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Research Article

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Maternal Serum Interleukin-6 as a Biomarker for Early detection of Preterm Labor

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Abstract

Preterm labor, defined as the onset of labor before 37 weeks of gestation, remains a significant challenge in obstetrics. It is a leading cause of neonatal morbidity and mortality worldwide. Maternal serum interleukin-6 (IL-6) has emerged as a promising biomarker that may aid in the early detection of preterm labor. This article explores the role of maternal serum IL-6 as a potential biomarker and its implications in the prediction of preterm labor.

Methods of Study: *This is case-control study of preterm birth conducted at a tertiary care center at Ain Shams University. We enrolled 88 women with singleton pregnancies divided into two groups. Group 1 (study group), Included 44 women after 28 weeks and 0 days and before 37 weeks and 0 days of gestational age diagnosed with preterm labor. Group II (The control group), Included 44 women singleton pregnancies after 28 weeks and 0 days and before 37 weeks and 0 days of gestational age and not in labor who delivered at term. Maternal placental tissues and maternal serum interleukin-6 (IL-6) were evaluated for cases and control groups. The primary outcome is the recorded higher elevation of interleukin -6 in case group with evidences of infection in the placental tissue. Secondary Outcome, preterm delivery of those who showed higher levels of maternal serum interleukin -6.*

Results: *Among 88 studies women, levels were statistically significantly higher among case group of preterm labor compared to women who delivered at term (mean IL-6: 138.27 vs. 7.55 pg/mL, P < 0.0001). Similarly, levels were statistically significantly higher among women who infected placental tissues compared to the none infected placental tissue (mean IL-6: 175.33 vs. 27.08 pg/mL, P< 0.0001). There was no statistical difference between cases and control groups regarding the mean age, number of deliveries, early pregnancy losses.*

Conclusions: *After controlling the potential confounders, we recorded in this study a positive correlation between the higher levels of biomarker of InterleukinL-6 among women who presented in our tertiary center with preterm labor, suggesting an imbalance of immune regulation could impact cervical length and could be used as a marker of an early prediction of preterm labor. The interleukin-6 shows mild elevation in all non-infected preterm labors with 13.35 pg/ml as a cut-off point and shows also higher elevation in infected preterm labor with 50.5 pg/ml as cut-off point. Therefore, Maternal Serum Interleukin-6 can be used as a marker to predict the infection induced preterm labor.*

Key Words: Preterm labor, interleukin-6, preterm birth, cervical length, pregnancy.

1. Introduction

Preterm labor, defined as the onset of labor before 37 weeks of gestation, remains a significant challenge in obstetrics. In the United States, approximately 12.7% of pregnancies are affected by preterm birth, which accounts for 75% of perinatal deaths and over 50% of the long-term health issues experienced by survivors [1]. It is a leading cause of neonatal morbidity and mortality worldwide. Identifying reliable biomarkers for the early prediction of preterm labor can help healthcare providers intervene in a timely manner and potentially improve maternal and neonatal outcomes. *The incidence of preterm labor is increasing, and there is no single reliable method for early diagnosis and prevention. The incidence of preterm labor* is 8%-12% has not changed over the last 40 years despite the use of tocolytic drugs [2]. In addition,

the cost of prematurity are steep specially for neonates born before 28 age weeks. Thus, the importance to find a one test to use as a biomarker for early detections of preterm labor before the actual start of inevitable labor [2].

From an evolutionary standpoint, the initiation of preterm labor during an infection can be seen as beneficial for survival, as it enables the mother to eliminate infected tissue and maintain her reproductive fitness. It is fascinating to observe how molecular mechanisms originally developed for defending against infections in primitive multicellular organisms, such as pattern recognition receptors in sponges, have been adapted in viviparous species to trigger labor when infection is present. This unique maternal defense mechanism, however, comes with a trade-off - premature birth. In terms of the fetus, inflammation may also serve a purpose near full-term, aiding in the defense against infection and promoting faster lung maturation

Maternal serum interleukin-6 (IL-6) has emerged as a promising biomarker that may aid in the early detection of preterm labor. This article explores the role of maternal serum IL-6 as a potential biomarker and its implications in the prediction of preterm labor. Interleukin-6 (IL-6) is a cytokine that plays a role in the inflammatory response. It is produced by a variety of cells, including macrophages, lymphocytes, and fibroblasts. Interleukin-6 is a pro-inflammatory cytokine involved in various physiological processes, including immune regulation, inflammation, and tissue repair. During pregnancy, the placenta and fetal membranes produce IL-6, which enters the maternal circulation. As a result, researchers have focused on exploring the utility of maternal serum IL-6 as a biomarker for predicting preterm labor.

The use of IL-6 as a biomarker for the early prediction of preterm labor has a number of potential advantages. IL-6 is a relatively inexpensive and easy-to-measure biomarker. It can be measured in a variety of settings, including prenatal care clinics and hospitals. IL-6 is also a relatively non-invasive biomarker. It can be measured from a blood sample, which is a less invasive procedure than amniocentesis or chorionic villus sampling.

The use of IL-6 as a biomarker for the early prediction of preterm labor has a number of potential limitations. IL-6 is not a perfect biomarker. It is not always elevated in women with preterm labor, and it is not specific to preterm labor, it can be elevated with other conditions as well, such as infection and inflammation. IL-6 is also a dynamic biomarker. Its levels can fluctuate over time, making it difficult to interpret single measurements. In term of application, it is not an easy test to run in emergency settlings, like the emergency rooms and private practices for immediate result. The blood sample need to be collected and measurement has to be conducted by laboratory settings. Despite its limitations, IL-6 is a promising biomarker for the early prediction of preterm labor.

A number of studies have investigated the use of IL-6 as a biomarker for the early prediction of preterm labor. The results of these studies have been mixed, with some studies showing that IL-6 is a sensitive and specific marker for preterm labor, while others have shown that IL-6 is not a reliable marker.

2. Evidence supporting the use of Maternal Serum IL-6 as a Biomarker:

A study conducted by [Heng et al.] found that elevated maternal serum IL-6 levels in the second trimester were significantly associated with an increased risk of preterm labor [3]. The authors concluded that measuring IL-6 levels could help identify women at higher risk of preterm labor and facilitate appropriate interventions.

In a prospective cohort study by [Poggi et al.], researchers observed that women who subsequently delivered preterm had higher levels of maternal serum IL-6 as early as 24-28 weeks of gestation compared to those who delivered at term. This finding suggests that IL-6 may serve as an early predictive biomarker for preterm labor [4].

A systematic review and meta-analysis by [Lee et al.] investigated the accuracy of various biomarkers, including IL-6, in predicting preterm birth [5]. The analysis demonstrated that elevated levels of maternal serum IL-6 were significantly associated with an increased risk of preterm birth, with a sensitivity of 64% and a specificity of 78%, supporting its potential as a predictive biomarker. The author of the meta-analysis concluded that IL-6 is a promising biomarker for the early prediction of preterm labor, and that further studies are needed to confirm these findings.

Further studies are needed to confirm the findings of the meta-analysis and to develop a reliable method for using IL-6 to identify women at risk for preterm labor. If successful, the use of IL-6 as a biomarker could lead to the development of new interventions to prevent preterm birth and improve neonatal outcomes.

