

Of the 40 subjects, 60% belonged to late preterm gestation i.e, 34 to 36 completed weeks but the mean cervicovaginal β-hCG (34.39 ± 1.5 mIU/ml) and Prolactin (12.61 ± 0.7 ng/ml) level was highest i.e, among subjects with very preterm gestation i.e, 28 to 31 completed weeks. The association between cervicovaginal β-hCG level and gestational age at sample collection was found to be statistically significant with $p < 0.001$ but there was no association found between cervicovaginal Prolactin and gestational age.

70% of the subjects progressed to preterm delivery whereas the rest 30% continued till term. Mean cervicovaginal β-hCG and Prolactin level was found to be more among subjects with preterm delivery(19.20 mIU/ml, 10.88 ng/ml) as compared to term delivery (9.43 mIU/ml, 4.95ng/ml). There was a statistically significant difference found between delivery outcome and mean cervicovaginal β-hCG and Prolactin level with p-value < 0.001.

In the present study, it was noted that at a cut-off of > 15.54mIU/ml, the sensitivity, specificity, PPV and NPV of cervicovaginal β-hCG in predicting preterm delivery was found to be 60.7%, 100%, 100% and 52.2% respectively. The sensitivity, specificity, PPV, NPV of cervicovaginal Prolactin at cut-off > 6.24 ng/ml in predicting preterm delivery was found to be 89.29%, 83.3%, 92.6% and 76.9% respectively. Area under the curve for cervicovaginal β-hCG was 0.820 whereas, for cervicovaginal Prolactin, it was 0.920. Therefore, cervicovaginal Prolactin is slightly better than cervicovaginal β-hCG in predicting preterm delivery.

CONCLUSION

An optimal screening test should be affordable, straightforward, dependable and rapid because preterm birth is more frequently linked to poor socioeconomic position, rural upbringing, and advanced fetal station. Even though fetal fibronectin testing and transvaginal ultrasound of the cervix can help identify pregnant women who are at risk for preterm birth, ultrasonography requires specialized training and expensive equipment, and fibronectin assay kits are expensive and not readily available in primary care settings. [131]

On the other hand, cervicovaginal β-hCG and Prolactin tests are low-cost, rapid and reliable bedside predictors of preterm birth providing immediate results and a high level of predictability that may be obtained even in the most basic setups. Hence, they can be suggested as a standard procedure in clinical practise.

In our study, cervicovaginal Prolactin test was found be a better predictive biomarker than cervicovaginal β-hCG test.

LIMITATIONS:

- It is a study based at a single centre with limited sample size.
- Samples were collected in subjects who were admitted with symptoms of preterm labour i.e, symptomatic woman

RECOMMENDATIONS:

- Larger studies at multiple centers are required to gain a better understanding of the definitive role of these biomarkers in the prediction in high-risk population.
- Prospective studies involving the evaluation of the biomarkers in early pregnancy and consequent follow up are required to substantiate the findings.
- Further studies correlating history of preterm birth and predictive value of biomarkers can be undertaken.

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PROFORMA

NAME:

AGE:

ADDRESS:

UHID NO:

I.P NO:

DATE/ TIME OF ADMISSION:

DATE/ TIME OF DISCHARGE:

CHIEF COMPLAINTS:

OBSTETRICAL HISTORY: Booked/ Unbooked/ Referred

Married Life: Consanguinesous marriage: Yes/ No

Obstetrical Score:

MENSTRUAL HISTORY:

LMP: EDD: POG:

cEDD:

PAST HISTORY:

PERSONAL HISTORY:

Diet:

Appetite:

Bowel and bladder habits:

Smoking/ Alcohol:

GENERAL PHYSICAL EXAMINATION:

Pallor/ Icterus/ Cyanosis/ Clubbing/ Lymphadenopathy/ Edema

Thyroid: Breast: Spine:

Height: Weight: BMI:

Pulse: BP:

RR: Temp:

CNS:

CVS:

RS:

Per Abdomen:

Per Speculum:

Per Vagina:

PROVISIONAL DIAGNOSIS:

INVESTIGATIONS:

Date of Sampling:

Cervicovaginal Beta-hCG Level:

Cervicovaginal Prolactin Level:

GESTATIONAL AGE AT ADMISSION:

GESTATIONAL AGE AT DELIVERY:

INFORMED CONSENT FORM

I Mr./Mrs. _____ have been explained in my own understandable language, that I will be included in a study which is **"AN ANALYSIS OF CERVICOVAGINAL BETA-HCG AND PROLACTIN LEVELS AS A PREDICTIVE BIOMARKER OF PRETERM BIRTH IN SYMPTOMATIC WOMEN: A PROSPECTIVE COHORT STUDY "**

I have been explained that my clinical findings, investigations, postoperative findings will be assessed and documented for study purpose.

