International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Jyothi VJ et al. Int J Reprod Contracept Obstet Gynecol. 2023 Apr;12(4):940-945
www.ijrcog.org
pISSN 2320-1770 | eISSN 2320-1789

DOI: https://dx.doi.org/10.18203/2320-1770.ijrcog20230791

Original Research Article

Evaluation of screening efficacy of IL6, IL8, CRP and salivary progesterone in predicting preterm pregnancy

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Received: 04 January 2023
Revised: 17 March 2023
Accepted: 18 March 2023

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ABSTRACT

Background: According to WHO preterm birth defined as births occurring before completion of 37 weeks, in a pregnancy beyond 20 weeks of gestation. As reported by W.H. O preterm birth has incidence of about 9.6% of all the live births, preterm births have high neonatal morbidity and mortality. In this review we look at association between CRP, IL6, IL8 and salivary progesterone in predicting the preterm delivery.
Methods: A hospital based prospective study to be conducted in a group of 100 women of 20- 24 weeks of gestation, they were analysed for IL6, IL8, CRP and salivary progesterone and followed them till delivery.
Results: On assessment of the biomarkers to predict the preterm and term pregnancy, we assessed for the blood level of CRP, IL6, IL8 and salivary progesterone among the pregnant women at 20-24 weeks of gestation and followed till the pregnancy outcomes. Among which 46% were with preterm pregnancy and 54% with term pregnancy during delivery. Among them, 20% had the previous preterm pregnancy and 80% were not. We found 70% with normal vaginal delivery, 24% with emergency LSCS and 6% with elective LSCS.
Conclusions: The present study documented the significant higher efficacy of IL6, IL8, CRP and salivary progesterone in predicting the preterm pregnancy. Progesterone levels in the saliva was markedly lower among the pregnancy with preterm delivery compared to term delivery outcome. The fetal outcome among the preterm delivery was significantly with morbidity and mortality compared to term delivery. The ROC curve showed the estimation of IL6, IL8, CRP and salivary progesterone at 20-24 weeks of gestation can predict the outcome of preterm pregnancy.

Keywords: CRP- c reactive protein, IL6, IL8 (interleukins), Preterm, Salivary progesterone

INTRODUCTION

According to WHO preterm birth defined as births occurring before completion of 37 weeks, in a pregnancy beyond 20 weeks of gestation. As reported by WHO preterm birth has incidence of about 9.6% of all the live births. Over 15 million preterm births occur globally every year. Preterm births have high neonatal morbidity and mortality.

Around the globe, preterm birth poses an important perinatal health problem, and has high socio-economic cost including neonatal intensive care, for the families and health-care systems.[1-3] Across the world, prematurity is among the most common causes of death in children of less than 5 years and contributes to adverse perinatal outcomes. preterm birth is the second most typical cause of death after pneumonia in children less than 5 years.[4]

Almost 1 million children, i.e., 1 in 10 babies, die every year due to preterm birth complications.5 Complications of preterm birth include bronchopulmonary dysplasia, respiratory distress syndrome, hyaline membrane disease, pneumothorax, pneumonia, sepsis, necrotizing enterocolitis, patent ductus arteriosus, retinopathy of prematurity, periventricular leukomalacia, intraventricular haemorrhage, unattended births, and deaths of a preterm new born.

Long term morbidities which include learning disabilities due to cerebral palsy, mental development delay, visual and hearing disabilities seen in association with preterm births. In India every year 3,519,100 preterm births are registered which counts to 13.0 preterm births per 100 births.[5] So screening should be done to predict preterm labour and preterm delivery to decrease perinatal morbidity thereby decreasing mortality also by taking early intervention and intensive ante-natal care can be provided.

To prevent preterm delivery at primary level risk factors must be identified antenatally. Antenatal risk factors in mothers can be predicted by clinical, biochemical, and biophysical parameters.

Clinical predictors are history of prior preterm birth, genital tract infection, multiple pregnancy, symptoms of preterm labour include vaginal discharge, bleeding, pelvic pressure and backache.