Early identification of women at risk for preterm labor enables healthcare providers to offer interventions such as close monitoring, administration of corticosteroids to promote fetal lung maturation, and preventive measures to prolong gestation. Maternal serum IL-6, if validated as a reliable biomarker, could be incorporated into routine prenatal care to enhance preterm labor prediction. Additionally, further studies are needed to establish standardized cutoff values and evaluate the cost-effectiveness of incorporating IL-6 testing into routine care.

Preterm birth is a multifactorial condition influenced by various maternal, uterine, placental, and fetal factors [6]. The underlying mechanisms involve complex interactions between hormonal, inflammatory, immune, and mechanical processes. Understanding these causes and mechanisms is essential for developing targeted interventions and preventive strategies to reduce the incidence of preterm birth and improve neonatal outcomes. Understanding the causes and underlying mechanisms that contribute to preterm birth is crucial for developing effective preventive strategies and

improving maternal and neonatal outcomes. This article explores the various causes and underlying mechanisms associated with preterm birth.

The limitations of maternal serum interleukin-6 (IL-6) in acute preterm labor include the following [7]:

I. Timing of measurement: Maternal serum IL-6 levels may vary depending on the stage and progression of labor. It may not accurately reflect the acute inflammatory response at the time of testing, as there can be fluctuations in cytokine levels during labor.

II. Specificity: Maternal serum IL-6 is not specific to preterm labor and can be elevated in various other conditions, such as infections or inflammatory processes unrelated to preterm labor. This lack of specificity may limit its diagnostic accuracy in identifying acute preterm labor specifically.

III. False negatives: Maternal serum IL-6 levels may not always be elevated in cases of acute preterm labor, leading to false negatives. This can occur due to variations in individual immune responses or other factors influencing IL-6 levels, resulting in missed diagnoses of preterm labor.

IV. Limited predictive value: While maternal serum IL-6 can indicate the presence of inflammation, it may not provide precise predictive value in terms of timing or likelihood of preterm birth. It serves as an indicator of inflammation but does not offer detailed information on the progression or severity of preterm labor.

V. Clinical interpretation: Interpreting maternal serum IL-6 levels requires careful consideration of other clinical factors, such as cervical examination and assessment of contractions. Relying solely on IL-6 levels may overlook other important signs or symptoms of preterm labor.

3. Etiologies of preterm birth

3.1. Maternal Factors

Several maternal factors have been identified as potential contributors to preterm birth:

1. Infection and inflammation: Infections of the reproductive tract, such as bacterial vaginosis, urinary tract infections, and sexually transmitted infections, can trigger an inflammatory response, leading to preterm labor.

2. Maternal age: Adolescents and women over 35 years of age have an increased risk of preterm birth.

3. Medical conditions: Chronic conditions like hypertension, diabetes, and preeclampsia have been associated with an elevated risk of preterm birth.

4. Lifestyle factors: Smoking, substance abuse, inadequate prenatal care, and maternal stress have also been linked to preterm birth.

5. Uterine Factors: The uterus plays a crucial role in maintaining pregnancy. Various uterine factors can contribute to preterm birth:

a) Uterine abnormalities: Structural abnormalities, such as uterine malformations or cervical incompetence, can increase the risk of preterm labor.

b) Uterine over distention: Multiple gestations, polyhydramnios (excessive amniotic fluid), or uterine fibroids can lead to uterine overdistention, triggering preterm contractions.

4. Placental Factors

I. Infections: Infections, such as urinary tract infections, bacterial vaginosis, and sexually transmitted infections, can trigger an inflammatory response that may lead to preterm birth. The infection can directly affect the uterus or cause systemic inflammation, which can induce early labor [8].

II. Chronic conditions: Chronic medical illnesses like hypertension, diabetes, and autoimmune diseases can increase the risk of preterm birth. These conditions can affect the placenta's function, impair fetal development, and contribute to complications such as preeclampsia or gestational diabetes, which are associated with preterm birth [9].

III. Maternal obesity: Obesity is a risk factor for preterm birth. It can lead to inflammation, insulin resistance, and hormonal imbalances, which may disrupt normal pregnancy processes and increase the likelihood of preterm labor [10].

IV. Maternal mental health disorders: Mental health conditions, such as depression, anxiety, and stress, have been associated with an increased risk of preterm birth. These disorders can activate the maternal stress response and affect hormonal balance, potentially leading to preterm labor [11].

V. Antepartum Hemorrhage:

a) Retro-placental hemorrhage: The placenta is essential for providing oxygen and nutrients to the fetus. Disruptions in placental function can contribute to preterm birth: The production of thrombin during decidual hemorrhage can activate contractions in the myometrium and break down the extracellular matrix in the chorioamniotic membranes, making them more prone to rupture. Mothers who show signs of heightened thrombin generation are at a higher risk of experiencing spontaneous preterm labor [12,13].

b) Accidental hemorrhage/placenta previa: Pregnancies affected by abnormal umbilical artery blood flow (ABUO) face an elevated likelihood of preterm delivery and induced labor. However, after accounting for preterm birth, there was no observed rise in perinatal mortality [14].

c) Placental insufficiency: Patients experiencing preterm labor often exhibit placental vascular abnormalities, including a lack of proper transformation of the uterine spiral arteries. Conditions that impair blood flow to the placenta, such as preeclampsia or placental abruption, can result in inadequate nutrient supply and oxygenation, leading to preterm birth [15].