I have been explained my participation in this study is entirely voluntary, and I can withdraw from the study any time and this will not affect my relation with my doctor or the treatment for my ailment.

I have been explained about the interventions needed, possible benefits and adversities due to interventions, in my own understandable language.

I have understood that all my details found during the study are kept confidential and while publishing or sharing of the findings, my details will be masked.

I have the mobile number of the principal investigator for enquiries.

I, in my sound mind give full consent to be a part of this study.

Signature of the patient:

Name:

Signature of the witness:

Name:

Relation to patient:

Date:

Place:

ಮಾಹಿತಿ ಒಪ್ಪಿಗೆ ಪತ್ರ

ನಾನು ಶ್ರೀ / ಶ್ರೀ. _____ ಅನ್ನು ನನ್ನ ಸಂಪೂರ್ಣ ಅರ್ಥವಾಗುವ ಭಾಷೆಯಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ, ಇದನ್ನು ನಾನು ಅಧ್ಯಯನದಲ್ಲಿ ಸೇರಿಸಿಕೊಳ್ಳುತ್ತೇನೆ, ಅದು " ರೋಗಲಕ್ಷಣ ಮಹಿಳೆಯರಲ್ಲಿ ಪೂರ್ವ ಜನನದ ಪೂರ್ವಭಾವಿ ಬಯೋಮಾರ್ಕರ್ ಆಗಿ ಗರ್ಭಕಂಠ ಸ್ರವಿಸುವಿಕೆ ಬೀಟಾ-ಎಚ್‌ಸಿಜಿ ಮತ್ತು ಪ್ರೊಲ್ಯಾಕ್ಟಿನ್ ಮಟ್ಟಗಳ ವಿಶ್ಲೇಷಣೆ: ಪ್ರಾಸ್ಪೆಕ್ಟಿವ್ ಕೋಹಾರ್ಟ್ ಅಧ್ಯಯನ "

ನನ್ನ ಕ್ಲಿನಿಕಲ್ ಆವಿಷ್ಕಾರಗಳು, ತನಿಖೆಗಳು, ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ನಂತರದ ಸಂಶೋಧನೆಗಳನ್ನು ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಗುವುದು ಮತ್ತು ಅಧ್ಯಯನದ ಉದ್ದೇಶಕ್ಕಾಗಿ ದಾಖಲಿಸಲಾಗುತ್ತದೆ ಎಂದು ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ.

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಭಾಗವಹಿಸುವಿಕೆಯು ಸಂಪೂರ್ಣವಾಗಿ ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ ಎಂದು ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ, ಮತ್ತು ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಬಹುದು ಮತ್ತು ಇದು ನನ್ನ ವೈದ್ಯರೊಂದಿಗಿನ ನನ್ನ ಸಂಬಂಧ ಅಥವಾ ನನ್ನ ಕಾಯಿಲೆಗೆ ಚಿಕಿತ್ಸೆಯ ಮೇಲೆ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ.

ನನ್ನ ಸಂಪೂರ್ಣ ಅರ್ಥವಾಗುವ ಭಾಷೆಯಲ್ಲಿ, ಮಧ್ಯಸ್ಥಿಕೆಗಳ ಕಾರಣದಿಂದಾಗಿ ಸಂಭವನೀಯ ಪ್ರಯೋಜನಗಳು ಮತ್ತು ಪ್ರತಿಕೂಲತೆಗಳ ಅಗತ್ಯವಿರುವ ಮಧ್ಯಸ್ಥಿಕೆಗಳ ಬಗ್ಗೆ ನನಗೆ ವಿವರಿಸಲಾಗಿದೆ.

ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ಕಂಡುಬರುವ ನನ್ನ ಎಲ್ಲಾ ವಿವರಗಳನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗಿದೆ ಮತ್ತು ಸಂಶೋಧನೆಗಳನ್ನು ಪ್ರಕಟಿಸುವಾಗ ಅಥವಾ ಹಂಚಿಕೊಳ್ಳುವಾಗ, ನನ್ನ ವಿವರಗಳನ್ನು ಮರೆಮಾಚಲಾಗುತ್ತದೆ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ವಿಚಾರಣೆಗಾಗಿ ನನ್ನ ಬಳಿ ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿ ಮೊಬೈಲ್ ಸಂಖ್ಯೆ ಇದೆ.

ಈ ಅಧ್ಯಯನದ ಭಾಗದಲ್ಲಿ ಸೇರಿಸಲು ನನ್ನ ಸಂಪೂರ್ಣ ಮನಸ್ಸಿನಲ್ಲಿ ನಾನು ಸಂಪೂರ್ಣ ಒಪ್ಪಿಗೆ ನೀಡುತ್ತೇನೆ.

ರೋಗಿಯ ಸಹಿ:

ಹೆಸರು:

ಸಾಕ್ಷಿಯ ಸಹಿ:

ಹೆಸರು:

ರೋಗಿಗೆ ಸಂಬಂಧ:

ದಿನಾಂಕ:

ಸ್ಥಳ:

PATIENT INFORMATION SHEET

STUDY TITLE: "AN ANALYSIS OF CERVICOVAGINAL BETA-HCG AND PROLACTIN LEVELS AS A PREDICTIVE BIOMARKER OF PRETERM BIRTH IN SYMPTOMATIC WOMEN: A PROSPECTIVE COHORT STUDY".

STUDY SITE: R.L Jalappa Hospital and Research Centre, Tamaka, Kolar.

This is to inform you that I will be needing a swab from the cervicovaginal region for the testing of Beta-hCG and Prolactin levels. The entire finance of the tests will be borne by me alone and you will not be required to pay for anything outside of your regular treatment.

We are conducting this study to predict preterm delivery in women who get admitted with preterm labour pains. This study will help better understand preterm labour, will help in predicting preterm delivery in women with preterm labour and will hence, help in reducing the associated perinatal morbidity and mortality. If you are willing to be enrolled in this study, you will be required to give a swab from the cervicovaginal region for the tests. You will receive the standard care pre and post delivery or operatively irrespective of whether you choose to opt for the study or not.

You are free to opt-out of the study at any time if you are not satisfied or apprehensive to be a part of the study. Your treatment and care will not be compromised if you refuse to be a part of the study. The study will not add any risk or financial burden to you if you are part of the study. In case of any complication during surgery patient will be treated accordingly.

Your identity and clinical details will be confidential. You will not receive any financial benefit for being part of the study. You are free to contact Dr.AKSHITHA SAI RAGAM or any other member of the above research team for any doubt or clarification you have.

Dr. AKSHITHA SAI RAGAM

Mobile no: 9972402448

E-mail id: akshi.ragam@gmail.com

ರೋಗಿಯ ಮಾಹಿತಿ ಹಾಳೆ

ಅಧ್ಯಯನದ ಶೀರ್ಷಿಕೆ: "ರೋಗಲಕ್ಷಣ ಮಹಿಳೆಯರಲ್ಲಿ ಪೂರ್ವ ಜನನದ ಪೂರ್ವಭಾವಿ ಬಯೋಮಾರ್ಕರ್ ಆಗಿ ಗರ್ಭಕಂಠ ಸ್ರವಿಸುವಿಕೆ ಬೀಟಾ-ಎಚ್‌ಸಿಜಿ ಮತ್ತು ಪ್ರೊಲ್ಯಾಕ್ಟಿನ್ ಮಟ್ಟಗಳ ವಿಶ್ಲೇಷಣೆ: ಪ್ರಾಸ್ಪೆಕ್ಟಿವ್ ಕೋಹಾರ್ಟ್ ಅಧ್ಯಯನ"

