Introduction

Preterm delivery (PTD) is the major obstetric and neonatal problem of the developing and developed world ***(Charles et al., 2012).***

Babies who are born before 37 weeks, and particularly those born before34 weeks, are at greater risk of having problems at birth and complications in infancy ***(Dodd et al., 2013).***

Traditional methods for predicting women destined to deliver preterm relied upon obstetrical history, demographic factors, or premonitory symptoms that were neither sensitive nor specific. The development of various biochemical and biophysical tools has helped to distinguish between women who will and will not deliver preterm ***(Errol et al., 2012).***

A number of biologic markers in serum, amniotic fluid, and cervical secretions have been evaluated for their potential to predict PTD. The most commonly used biochemical approach for differentiating women who are at high risk for impending PTD from those who are not at high risk is measurement of fetal fibronectin (fFN) in the cervicovaginal secretions. The test is performed alone or in conjugation with sonographic assessment of cervical length ***(Charles et al., 2012).***

Progesterone is a steroid hormone initially produced by the corpus luteum. It is critical for the maintenance of early pregnancy until the placenta takes over this function at 7 to 9 weeks of gestation, and its name is derived from this function: pro-gestational steroidal ketone. Indeed, removal of the source of progesterone (the corpus luteum), or administration of a progesterone receptor antagonist, readily induces abortion before 7 weeks (49 days) of gestation ***(Errol et al., 2012).***

The role of progesterone later in pregnancy, however, is less clear. Progesterone appears be important in maintaining uterine quiescence in

the latter half of pregnancy, possibly by limiting the production of stimulatory prostaglandins and inhibiting the expression of contraction-associated protein genes within the myometrium, including ion channels, oxytocin and prostaglandin receptors, and gap junctions ***(Errol et al., 2012).***

These observations provide the rationale behind the use of progesterone as biochemical marker to predict preterm labor and birth.

Aim of the Study

The aim of study to evaluate salivary progesterone as a predictor of preterm birth (PTB).

Rational of study

In women at risk of PTB salivary progesterone may be decreased before onset of labor.

Research question

In women with risk for PTB, does salivary progesterone decrease before onset of labor compared to women who will not deliver preterm?

Chapter (I): Preterm Birth

Preterm birth refers to a delivery that occurs before $37^{0/7ths}$ weeks of gestation. It may or may not be preceded by preterm labor ***(Lockwood, 2012).***

Classification - Subtypes of preterm birth are variably defined.

By gestational age

- Moderate preterm: 32 to <37 weeks
- Late preterm: $34^{0/7ths}$ to $36^{6/7ths}$ weeks
- Very preterm: 28 to <32 weeks
- Extremely preterm: <28 weeks

By birth weight

- Low birth weight (LBW): <2500 grams
- Very low birth weight (VLBW): <1500 grams
- Extremely low birth weight (ELBW): <1000 grams ***(Lockwood, 2012):***

Preterm labor precedes almost half preterm births ***(Lykke et al., 2009).***

Preterm labor is defined as the presence of uterine contractions of sufficient frequency and intensity to cause progressive effacement and dilatation of the cervix prior to term gestation (between 20 and 37 week) ***(Ross and Eden, 2010).***

Preterm delivery occurs in 5-13% of pregnancies before 37 weeks' gestation. Preterm delivery is a major cause of perinatal morbidity and mortality. Most of the damage and death cases occur in infants delivered

before 34 weeks. The incidence of early preterm delivery (<34 gestational weeks') is 1-3.6% ***(Goldenberg et al., 2008).***

The complications of preterm birth (PTB) cause approximately 70% of neonatal deaths and nearly half of all long term neurological morbidity ***(Mathews and MacDorman, 2010).***

Preterm labor and birth are responsible for the majority of neonatal morbidity and mortality including cerebral palsy, blindness, and deafness, resulting in an annual cost of over 26 billion dollars in 2005 ***(March of Dimes Website, 2012).***

Risk Factors:

Defining risk factors for prediction of preterm birth is a reasonable goal for several reasons. First, identification of at risk women allows initiation of risk-specific treatment. Second, the risk factors might define a population useful for studying specific interventions. Finally, identification of risk factors might provide important insight into mechanisms leading to preterm birth ***(Goldenberg et al., 2008).***