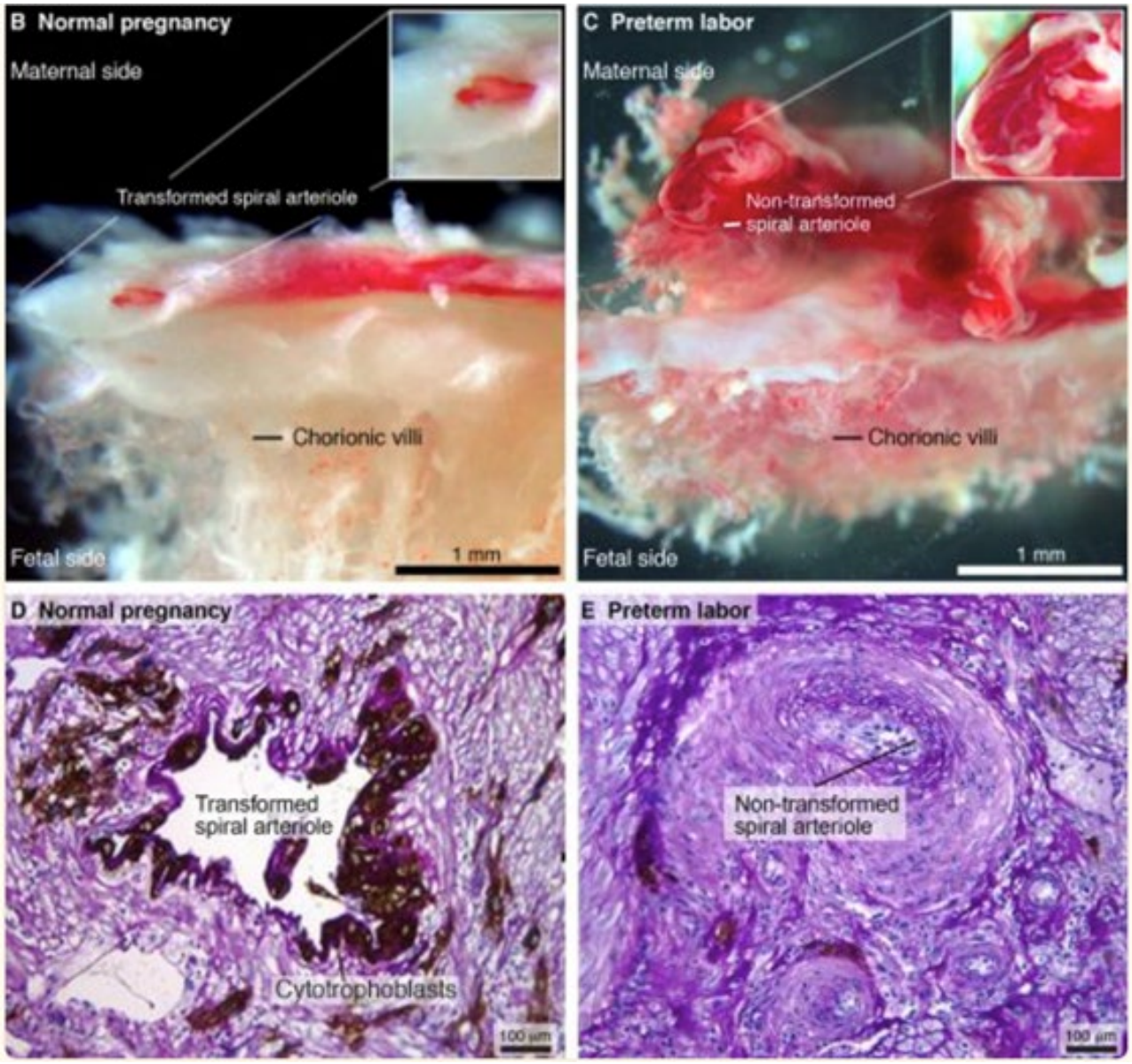


Figure 1: Placental insufficiency. (A) The diagram illustrates the interface between the mother and fetus during a normal pregnancy. A physiologically transformed uterine spiral artery with a wide opening transports blood to the intervillous space of the placenta, ensuring adequate blood supply to the villi. (B) A spiral artery with an expanded opening that allows for sufficient perfusion of the intervillous space. (C) In a patient with spontaneous preterm labor, a narrow spiral artery with failed physiological transformation has a constricted opening. (D) Histological staining of the maternal-fetal interface in a normal pregnancy using PAS staining reveals a transformed spiral artery lined with cytokeratin 7-positive cytotrophoblasts (brown) that form the inner surface of the artery (200x magnification). (E) In a patient experiencing preterm labor, there is a failure of proper transformation of the spiral artery, resulting in a narrow lumen, and the cytotrophoblasts have not invaded the muscular wall (200x magnification) [16,17].

5. Infection and inflammation: The normal flora is characterized by an anaerobes-to-aerobes ratio of 2:1 to 5:1. The production of lactic acid by lactobacilli keeps the pH of the vagina below 4.5, which inhibits the growth of other pathogenic organisms. Under normal conditions, when the pH is low, lactobacilli produce hydrogen peroxide (H2O2), which is toxic to bacteria in two ways: by producing toxic hydroxic radicals and by combining with the chlorine ions in the vagina to synthesize chloridinium ions. When the pH is high, as seen when the alkalinity is increased (e.g. bleeding in pregnancy, sexual intercourse, or vaginal douching), when alterations in the endocrine status take place, or during antibiotic treatment, lactobacilli produce a lower amount of H2O2, thus permitting the overgrowth of other organisms [Goldenberg et al., 2000]. Bacteria ascending from the vagina and cervix subsequently colonize the fetal membranes and decidua. Infections affecting the placenta, such as chorioamnionitis, can trigger an inflammatory response and preterm labor. Approximately one out of every three preterm infants is born to mothers who have an intra-amniotic infection, which often presents with minimal symptoms [17].

The microorganisms detected in the amniotic fluid are similar to those found in the lower genital tract, suggesting that the most common route of infection is through ascending transmission. Additionally, bacteria associated with periodontal disease have been identified in the amniotic fluid, indicating that dissemination through the bloodstream with transplacental passage is also possible [18]. The process of preterm labor triggered by microbial infection is mediated by an inflammatory response. Microorganisms and their byproducts are recognized by pattern recognition receptors, such as toll-like receptors (TLRs), which stimulate the production of chemokines (e.g., IL-8, IL-1, CCL-2), cytokines (e.g., IL-β, TNF-α) prostaglandins, and proteases, ultimately activating the common pathway leading to the onset of labor [19,20].

Biochemical changes in the cervix, amniotic fluid, provide insights into the physiological and pathological processes associated with preterm birth.

Vaginal Infections that causes an elevation in pH observed in cervical secretions, may indicate alterations in the local microenvironment. Changes in pH levels can impact the balance of microbial colonization and influence the integrity of cervical tissues, potentially affecting the risk of preterm birth.

Increased concentrations of di-amides, polyamines, and enzymes suggest potential dysregulation of specific biochemical pathways involved in tissue remodeling, inflammation, immune responses, or hormonal regulation. These changes may disrupt the delicate balance required for maintaining a healthy pregnancy and contribute to the onset of preterm labor [17].

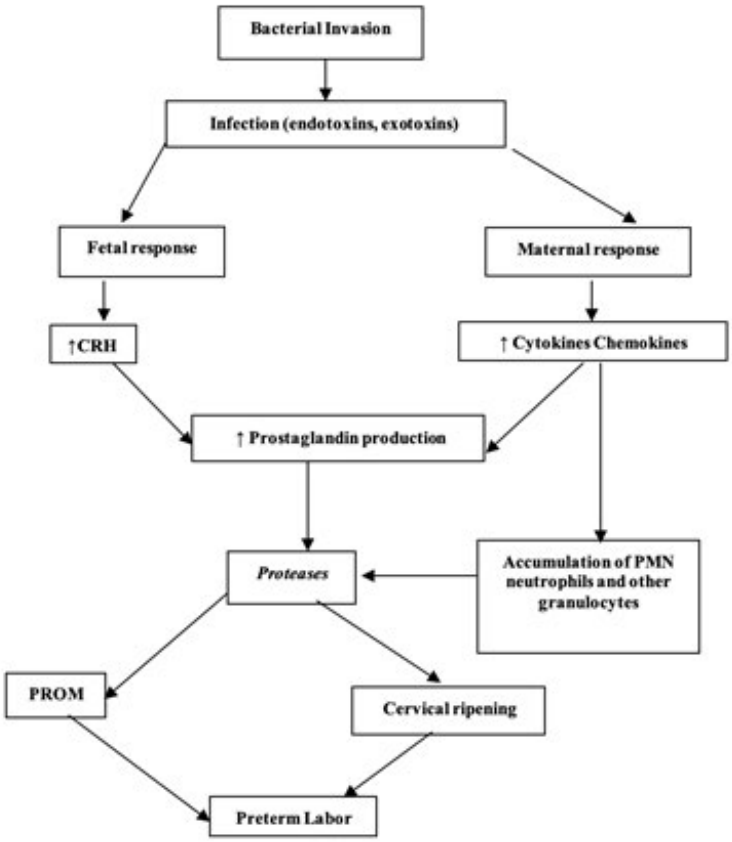


Figure-2: Pathogenic mechanisms implicated in infections associated preterm labor. (CRH) coiticotropin releasing hormone, (PMN) polymorphonuclear neutrophils, (PROM) premature rupture of the fetal membranes [21].