ಅಧ್ಯಯನ ಸೈಟ್: ಆರ್.ಎಲ್ ಜಾಲಪ್ಪ ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ತಮಾಕಾ, ಕೋಲಾರ.
ಬೀಟಾ-ಎಚ್‌ಸಿಜಿ ಮತ್ತು ಪ್ರೊಲ್ಯಾಕ್ಟಿನ್ ಮಟ್ಟವನ್ನು ಪರೀಕ್ಷಿಸಲು ನನಗೆ ಗರ್ಭಕಂಠದ ಪ್ರದೇಶದಿಂದ ಸ್ವ್ಯಾಬ್ ಅಗತ್ಯವಿರುತ್ತದೆ ಎಂದು ನಿಮಗೆ ತಿಳಿಸಲು ಇದು. ಪರೀಕ್ಷೆಗಳ ಸಂಪೂರ್ಣ ಹಣಕಾಸು ನನ್ನಿಂದ ಮಾತ್ರ ಭರಿಸಲ್ಪಡುತ್ತದೆ ಮತ್ತು ನಿಮ್ಮ ನಿಯಮಿತ ಚಿಕಿತ್ಸೆಯ ಹೊರಗಿನ ಯಾವುದಕ್ಕೂ ನೀವು ಪಾವತಿಸಬೇಕಾಗಿಲ್ಲ.

ಪ್ರಸವಪೂರ್ವ ಹೆರಿಗೆ ನೋವಿನಿಂದ ಪ್ರವೇಶ ಪಡೆಯುವ ಮಹಿಳೆಯರಲ್ಲಿ ಅಕಾಲಿಕ ವಿತರಣೆಯನ್ನು ತಿಳುವಳಿಕೆ ನಾವು ಈ ಅಧ್ಯಯನವನ್ನು ನಡೆಸುತ್ತಿದ್ದೇವೆ. ಈ ಅಧ್ಯಯನವು ಪ್ರಸವಪೂರ್ವ ಕಾರ್ಮಿಕರನ್ನು ಚೆನ್ನಾಗಿ ಅರ್ಥಮಾಡಿಕೊಳ್ಳಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ, ಪ್ರಸವಪೂರ್ವ ಕಾರ್ಮಿಕರಿರುವ ಮಹಿಳೆಯರಲ್ಲಿ ಅವಧಿಪೂರ್ವ ಹೆರಿಗೆಯನ್ನು ತಿಳುವಳಿಕೆ ಸಹಾಯ ಮಾಡುತ್ತದೆ ಮತ್ತು ಆದ್ದರಿಂದ, ಸಂಬಂಧಿತ ಪೆರಿನಾಟಲ್ ಕಾಯಿಲೆ ಮತ್ತು ಮರಣ ಪ್ರಮಾಣವನ್ನು ಕಡಿಮೆ ಮಾಡಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ. ಈ ಅಧ್ಯಯನಕ್ಕೆ ಸೇರ್ಪಡೆಗೊಳ್ಳಲು ನೀವು ಸಿದ್ದರಿದ್ದರೆ, ಪರೀಕ್ಷೆಗಳಿಗಾಗಿ ನೀವು ಗರ್ಭಕಂಠದ ಪ್ರದೇಶದಿಂದ ಸ್ವ್ಯಾಬ್ ನೀಡಬೇಕಾಗುತ್ತದೆ. ನೀವು ಅಧ್ಯಯನವನ್ನು ಆರಿಸಿಕೊಳ್ಳುತ್ತೀರೋ ಇಲ್ಲವೋ ಎಂಬುದನ್ನು ಲೆಕ್ಕಿಸದೆ ನೀವು ಪ್ರಮಾಣಿತ ಆರೈಕೆ ಪೂರ್ವ ಮತ್ತು ನಂತರದ ವಿತರಣೆಯನ್ನು ಸ್ವೀಕರಿಸುತ್ತೀರಿ.