The exact mechanism(s) of preterm labor is largely unknown but is believed that a variety of maternal and obstetric characteristics are known to increase the risk. Also, the fetus plays a role in the initiation of labor. In a simplistic sense, the fetus recognizes a hostile intrauterine environment and precipitates labor by premature activation of a fetal placental parturition pathway ***(Ross and Eden, 2009).***

(A) Maternal characteristics:

***1. Age*:**

Teenage childbearing has repeatedly been associated with increased risks for adverse pregnancy outcomes, like preterm birth, low birth weight, and death in the neonatal or postneonatal periods ***(Haldre et al., 2007).***

2. Weight:

Women lower than normal maternal body weight have been shown to be at increased risk for adverse prenatal outcomes such as prematurity and intrauterine growth restriction ***(Yekta et al., 2006).***

***3. Maternal general condition*:**

Maternal medical disorders, such as thyroid disease, asthma, diabetes, and hypertension, are associated with increased rates of preterm delivery ***(Goldenberg et al., 2008).***

Anemia is associated with suboptimal pregnancy outcome mainly due to lower birth weight and preterm delivery ***(Levy et al., 2005).***

4. Race:

The risk of preterm birth for white women in the United States is 11.5%, the risk for black women is 17.9%. This racial disparity in the occurrence of preterm birth is even more profound at the earliest gestational ages of delivery. The prevalence of very preterm birth (<32 weeks gestational age) is 1.6% for white women, whereas it is 4.0% for black women ***(Palomar et al., 2007).***

(B) Psychological characteristics and life style factor:

1. Psychological factors:

Clinical depression during pregnancy has been reported in up to 16% of women, with up to 35% having some depressive symptoms. Mothers experiencing high levels of psychological or social stress are at increased risk of preterm birth (generally >2 fold) even after adjustment for the effects of sociodemographic, medical, and behavioural risk factors. Furthermore, exposure to objectively stressful conditions, such as housing instability and severe maternal hardship, has also been

associated with preterm birth. Although the mechanism of association between psychological or social stress and increased risk of preterm birth is unknown, a role for corticotrophin releasing hormone has been proposed ***(Goldenberg et al., 2008).***

2. Cigarette smoking:

Both nicotine and carbon monoxide are powerful vasoconstrictors, and are associated with placental damage and decreased uteroplacental blood flow. Both pathways lead to fetal growth restriction and preterm births. Smoking is also associated with a systemic inflammatory response and can increase spontaneous preterm birth through that pathway ***(Bermudez et al., 2000).***

3. Alcohol, coffee and drug abuse:

Alcohol abuse has been linked not only to preterm labor, but also to substantially increased risk of brain injury in preterm infants ***(Sokol et al., 2007).***

Drug dependency is a risk factor of preterm labor. Dose related coffee consumption in the first trimester was associated with 2 fold increase risk of preterm rupture of membranes (PROM) ***(William et al., 1992).***

4. Socioeconomic:

Low socioeconomic status is associated with preterm labor (PTL) but probably because of other factors that keep women in/near poverty, for example poor women are poorly nourished, and have a higher prevalence of tobacco, alcohol, and illicit drug abuse and have less access to prenatal care ***(Reedy, 2007).***

5. Nutrition:

Women with low serum concentrations of iron, folate, or zinc have more preterm births than those with measurements within the normal range ***(Goldenberg et al., 2008).***

(C) Obstetric history:

1. Previous preterm labor:

There is strong association between prior preterm delivery and recurrence risk in the next birth. This association is affected by 3 risk factors: the frequency of prior preterm deliveries, the order in which the prior preterm delivery occurred, and severity of preterm delivery as measured by the gestational age at delivery.