6. Fetal Factors

The health and well-being of the fetus can influence the timing of delivery:

• **Fetal growth restriction:** Inadequate growth and development of the fetus, often due to placental insufficiency, can lead to preterm birth [22-24].

• **Multiple gestations:** This overdistension can lead to increased uterine irritability and a higher frequency of contractions, potentially triggering preterm labor. Multiple gestations often have a higher risk of placental problems, such as placental abruption, placenta previa or placental insufficiency. Multiple gestations are associated with a higher risk of various pregnancy complications, including preeclampsia, gestational diabetes, and intrauterine growth restriction. These complications can contribute to an increased risk of preterm birth [25].

7. Underlying Mechanisms

Several underlying mechanisms contribute to the initiation of preterm labor:

1. Hormonal changes: The onset of labor involves complex hormonal interactions, including an increase in oxytocin and a decrease in progesterone levels.

2. Inflammation and immune activation: Inflammatory processes and immune activation, triggered by infections or other factors, can promote uterine contractions and cervical ripening.

3. Mechanical stretch: Over distention of the uterus, has been linked to spontaneous preterm birth in cases involving multiple gestations and polyhydramnios, where there is an excessive amount of amniotic fluid. In studies conducted on non-human primates, the inflation of intra-amniotic balloons has been shown to induce uterine contractions, leading to preterm labor, along with an associated "inflammatory pulse." This inflammatory response is characterized by elevated concentrations of inflammatory cytokines, including IL-β, TNFα, IL-8, and IL-6, in the maternal plasma [26]. This finding aligns with observations that stretching the human myometrium leads to the over expression of inflammatory cytokines.

4. Genetic factors: Several genetic variants have been identified that are associated with an increased susceptibility to preterm birth. These variants involve genes related to immune responses, inflammation, hormonal regulation, and the structure of the cervix and fetal membranes. It is important to note that while genetic factors contribute to the risk of preterm birth, they do not act in isolation. Environmental factors, such as maternal health, lifestyle, and exposure to certain infections or stressors, interact with genetic predispositions, further influencing the likelihood of preterm birth.

a. Immune system function: One of the genetic risk factors for preterm birth involves genes associated with immune system function. Variations in genes involved in the immune response, such as those related to the production and regulation of cytokines, can influence the susceptibility to infections or inflammatory conditions that may trigger preterm labor [27].

b. Hormonal regulation is another aspect influenced by genetic factors. Genetic variants in genes involved in the production and regulation of hormones, such as progesterone and oxytocin, can impact the timing and strength of uterine contractions, potentially leading to preterm birth [28].

c. Structural changes in the cervix and fetal membranes can also contribute to preterm birth risk. Genetic variations in genes responsible for the formation and maintenance of cervical tissue and fetal membrane integrity can affect the structural strength and ability to withstand the pressures of pregnancy, making them more prone to premature rupture or cervical insufficiency [29].

8. Screening and prevention of preterm birth

Two primary predictors of preterm labor have emerged: the identification of a sonographic short cervix during the mid-trimester and a history of previous spontaneous preterm birth [30]. In terms of prevention strategies, the administration of vaginal progesterone to asymptomatic women with a short cervix during the mid-trimester has yielded promising outcomes. It reduces the incidence of preterm birth before 33 weeks by 45% and lowers the occurrence of neonatal complications, including neonatal respiratory distress syndrome [31]. For women with a prior history of spontaneous preterm birth, the use of 17-alpha hydroxyprogesterone caproate has demonstrated a 34% reduction in the rate of preterm birth before 37 weeks and a decrease in the need for oxygen supplementation for infants [32]. Additionally, in patients with a previous spontaneous preterm birth and a short cervix, cervical cerclage has been found to decrease the rate of preterm birth before 35 weeks by 30% and improve composite perinatal mortality/morbidity. However, it is worth noting that vaginal progesterone exhibits comparable effectiveness to cervical cerclage in these patients, offering the advantage of not requiring anesthesia or a surgical procedure.

9. Diagnosis of Preterm Labor

The American College of Obstetricians and Gynecologists (ACOG) has published a Practice Bulletin that provides guidance on the diagnosis of preterm labor. Here is a summary of the key points covered in the bulletin:

• **Clinical Evaluation:** The diagnosis of preterm labor involves a thorough clinical evaluation, including assessing the patient's symptoms, medical history, and physical examination. Symptoms such as regular uterine contractions, pelvic pressure, or changes in vaginal discharge should be carefully evaluated.

• **Cervical Assessment:** Cervical examination is an important component of the diagnostic process. Assessment of cervical dilation, effacement, and consistency can provide information about the likelihood of preterm labor. Transvaginal ultrasound measurement of cervical length has been shown to be a useful tool in predicting preterm birth.

• **Transvaginal ultrasound measurement of cervical length and assessment** is indicated to evaluate the risk of preterm birth. Here are some common indications for this procedure:

1. History of Preterm Birth: Women with a history of previous preterm birth are at an increased risk of recurrent preterm labor in subsequent pregnancies.

2. Suspected or threatened Preterm Labor: In cases where a woman presents with symptoms suggestive of preterm labor, such

as regular uterine contractions or pelvic pressure, transvaginal ultrasound measurement of cervical length can provide valuable information. A shortened cervical length is associated with an increased risk of preterm birth.

3. Multiple Gestations.

4. Low-Risk Pregnancies: In certain clinical situations, healthcare providers may choose to perform transvaginal ultrasound measurement of cervical length in low-risk pregnancies. This may include situations where there are concerns about potential cervical insufficiency or if there is a need for reassurance regarding the risk of preterm birth.

5. Fetal Fibronectin Testing: Fetal fibronectin (fFN) is a protein produced by fetal cells, acts as a glue-like substance attach the fetal membranes to the uterine lining. The presence of fFN in cervical secretions during pregnancy has been investigated as a potential marker for predicting preterm labor. A positive fFN test result indicates the presence of fFN in the cervix, which suggests that the fetal membranes may be at risk of separation from the uterine lining, potentially leading to preterm labor [33]. A negative fFN test result can provide reassurance that preterm birth is unlikely within the next 1-2 weeks, while a positive result does not definitively indicate preterm labor but suggests an increased risk.

6. Biomarkers and Proteomic Profiling: The use of additional biomarkers or proteomic profiling to diagnose preterm labor is an area of ongoing research. While several markers such as inflammatory markers and recently cell-free fetal (cff) DNA have shown promise, further studies are needed to determine their clinical utility.

7. (Cell-free Fetal DNA): Recently, there has been a proposal suggesting a potential role for cell-free fetal (cff) DNA as a signaling factor in the initiation of labor (Phillippe et al, 2014). In pregnant women, cff DNA is naturally present in the plasma, and its concentrations increase as gestational age progresses, reaching a peak towards the end of pregnancy just before the onset of labor. Notably, cff DNA differs from adult cell-free DNA as it is hypomethylated and has the ability to activate Toll-like receptor 9 (TLR-9), thereby inducing an inflammatory response [34,35]. The subsequent effects may involve the activation of the common pathway leading to labor. Interestingly, individuals who exhibit elevated levels of cff DNA during the midtrimester are at higher risk of spontaneous preterm birth later in the gestational period [36-38]. The concept that cff DNA could serve as a mediator of a fetal/placental/maternal communication mechanism to signal the onset of labor in both normal pregnancies and instances of preterm labor following an insult is an intriguing hypothesis that warrants further investigation [39].