Biochemical predictors are fetal fibronectin in cervico-vaginal discharge, inflammatory markers present in maternal serum like C-reactive protein (CRP), TNF -alpha, IL6, IL8. Cytokines are protein and polypeptide products secreted by cells that regulate intracellular cell functions. They have diverse actions including growth factor effects, chemotaxis, and angiogenesis. Their actions are regulated through specific membrane receptors, which will activate intracellular pathways. These inflammatory mediators help in regulation of immune response to fight against infections and thus, help to maintain pregnancy. However, the inflammatory response to infection can have a detrimental effect on the pregnancy and the fetus.[6] Interleukins (ILs) are cytokines and are mediators of inflammation and large scale research was done to determine their relationship with PTL since decades. Interleukins such as IL1, IL2, IL4, IL5, IL6, IL8, IL10, IL17, and IL18 have been studied by many researchers and found association with PTD.

Aims

Study aimed to evaluate the screening efficacy of IL6, IL8, C-reactive protein and salivary progesterone in predicting preterm pregnancy.

Objectives

To identify the association between CRP, IL6, IL8 and salivary progesterone with preterm delivery in antenatal patients. To evaluate the screening efficacy of IL6, IL8, C reactive protein and salivary progesterone in predicting preterm pregnancy.

METHODS

A hospital based prospective study to be conducted in a group of 100 women of 20-24 weeks of gestational age attending the outpatient and or admitted in antenatal ward in the department of obstetrics in Narayana medical college over a period of two years (June 2020-May 2022).

Inclusion criteria

Gestational age between 20-24 weeks of gestation, with intact membranes, singleton pregnancy.

Exclusion criteria

Patients in active labour, ruptured membranes, multifetal gestation, preeclampsia, placental abruption, use of tocolytic drugs before sample collection, fetus or amniotic fluid anomaly, uterus or cervical anomaly, cardiovascular diseases.

The pregnant woman in the study group were subjected to a detailed history, general, abdominal, and pelvic examination.

The gestational age was calculated from the date of the LMP and confirmed by an ultrasound scan in early pregnancy. Routine antenatal investigations and specific investigations for prediction of preterm like IL6, IL8, CRP and salivary progesterone were done. Blood sample for estimation of IL6, IL8, CRP was collected in a test tube and allow the sample to clot. The serum was removed from the clot and then the samples were kept frozen until tested by the lab. The level of CRP was measured through a quantitative immunoassay test. IL6, IL8 and salivary progesterone measured by ELISA. The patients were then followed up till delivery.

Methods of estimation

Maternal serum concentration of CRP was measured quantitatively. This assay works on the principle that anti-CRP antibody reacts with CRP present within the sample and results in visible agglutination. The degree of agglutination was detected as a reducing in the intensity of transmitted light at 572 nm (turbidimetry), which was proportional to the amount of CRP within the sample. The concentration of CRP in the sample was determined by interpolation from a calibration curve prepared from calibrators of known concentration. The inter-assay coefficient of variation varied between 1.2 and 6.9%.

The human IL6, IL8 solid phase sandwich ELISA (enzyme linked immuno sorbent assay) is designed to measure the amount of the target bound between a matched antibody pair. A target specific antibody has been coated in the wells of the microplate. Samples, standards, controls are added to these wells and bind to the immobilised (capture) antibody. With addition of the second (detector) antibody the sandwich is formed and a substrate solution is added that reacts with enzyme antibody target complex to produce measurable signal. The intensity of the signal is directly proportional to the concentration of the target present in the original specimen.

Before collection of saliva samples, mouth should be washed with water 10 minutes ahead. Unstimulated passive drool samples (with a minimum of three) were collected in a sterile wide-mouthed glass or plastic container can be used to collect passive drool samples (unstimulated) within 2 hours, at an interval of 30 minutes, three minimum samples were collected and then pooled together. The sample was kept in the refrigerator for 30 minutes and allow them to freeze, at or below -20°C until samples were analysed. Each frozen sample was thawed and centrifuged at 1502 gm for 5 minutes. A clear colourless supernatant solution forms later that will be used for the quantitative assessment of salivary progesterone using an ELISA kit and following protocol of manufacturer.