Women with 2 prior preterm deliveries had the highest overall risk (42%) for recurrent preterm delivery. This risk ranged from 38-57%, in inverse relation to the gestational ages of the prior preterm births. The overall recurrence risk for women with one prior preterm delivery was less than the half the magnitude of that with two prior preterm deliveries. Although this risk was higher in women with a second birth preterm (21%) than in those with a first birth preterm (13%) ***(McManemy et al., 2007).***

2. Previous abortion:

There is a relationship between the number of the prior abortions, whether spontaneous or induced and prevalence of preterm labor. The relative risk of one previous abortion either spontaneous or induced is 1.66 & 1.55 respectively, and for two previous abortions either spontaneous or induced is 2.49 & 2.46 respectively, and for three or more abortions either spontaneous or induced is 5.89 & 5.58 respectively ***(Lumley, 1993).***

3. Inter-pregnancy interval:

An inter-pregnancy interval of less than 6 months confers a greater than two fold increased risk of preterm birth after adjustment for confounding variables. Furthermore, women whose first birth was preterm are far more likely to have a short interval than women who had a term first birth, thus compounding the risk. Although the mechanism is not clear, one potential explanation is that the uterus takes time to return to its normal state, including resolution of the inflammatory status associated with the previous pregnancy. Maternal depletion might be another cause because pregnancy consumes maternal stores of essential vitamins, minerals, and amino acids. A short interval decreases the opportunity to replenish these nutrients ***(Goldenberg et al., 2008).***

4. Use of assisted reproductive techniques:

A high number of preterm multiple gestations associated with assisted reproductive technologies is also an important contributor to the overall increase in preterm births. Singleton pregnancies after in-vitro fertilization are also at increased risk of preterm birth ***(Goldenberg et al., 2008).***

5. Cervical cone biopsy sample or loop electrocautery excision:

History of cervical cone biopsy sample or loop electrocautery excision procedures secondary to premalignant cervical disorders has also been associated with an increase in spontaneous preterm delivery ***(Goldenberg et al., 2008).***

(D) Factors in the current pregnancy:

1. Bleeding in pregnancy

Vaginal bleeding caused by placental abruption or placenta previa is associated with a very high risk of preterm delivery, but bleeding in the

first and second trimesters that is not associated with either placental abruption or placenta previa is also associated with subsequent preterm birth ***(Krupa et al., 2006).***

Early pregnancy bleeding is also associated with preterm birth. The association between first trimester bleeding, common genitourinary tract infection and preterm birth was confirmed. Prematurity is associated with microbe related conditions that lead to cervicitis, cervical erosions and/or endometritis and they may cause bleeding indirectly. Bleeding may impair maternal cervical and lower uterine segment host defense mechanisms and may enhance the ascent of micro-organisms into the lower uterine segment early in pregnancy ***(French et al., 1999).***

2. Pre-eclampsia:

Sibai reported that an increased incidence of late preterm birth among women with preeclampsia or gestational hypertension (GH), the rate of late preterm birth was reported to range from10%-11% among women with preeclampsia and from 4%-6% among women with GH ***(Sibai, 2006).***

3. Multiple gestations:

In 1999 1.44% of all maternities in UK involved multiple pregnancies. The vast majority were twin gestations. The median gestation at delivery for twins is approximately 35 weeks and triplets 33 weeks. Presently, associated reproductive techniques are responsible for 35% of twin pregnancies and 77% of triplets, leading to increasing burden of preterm births. Multi-fetal reduction has been shown to reduce risk in higher order pregnancies and should always be considered. Preterm births of twins is associated with an increased risk of preterm delivery in a subsequent singleton pregnancy ***(Facco et al., 2007).***

4. Surgical operations:

Maternal abdominal surgery in the second and third trimesters can stimulate contractions culminating in preterm delivery ***(Goldenberg et al., 2008).***

(E) Infections:

Bacterial vaginosis (BV) in pregnancy is also associated with PTL, particularly when the BV is diagnosed before 16 weeks' gestation ***(Reedy, 2007).***

Bacterial vaginosis (BV) affects 6–32% of pregnant women, it is characterised by an imbalance in the vaginal microflora, it may be symptomless or it may be accompanied by increased vaginal discharge, which may be foul smelling with a fishy odour. In women with BV, there are usually no clinical signs of infection in the vaginal mucosa ***(Svare et al., 2006).***

Pathophysiology:

The pathological processes implicated in the preterm birth syndrome include:

1. Intrauterine infection.
2. Uterine ischaemia.
3. Uterine over distension.
4. Abnormal allogenic recognition.
5. Allergic-like reaction.
6. Cervical disease.
7. Endocrine disorders **(Fig. 1)** ***(Romero et al., 2006)***