10. Management of Preterm Labor

The American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin (number 212, published in July 2019) the treatment of preterm labor provides recommendations for managing and preventing preterm birth. The bulletin emphasizes the importance of accurately diagnosing preterm labor and assessing the risks and benefits of various interventions. Here is a summary of the key points:

10.1. Tocolytic Therapy: Tocolytic medications can be used to inhibit uterine contractions and delay delivery. The bulletin recommends using medications such as beta-agonists, calcium channel blockers, and nonsteroidal anti-inflammatory drugs (NSAIDs) for selected patients based on gestational age, contraindications, and potential side effects.

10.2. Antenatal Corticosteroids: Administration of antenatal corticosteroids, such as betamethasone or dexamethasone, is recommended for women at risk of preterm birth between 24 and 34 weeks of gestation. These steroids help promote fetal lung maturity and reduce the risk of respiratory distress syndrome.

10.3. Magnesium Sulfate: Magnesium sulfate can be used for neuroprotection in women at risk of preterm birth before 32 weeks of gestation to reduce the risk of cerebral palsy in the newborn.

10.4. Antibiotics: Antibiotic therapy may be considered for women with preterm labor and intact membranes who are at risk of intrauterine infection.

10.5. Cerclage: In certain cases, cervical cerclage (suturing the cervix) may be performed for women with a history of cervical insufficiency or short cervix to reduce the risk of preterm birth.

10.6. Delivery Timing: Decisions regarding the timing and mode of delivery should be based on the gestational age, maternal and fetal status, and potential risks to the mother and baby.

In the current study, we assessed whether Interleukin-6 biomarkers of inflammation, including were associated with preterm delivery between 24-36 weeks of gestation and its correlation with the evidenced histological signs of acute infection or inflammatory signs in placental tissue. We hypothesized that inflammatory maker of interleukin-6 is implicated in preterm labor and can be used as a marker for an early detection of preterm labor.

11. Materials and Methods

11.1 Study Method and Patient Details

The current study is a primary analysis of a case and control study of preterm birth conducted among 88 women of singleton pregnancies enrolled in case control study at Ain Shams University maternity hospital and and Misr University For Science and Technology Hospital from 2006 to 2008. The primary outcome of this study was preterm birth, which was defined as any delivery at or after 28 weeks and 0 days and prior to 37 weeks and 0 days. Secondary Outcome, higher levels of maternal serum interleukin -6 in case group with evidences of infection in the placental tissue. The study was approved by the Institutional Review Board at Ain Shams University Hospital, Cairo, Egypt. Total of 88 women enrolled and divided into two groups: Case group, consist of Forty-Four singleton pregnant women after 28 weeks and 0 days and 37 weeks and 0 days of gestational age who presented to the outpatient clinic or were hospitalized in inpatient treatment for diagnosis of preterm labor. The diagnosis of preterm labor was given for the clinical findings such as in persistent regular uterine contractions (at least three uterine contractions/10 minutes during 30 min observation) combination with cervical changes in form of Cervical length 2 cm or less or Cervical dilatation 1 cm or more. Control group, Forty-Four healthy pregnant women who

presented to the outpatient clinic at the same gestational age were selected as the control group. The participating women completed a demographic questionnaire, as well as supplied blood samples for biomarker analysis (interleukin-6) and placental tissue were collected after delivery.

In the study and control groups, age of gestation was determined using the last menstrual period in patients who sure of their last menstrual cycle for women with regular menstrual cycles. In cases where the LMP is uncertain, when there are discrepancies between the LMP and ultrasound findings or irregular menstrual cycle. In early pregnancy, ultrasound measurements of the fetal crown-rump length (CRL) are used to estimate gestational age. Later in pregnancy, other fetal measurements, such as biparietal diameter and femur length, can be used.

12. Diagnosis of preterm labor can be made on the bases of the clinical physical findings in combination with trasvaginal ultrasound and fetal fibrinecton:

I. Uterine Contractions: Regular, painful contractions occurring more frequently than every 10 minutes, persisting for at least one hour, and accompanied by progressive cervical changes (such as effacement and dilation) may suggest preterm labor. Presence of contractions was identified in all pregnant women via manual follow-up or nonstress test (NST).

II. Cervical Changes: Progressive cervical changes, including cervical effacement (thinning) and dilation (opening) more than 4 cm, can be indicative of preterm labor assessed through a digital examination or transvaginal ultrasound.

III. Rupture of Membranes: Spontaneous rupture of the amniotic sac, resulting in the release of amniotic fluid, may be a sign of preterm labor. This can be assessed by observing pooling of amniotic fluid in the vaginal vault or performing tests such as the Nitrazine or fern test.

IV. Pelvic Pressure or Pelvic Pain: Persistent sensations of pressure or menstrual like cramping pain in the lower abdomen, pelvis, or back may be indicative of preterm labor.

All pregnant women with final diagnosis of preterm labor were admitted for inpatient treatment in our hospital which includes pregnant women with PPROM with > 4 cm cervical effacement. Pregnant women with other causes of preterm labor were excluded from our study. Other reasons of preterm labor such as abruptio placenta, placenta previa, pre-eclampsia, eclampsia, intrauterine growth retardation, chorioamnionitis, pregnancy achieved by Assisted Reproductive Technology (ART) or Rh immunization were excluded from this study.

Blood samples were collected and the Plasma interleukin-6 (IL-6) measured using Enzyme- Linked Immuno-Sorbent Assay test (ELISA test). Samples of Maternal placental tissues and membranes were collected and examined grossly. The placental and Samples of the membranes were stained by hematoxylin and eosin (H and E) stain and examined histologically for signs of acute inflammations and infection in chorionic plate, intervillous spaces and intervillous stroma. Histological diagnosis of acute infection was made based on presence of number of neutrophils and extent of tissue invasion in amnion and chorionic plate.

Pelvic examination was performed using a sterile speculum (Simpson speculum) for all participating pregnant women at the study and control groups, and vaginal and cervical status was evaluated. A study information form was issued to all pregnant women and patient demographics (age, body weight, gravidity, parity, abortion, live children, examination findings, current medications, Bishop's score, vital findings, and laboratory examinations) were recorded.

12.1. Study variables

The study received approval from the Institutional Review Board (IRB) and ethical clearance from the ethics committees of Ain Shams University and Misr University for Science and Technology. Informed consent was obtained from all participants. Demographic variables, including age, race/ethnicity, education, parity, body mass index (BMI), tobacco and alcohol use, and assisted reproductive technology, were assessed at the beginning of the study.