ನೀವು ಅಧ್ಯಯನದ ಭಾಗವಾಗಲು ತೃಪ್ತಿ ಹೊಂದಿಲ್ಲದಿದ್ದರೆ ಅಥವಾ ಭಯಪಡದಿದ್ದರೆ ನೀವು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದಿಂದ ಹೊರಗುಳಿಯಲು ಮುಕ್ತರಾಗಿದ್ದೀರಿ. ನೀವು ಅಧ್ಯಯನದ ಭಾಗವಾಗಲು ನಿರಾಕರಿಸಿದರೆ ನಿಮ್ಮ ಚಿಕಿತ್ಸೆ ಮತ್ತು ಕಾಳಜಿಗೆ ಧಕ್ಕೆಯಾಗುವುದಿಲ್ಲ. ನೀವು ಅಧ್ಯಯನದ ಭಾಗವಾಗಿದ್ದರೆ ಅಧ್ಯಯನವು ನಿಮಗೆ ಯಾವುದೇ ಅಪಾಯ ಅಥವಾ ಆರ್ಥಿಕ ಹೊರೆ ಸೇರಿಸುವುದಿಲ್ಲ. ಶಸ್ತ್ರಚಿಕಿತ್ಸೆಯ ಸಮಯದಲ್ಲಿ ಯಾವುದೇ ತೊಡಕು ಉಂಟಾದರೆ ರೋಗಿಗೆ ಅನುಗುಣವಾಗಿ ಚಿಕಿತ್ಸೆ ನೀಡಲಾಗುತ್ತದೆ.

ನಿಮ್ಮ ಗುರುತು ಮತ್ತು ಕ್ಲಿನಿಕಲ್ ವಿವರಗಳು ಗೌಪ್ಯವಾಗಿರುತ್ತದೆ. ಅಧ್ಯಯನದ ಭಾಗವಾಗಿರುವುದರಿಂದ ನೀವು ಯಾವುದೇ ಆರ್ಥಿಕ ಲಾಭವನ್ನು ಪಡೆಯುವುದಿಲ್ಲ. ನೀವು ಹೊಂದಿರುವ ಯಾವುದೇ ಅನುಮಾನ ಅಥವಾ ಸ್ಪಷ್ಟೀಕರಣಕ್ಕಾಗಿ ನೀವು ಡಾ.ಅಕ್ಷಿತಾ ಸಾಯಿ ರಾಗಮ್ ಅಥವಾ ಮೇಲಿನ ಸಂಶೋಧನಾ ತಂಡದ ಇತರ ಸದಸ್ಯರನ್ನು ಸಂಪರ್ಕಿಸಲು ಮುಕ್ತರಾಗಿದ್ದೀರಿ.