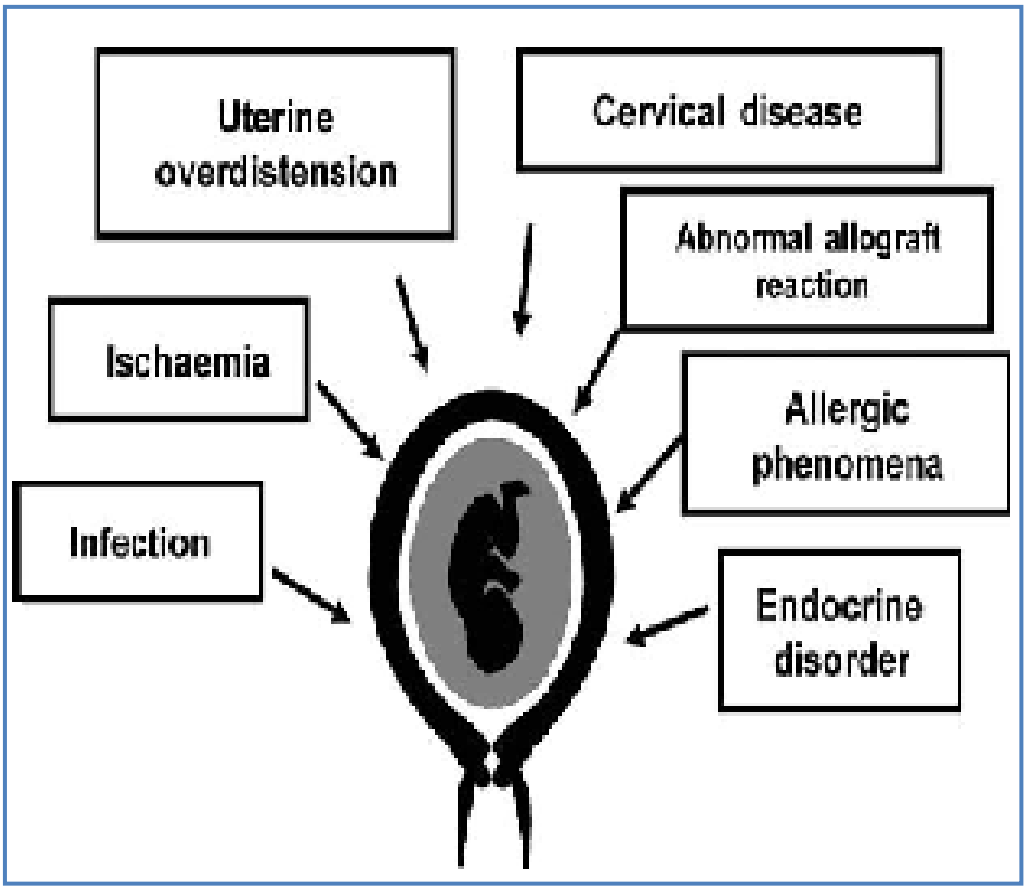

Fig. (1): The pathological processes implicated in the preterm birth syndrome ***(Romero et al., 2006)***

I. Infection:

Intrauterine infection has emerged as a frequent and important mechanism of disease in preterm birth ***(Goncalves et al., 2002).***

Pathways of intra-amniotic infection:

1) Microorganisms may gain access to the amniotic cavity and fetus using any of the following pathways: (1) Ascending from the vagina and the cervix.
2) Haematogenous dissemination through the placenta (transplacental infection).
3) Retrograde seeding from the peritoneal cavity through the fallopian tube.
4) Accidental introduction at the time of invasive procedures, such as amniocentesis, percutaneous fetal blood sampling, chorionic villus sampling, or shunting ***(Goldenberg et al., 2000).***

Infection results in the preterm labor through the aggregation of leucocytes in the uteroplacental system and fetal cell. This recruitment followed by the production of huge amount of inflammatory cytokines whether by leucocytes or the stimulated uteroplacental system cells. Cytokines stimulate the cascade of preterm labor as follows:

On Myometrium:

Proinflammatory cytokines stimulate the production interleukins (ILs), Tumor necrosis factor-α (TNF–α) and prostaglandins by the uteroplacental system. These substances stimulate COX-2 (cyclo-oxygenase-2) enzyme and inhibit prostaglandin dehydrogenase (PGDH) enzyme. The net result is excessive production of PGE2 and PGF2α which in turn stimulate myometrial contractions. IL6 also increase myometrial oxytocin secretion and their receptors response. Finally IL2 has direct myometrial stimulant effect. This cascade of event result finally in progressive uterine contractions **(Fig. 2)** ***(Park et al., 2005).***

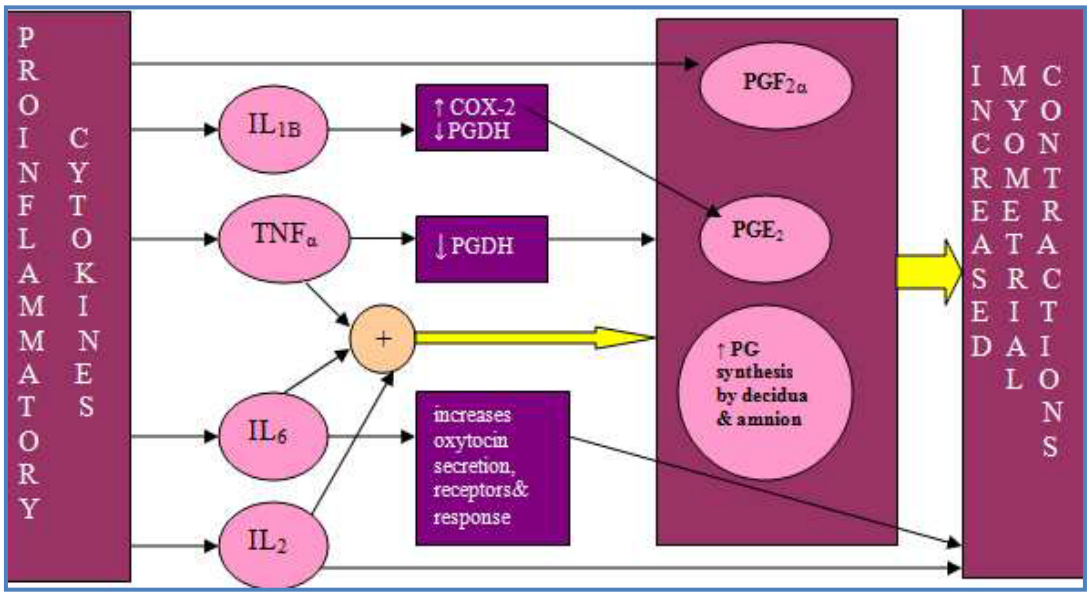

Fig. (2): Effect of inflammatory cytokines on myometrium ***(Park et al., 2005)***

On the Cervix:

Infection stimulates aggregation of leucocytes in cervical tissues. Leucocytes produce IL6 which in turn stimulates the production of other cytokines namely IL8, IL1B and TNF-α. These substances stimulate the production of matrix metalloproteinase (MMP), elatase, protease and prostaglandin synthase (PGHS) and they also inhibit the production of tissue inhibitory metalloproteinase (TIMP). The net result is excessive degradation of collagen fibers, excessive degradation of extracellular matrix and release of prostaglandins. The previous changes result in progressive cervical ripening **(Fig. 3)** ***(Park et al., 2005).***