Pregnant women in both the study and control groups were free from cervical or uterine anomalies, heart disease, asthma, diabetes, liver disease, kidney disease, and connective tissue disease. Those with multiple gestations, pre-existing medical conditions, significant uterine myoma, notable cervical or uterine anomalies, or any significant infections such as human immunodeficiency virus (HIV) or confirmed urinary tract infection, were excluded from the study. Additional exclusion criteria included maternal tachycardia (heart rate > 100 beats per minute), fetal tachycardia (heart rate > 160 beats per minute), maternal leukocytosis (white blood cell count > 15,000 mm3), maternal body temperature exceeding 37.8°C, presence of intra-amniotic infection as indicated by amniotic fluid culture, and patients below 17 years or above 45 years of age.

12.2. Sample Preparations

12.2.1. Maternal Serum

Maternal serum samples were obtained from 7–8 mL of whole blood drawn with a 10 mL syringe via puncture of the antecubital veins at room temperature (24°C) in a sitting position and irrespective of fasting status. Whole blood samples were centrifuged at 5000 rpm for 5 minutes at 4°C within 1 hour of collection in order to separate serum after blood clotting. Serum samples were stored at -30°C to allow study once an adequate number of patients were enrolled.

12.2.2. Placental Tissues

Placental Tissues were collected from 88 cases (case and control group). The placental samples were stained by hematoxylin and eosin (H and E) stain and examined histologically for signs of acute inflammations and infection in chorionic plate, intervillous spaces and intervillous stroma. Each sample was examined at the pathological laboratory of Ain Shams University for histological

signs of inflammatory signs such as (Infiltration of inflammatory cells). This involves the presence of various immune cells, such as neutrophils, lymphocytes, macrophages, and plasma cells, within the affected tissue).

12.2.3. IL-6 Immunoassay

The IL-6 levels of maternal serum samples were determined via solid phase enzyme amplified sensitivity immunoassay using a commercial kit (DioSource Europa SA, Nivelles, Belgium) in accordance with the procedure specified by the manufacturer. Automated microplate reader used to analyze the Enzymatic reaction. The plasma samples were analyzed for inflammatory biomarkers at the Ain Shams University Laboratory (Cairo, Egypt). Results were expressed as pg/mL. The blood samples with interleukin-6 level of 13.35 pg/mL is considered negative test and indicative of placental tissue without infection or inflammation. The blood samples with interleukin-6 level of 50 pg/mL is an indicative for infected or inflamed placental tissues.

12.3. Measurement of The Human Interleukin-6 (IL6)

Reagent and sample preparation:

• Ensure that all reagents and samples are equilibrated to room temperature (18-25°C) before initiating the experiment.

• **For the antibody coated plate,** assess the number of strips necessary for testing the desired number of samples, as well as 18 additional wells for running standards and blanks in duplicate. Remove any unused strips from the plate-frame and store them in the provided plastic pouch containing a desiccant at temperatures ranging from 2-8°C for up to 1 month.

• **Dilution of test standard:** To prepare the test standard dilution, combine 150µl of the standard solution (1000 pg/ml) with 150µl of the sample diluent.

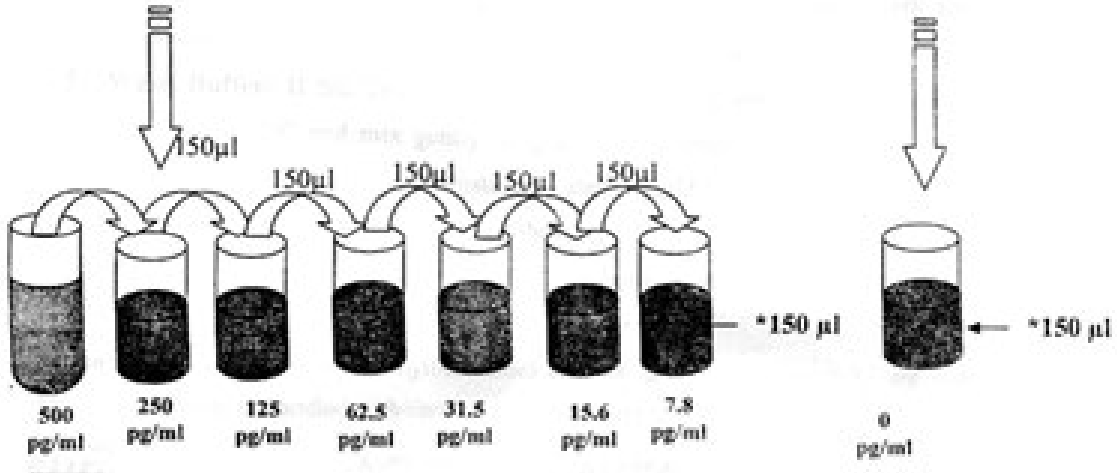


Figure 3: Dilution of the test standard

12.4. Test procedure summary

- Add 50µl ready for use green colored biotin antibody promptly to each well.
- Incubate 1 hour 30 min in room temperature
- Wash 5x with 1x wash buffer.
- Add 50µl TMB one-step substrate reagent to each well.
- Incubate 20 min at room temperature.
- Add 25µl stop solution to each well.
- Read at 450 nm against 630 nm immediately.

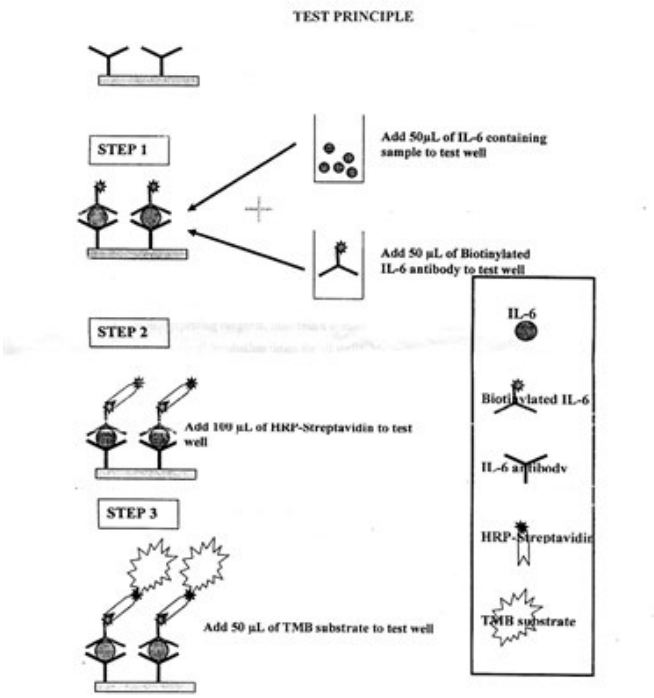


Figure 4: ELIZA test principle for measuring the maternal serum IL-6.

12.5. Statistical Analysis

The data were coded, entered and processed Statistical analyses were performed using IBM-PC compatible computer using SPSS (version 15) software. The level $P < 0.05$ was considered the cut-off value for significance. Data were expressed as mean and median. Inter-group comparisons were made with Student's t-test was used to assess the statistical significance of the difference between two population means in a study involving independent samples. The comparisons were made also with regard of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and receiver operating characteristic (ROC) curve was used to illustrate the diagnostic properties of a test on a numerical scale.

12.6. Results and statistical analysis

The results of this study showed that the levels of interleukin-6 (Figure 1) were higher in the case group (138.27±113.21) compared to the control group (7.55±2.07) as pretested in Table 1. Whereas, the levels of maternal serum interleukin-6 were higher in the infected cases (175.33± 107.32) compared to non-infected cases (27.08± 10.44) as pretested in Table 2.