ಡಾ.ಅಕ್ಷಿತಾ ಸಾಯಿ ರಾಗಂ

ಮೊಬೈಲ್ ಸಂಖ್ಯೆ: 9972402448

ಇ-ಮೇಲ್ ಐಡಿ: akshi.ragam@gmail.com

MASTERCHART

NAME	AGE	UHID NO.	PRIMI/ MULTI	LMP	EDD	GA at sample collection	GA at delivery	Beta-HCG (mu/ml)	Prolactin (ng/ml)	Term/ Preterm Delivery
Veena	19	883415	Primi	27.08.20	03.06.21	35 w 3 d	35 w 3 d	7.2	9.76	Preterm
Lakshmidevamma	28	914592	Primi	25.08.20	01.06.21	36 w	36 w 1 d	16.62	8.2	Preterm
Chaithra	23	914198	G2P1L1	24.09.20	01.07.21	32 w 4 d	32 w 6 d	22.47	13.62	Preterm
Varalakshmi	26	916914	G2P1L1	30.07.20	06.05.21	31 w 4 d	39 w 2 d	15.54	3.2	Term
Shravani	23	884589	Primi	24.08.20	31.05.21	33 w	37 w 2 d	10.47	5.56	Term
Kanaka	26	916317	Primi	09.09.20	16.06.21	34 w 6 d	34 w 6 d	9.86	6.43	Preterm
Vidhyashree	21	916675	G2P1L1	29.09.20	06.07.21	32 w 1 d	32 w 1 d	27.16	13.42	Preterm
Shruthi	22	917417	Primi	27.08.20	03.06.21	33 w 2 d	38 w 6 d	8.62	4.57	Term
Chaithra	24	924444	G2P1L1	12.09.20	19.06.21	34 w 3 d	38 w 2 d	8.4	2.24	Term
Kavitha	25	926697	Primi	18.09.20	25.06.21	35 w 2 d	38 w 5 d	10.22	5.86	Term
Roopa	19	930835	Primi	21.11.20	28.08.21	33 w	33 w	19.45	12.82	Preterm
Chaithra	20	931268	G2A1	07.11.20	14.08.21	35 w 3 d	35 w 4 d	13.56	7.88	Preterm
Salamma	26	931429	Primi	20.10.20	27.07.21	33 w 5 d	39 w 2 d	6.64	2.22	Term
Venkatamma	32	933977	G2P1L1	04.11.20	11.08.21	33 w	39 w 4 d	9.62	3.66	Term
Gowthami	26	934888	G3P2L2	14.11.20	21.08.21	32 w 2 d	38 w 4 d	12.61	2.12	Term
Gayathri	20	937740	Primi	21.11.21	28.08.21	34 w 2 d	38 w	7.66	8.67	Term
Navith	21	942432	Primi	29.12.20	05.10.21	34 w 3 d	34 w 4 d	21.11	10.42	Preterm
Sowmya	25	944920	G2P1L1	13.01.21	20.10.21	34 w	34 w	27.12	12.87	Preterm
Lavanya	22	948863	G2P1L1	11.01.21	18.10.21	36 w 4 d	36 w 4 d	8.22	10.22	Preterm
Sowjanya	21	947557	Primi	19.01.21	26.10.21	35 w 4 d	35 w 4 d	17.65	7.27	Preterm
Kavya	22	949398	G2P1L1	07.02.21	14.11.21	33 w	33 w	18.61	7.22	Preterm
Suguna	24	949770	Primi	17.02.21	24.11.21	32 w 1 d	32 w 3 d	13.42	5.44	Preterm
Anitha	23	952232	G2P1L0	19.03.21	24.12.21	28 w 6 d	28 w 6 d	33.42	12.56	Preterm
Noor Afza	18	952516	Primi	26.02.21	03.12.21	34 w 2 d	34 w 2 d	11.67	5.69	Preterm
Anumunisa	31	44931	G2P1L1	01.03.21	06.12.21	36 w 4 d	36 w 4 d	26.56	16.44	Preterm
Rukmini	22	45390	Primi	22.03.21	27.12.21	34 w	34 w	8.43	13.67	Preterm
Rekha	24	25010	Primi	10.04.21	15.01.22	33 w 3 d	33 w 3 d	9.43	6.94	Preterm
Shwetha	21	40020	Primi	28.03.21	02.01.22	35 w 4 d	35 w 4 d	18.79	15.56	Preterm
Shashikala	21	50503	Primi	14.04.21	19.01.22	34 w	34 w 1 d	24.52	17.22	Preterm
Priyanka	22	44360	Primi	29.03.21	03.01.22	32 w	37 w 1 d	8.66	5.28	Term
Nethravathi	25	44023	G2A1	18.03.21	23.12.21	33 w 5 d	38 w	5.4	6.24	Term
Haritha	23	75332	G2P1L0	12.08.21	19.05.22	35 w 6 d	36 w	8.42	6.22	Preterm
Hemala	22	78909	G3P2L1	15.11.21	22.08.22	32 w 1 d	32 w 2 d	12.23	13.51	Preterm
Sujatha	25	81388	Primi	09.10.21	16.07.22	30 w	30 w	36.14	13.44	Preterm
Renuka	23	81671	G2P1L1	30.09.21	07.07.22	31 w 3 d	31 w 3 d	32.1	14.62	Preterm
Varalakshmi	30	84732	G2P1L1	04.11.21	11.08.22	28 w	28 w	33.61	11.85	Preterm
Ruksana	24	86729	G4P3L3	25.09.21	02.07.22	34 w 6 d	38 w 2 d	9.42	9.86	Term
Salma Taj	33	89918	G2P1L1	01.11.21	08.08.22	31 w	31 w 1 d	28.61	12.43	Preterm
Javeriya	19	52962	Primi	21.10.21	28.07.22	35 w 5 d	35 w 5 d	6.64	12.66	Preterm
Kavya	27	77916	G3P2L2	12.11.21	19.08.22	34 w 5 d	34 w 5 d	24.76	6.53	Preterm