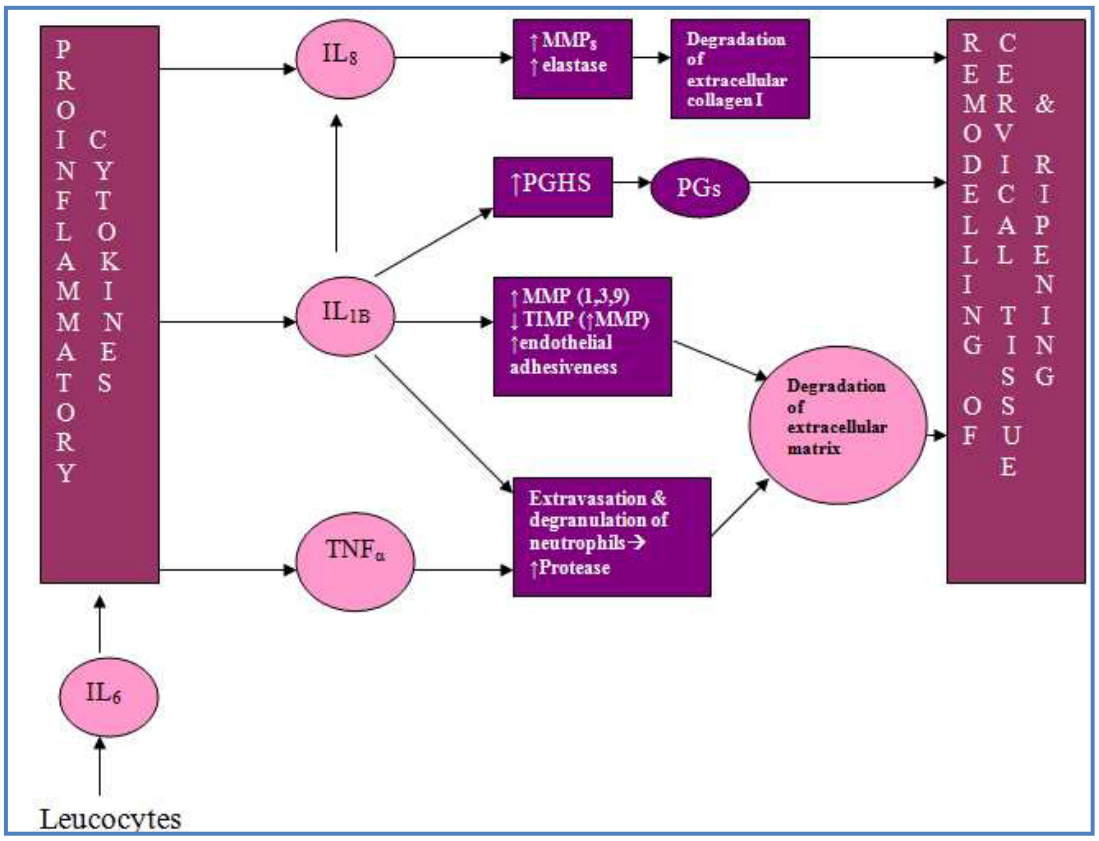

Fig. (3): Effect of inflammatory cytokines on cervix ***(Park et al., 2005)***

On Membranes:

IL1B and TNFα increase production of MMP and PGE2 by amniotic sac cells. PGE2 also increase production of MMP by amniotic sac cells. MMP causes progressive degradation of collagen fibers in amniotic

membrane. TNF-α and PGE2 stimulate apoptosis and cell necrosis of amniotic membrane. The previous changes ultimately result in weakening of amniotic membrane and premature rupture of membranes ***(Park et al., 2005).***

II- Uteroplacental ischemia:-

Women in spontaneous preterm labor can be classified into two groups: those with inflammatory lesions of the placenta and membranes and those without evidence of inflammation. The most common pathological features in the placenta of women who belong to the no inflammatory group are maternal and fetal vascular lesions.

Maternal lesions observed in the placenta of patients with a spontaneous preterm delivery include failure of physiological transformation of the myometrial segment of the spiral arteries, atherosis, thrombosis of the spiral arteries (a form of decidual vasculopathy), and a combination of these lesions. Fetal lesions may include a decrease in the number of arterioles in the villi and fetal arterial thrombosis. Maternal vascular lesions could lead to preterm labor by causing uteroplacental ischaemia ***(Romero et al., 2006).***

Evidence supports a role for uteroplacental ischaemia as a mechanism of disease leading to preterm labor:

1) Women in preterm labor with intact membranes and those with PROM who delivered preterm have a higher percentage of failure of physiological transformation in the myometrial segment of the spiral arteries than women who deliver at term ***(Kim et al., 2003).***
2) Abruptio placenta, a lesion of vascular origin, is more frequent in women who deliver preterm with intact membranes or with rupture of membranes than in those who deliver at term ***(Romero et al., 2006).***