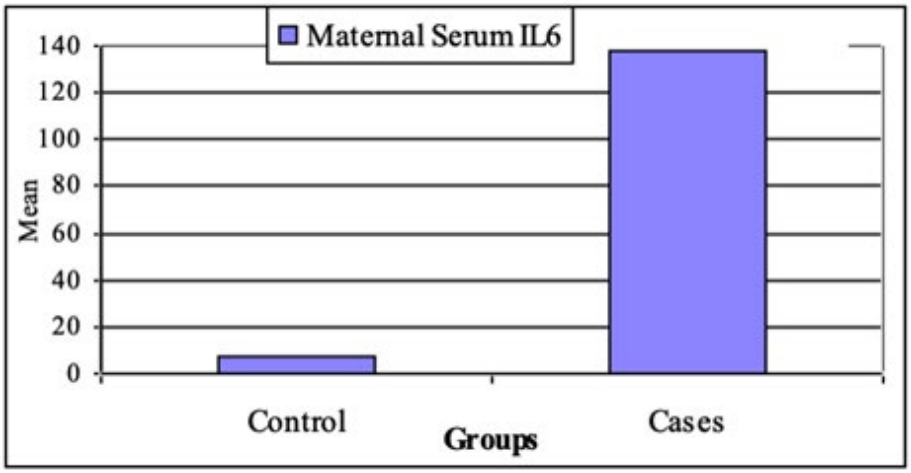


Figure 5: Comparison between cases and control according to IL6

Maternal Serum IL6				
Groups	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**
Control	7.55	2.07	3.80	13.80
Cases	138.27	113.21	13.70	450.00

Table 1: Maternal Serum Interleukin-6 (IL-6) demonstrate the comparison between cases and controls.

Maternal Serum IL6				
Infection	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**
-ve (10)	27.08	10.44	13.70	43.00
+ve (30)	175.33	107.32	58.00	450.00

Table 2: Placental tissue Histopathology demonstrate the comparison between cases and controls.

These findings suggest that interleukin-6 may play a role in the pathogenesis of preterm labor and could potentially serve as a diagnostic marker for identifying women at risk of preterm birth. There was no statistically significant difference between cases and control as regards the mean age, and deliveries with p-value of ($P > 0.05$). While there was a highly statistically significant difference between cases and control as regards to the time of delivery with p-value pf ($P < 0.0001$). While there was a highly significant difference ($P < 0.0001$) between cases and control as regards the time of delivery cases showed a statistically lower mean compared to control as presented in Table 3.

	Groups	**Mean**	**±SD**	**Student's t test (T)**	**Probability**	**Sig.**
Age	Control	27.95	5.65	0.77	0.44	None significant
	Cases	26.98	6.17			
Deliveries	Control	1.89	1.62	1.54	0.13	None significant
	Cases	1.36	1.57			
Time of delivery	Control	38.84	1.29	14.12	<0.0001	Highly significant
	Cases	32.41	2.73			

Table 3: Comparison between cases and control according to age, parity and time of delivery.

On the other hand, There was no statistically significant difference between infected cases and non-infected cases as regards the mean age and deliveries versus time of delivery with P-value of ($P > 0.05$) (Table 4). Table 4 displays the sensitivity, specificity, positive predictive value, and negative predictive value of maternal serum IL-6 in detecting infection associated with premature delivery. The optimal cut-off point for detecting infection with preterm labor was determined to be 50.5. At this cut-off level, the sensitivity, specificity, positive predictive value, and negative predictive value were all 100%. Alternatively, at a cut-off value of 39.5, the sensitivity and negative predictive value were 10%, while the specificity was 90% and the positive predictive value was 96.77%, resulting in an overall diagnostic accuracy of 97.5%. Similarly, at a level of 60.5, the specificity and positive predictive value were 100% and 90.91% respectively, the negative predictive value was 96.67%, and the sensitivity and overall diagnostic accuracy were both 97.5%. Therefore, the optimal cut-off point to detect infection associated with premature delivery is 50.5.

	infection	**Mean**	**±SD**	**Student's t test (T)**	**Probability**	**Sig.**
Age in years	-ve	28.50	5.52	1.12	0.27	NS
	+ve	26.27	6.42			
Deliveries	-ve	1.71	1.68	1.01	0.32	NS
	+ve	1.20	1.52			
Time of delivery	-ve	31.57	2.74	1.41	0.17	NS
	+ve	32.80	2.68			

Table 4: Comparison between infected cases and non-infected cases according to age, parity and time of delivery.

The results of this study also showed that there was a significant (P < 0.0001) difference between cases and control cases (138.27±113.21) showed a statistically higher mean compared to control (7.55±2.07) as presented in Table-5. Similarly, infected (175.33±107.32) cases showed a statistically significant higher mean compared to non-infected cases (27.08±10.44) with calculated p-value of (P < 0.0001) as presented in indicating a highly significant difference (Table 6). The predictive ability of maternal serum IL-6 is considered reliable, as indicated in Table 7 with a P-value of < 0.0001.

Groups	Maternal Serum IL6				
	Mean	±SD	Student's t test (T)	Probability	Sig.
Control	7.55	2.07	7.30	<0.0001	Highly significant
Cases	138.27	113.21			

Table 5: Comparison between cases and control according to IL-6.

Infected cases showed a statistically higher mean compared to non-infected cases P < 0.05

Groups	Maternal Serum IL6				
	Mean	±SD	Student's t test (T)	Probability	Sig.
Negative (10)	27.08	10.44	4.32	0.0001	Highly significant
Positive (30)	175.33	107.32			

Table 6: Comparison between infected cases and non-infected cases according to IL6.

In this study, two receivers operating characteristic (ROC) curves were generated to evaluate the diagnostic ability of maternal serum interleukin-6 (IL-6) levels in predicting premature delivery.

The first ROC curve, shown in, demonstrated that IL-6 had a high predictive ability for premature delivery (Figure 7). The AUC was calculated to be 0.99, indicating excellent accuracy. The standard error of the AUC was 0.001, suggesting a high level of precision. The 95% confidence interval (CI) for the AUC ranged from 0.99 to 1.0, further supporting the reliability of IL-6 as a diagnostic marker for premature delivery.

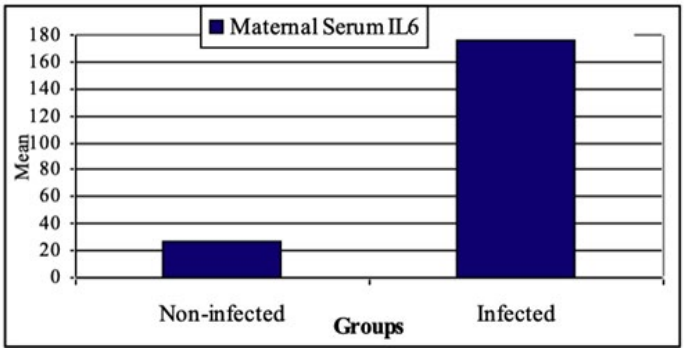


Figure-6: Comparison between infected cases and non-infected cases according to IL6.

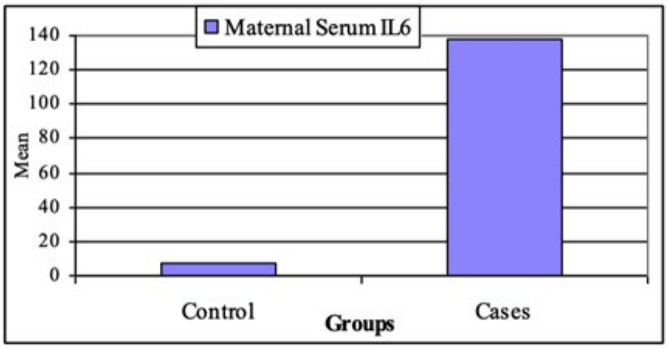
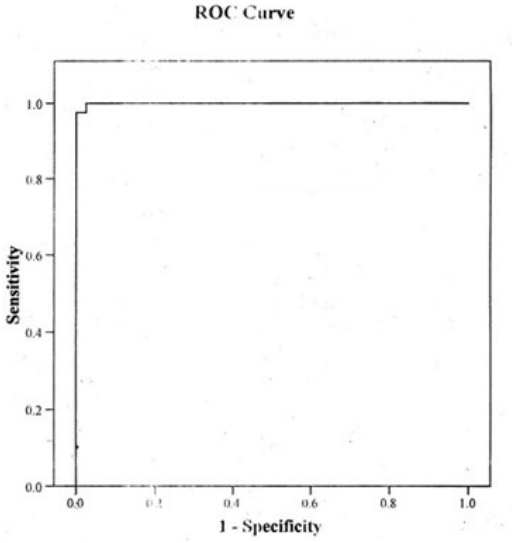


Figure-7: Receiver operating characteristic (ROC) curve for cut-off levels of IL6 in the diagnosis of premature delivery.



	Area under the curve	st error	95% CI
Maternal S. IL6	0.99	0.001	0.99 TO 1.0

Table (7): Receiver Operating Characteristic (ROC) Curve for cut-off levels of IL-6 in the diagnosis of premature delivery. The predictive ability of maternal serum IL-6 is reliable P < 0.0001.

13. Coordinates of the Curve

Comparing the sensitivity, specificity, positive predictive value, and negative predictive value of maternal serum IL-6 for premature labors at different cut-off levels. The best cut-off points to detect premature delivery were found to be 13.35 and 14.85, both exhibiting similar diagnostic ability. A maternal serum IL6 level of 13.35 and 14.85 are the best cut-off points to detect premature delivery and both have the same diagnostic ability but 13.35 level is more sensitive & less specific than 14.85 level and 14.85 is less sensitive and more specific.

Maternal Serum IL6	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
12	100.00	95.00	95.24	100.00	97.50
13.35	100.00	97.50	97.56	100.00	98.75
13.75	97.50	97.50	97.50	97.50	97.50
14.85	97.50	100.00	100.00	97.56	98.75
PPV: positive predictive value. NPV: negative predictive value					

Table 8: Sensitivity, specificity, PPV, NPV and diagnostic accuracy at different cut-off levels for premature labor.

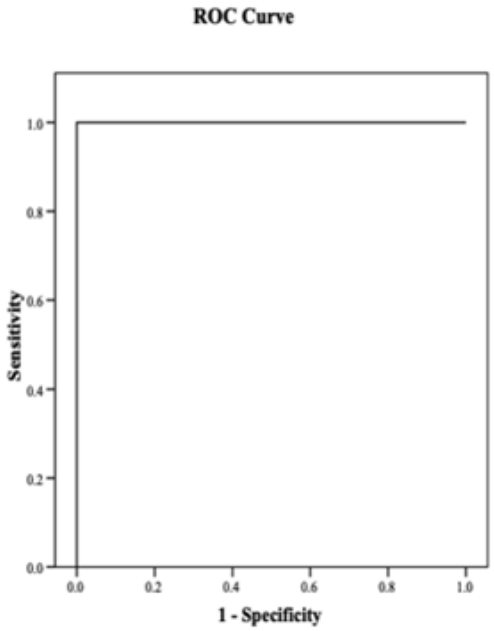


Figure 8 : Receiver Operating Characteristic (ROC) Curve for cut-off levels of IL-6 in the diagnosis of infection with premature delivery

Area under the curve (AUC) was calculated, and any value greater than 1 was indicative of good diagnostic performance and a statistically significant P-value is $P < 0.05$ (Table-11).

Table 11: Receiver Operating Characteristic (ROC). Curve for cut-off levels of IL-6 in the diagnosis of infection with premature delivery. The predictive ability of maternal serum IL-6 is perfect as area under the curve is 1 with p-value of ($P < 0.05$).

	Area under the curve	st error	95% CI
Maternal S. IL-6	1.00	0	1.0 - 1.0

Table 9: Receiver Operating Characteristic (ROC).

Lastly, we identified that the level of the maternal serum IL-6 of 50.5 is the optimal cut-off point to detect infection that will cause premature delivery (Table 12).

A maternal serum IL6 level of 50.5 is the best cut-off point to detect infection with premature delivery.

Maternal Serum IL6	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
39.5	100.00	90.00	96.77	100.00	97.50
50.5	100.00	100.00	100.00	100.00	100.00
60.5	96.67	100.00	100.00	90.91	97.50
PPV: positive predictive value. NPV: negative predictive value					

Table 10: Sensitivity, specificity, PPV, NPV and diagnostic accuracy at different cut-off levels for infection.

14. Results

A strength of the current study to our knowledge, the current study is the first study to investigate whether IL-6 biomarkers can be used as a marker for an early prediction of preterm labor.

The current study measured inflammatory biomarker of Interleukin-6 in 24-36 weeks of gestation among 88 pregnant women. We found a significant association between the cytokines IL-6 incidence of preterm labor regardless of evidenced placental tissue infection. These results provide further evidence that an imbalance of immune regulation could impact cervical length, highlighting a critical importance of use of interleukin-6 as marker for early prediction of preterm labor.

However, among 41 women with a previous preterm birth, patients with preterm diagnosis was associated with pro-inflammatory

cytokines (IL-6). These findings suggest that the maternal serum interleukin-6 is promising biomarker for early detection of preterm labor. Interleukin-6 have been shown to be highly correlated with early preterm labor, suggesting they may represent promising biomarker for early detection of preterm labor.

15. Conclusion

In conclusion, After controlling the potential confounders, we recorded in this study a positive correlation between the higher levels of biomarker of InterleukinL-6 among women who presented in our tertiary center with preterm labor, suggesting an imbalance of immune regulation could impact cervical length and could be used as a marker of an early prediction of preterm labor. The elevate maternal serum of interleukin-6 in patients with evidence of infection of the placental tissue showed a 50.5pg/ml as cut-off point with good diagnostic value. Therefore, Maternal Serum Interleukin-6 can be used as a marker to predict the infection induced preterm labor. Interpreting maternal serum IL-6 levels requires careful consideration of other clinical factors, such as cervical examination and assessment of contractions. Relying solely on IL-6 levels may overlook other important signs or symptoms of preterm labor. Maternal serum IL-6 is not specific to preterm labor and can be elevated in various other conditions, such as infections or inflammatory processes unrelated to preterm labor.

Ethics

The author has no conflict of interest. We obtained ethical approval for this study from the Research Ethics Committee, Ain Shams University. Before participation, we briefed about the objective and purpose of this study to the participants. Also, we took written consent from each participant before engaging in this study.

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