RESULTS

A total of 100 pregnant women fulfilling inclusion criteria are included in the present study after obtaining informed consent and were analysed for IL6, IL8, CRP and Salivary progesterone and followed them till delivery, of those 46% had a preterm delivery and 54% had a term delivery.

Table 1: Distribution of subjects according to gestational age at delivery.

		Frequency	Percent
Gestational age	Preterm pregnancy	46	46.0
	Term pregnancy	54	54.0
	Total	100	100.0

The mean age of the participants in present study found to be 25±3.70 years of age. mean age comparison of preterm and terms using t test. Showed pregnant women who underwent preterm delivery was 25.41 with standard deviation of 4.04. Mean age of pregnant women who underwent term delivery was 26.44 with standard deviation of 3.37. P value showed 0.16 which shows that age is not a significant criterion.

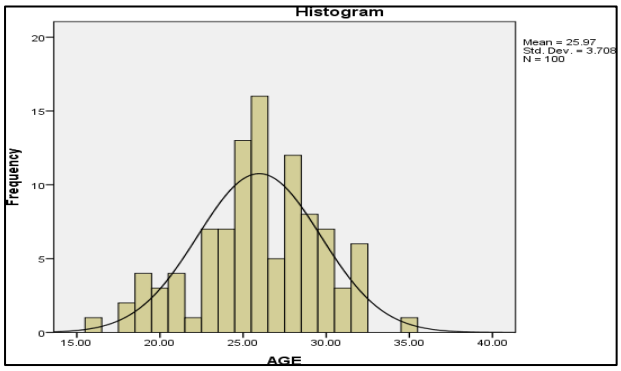

Figure 1: Histogram showing mean age of a pregnant women.

In our study group there was almost equal distribution of gravida of patients. 25 women (50%) of multigravida underwent preterm delivery and 26 women (50%) underwent term delivery. 21 women (42%) of primigravida underwent preterm delivery and 28 women (48%) underwent term delivery.

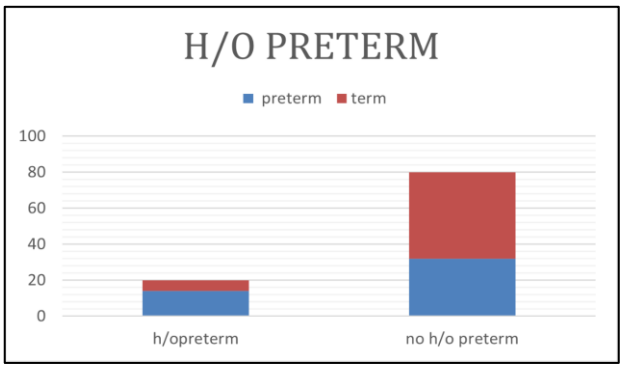

Figure 2: Distribution of subjects according to history of preterm.

Distribution of subjects according to mode of delivery, we found 70% with normal vaginal delivery, 24% with emergency LSCS and 6% with elective LSCS. Table 9 shows indications of LSCS among the study groups, we documented the presence of fetal distress in 40% of pregnancy, 16.7% with malpresentation, 13.3% with breech and CPD, 10% with transverse lie and 6.7% with the compound presentation. On assessment of perinatal morbidity, 33% were with presence of perinatal morbidity among which 21% were with RDS and 12% with birth asphyxia. Table 11 shows comparison of perinatal morbidity among the study groups and found birth asphyxia, RDS were significantly higher in preterm pregnancy compared to term.

On assessment of the biomarkers to predict the preterm and term pregnancy, we assessed for the blood level of CRP, IL-6, IL-8, and salivary progesterone among the pregnant women at 20-24 weeks of gestation and followed till the outcome of pregnancy. Among which 46% were underwent preterm delivery and 54% with term pregnancy. The serum CRP was significantly higher among the preterm pregnancy compared to term pregnancy. The mean CRP in preterm pregnancy was 97.65 and in term pregnancy was 25.35 mg/dl ($p<0.05$). On assessment of ROC curve the predictability of the CRP with higher mean was with preterm delivery outcome with AUC=0.842, with 95% CI, lower=0.759 and upper =0.925 ($p<0.05$).

The serum IL6 and IL8 were also significantly higher among the preterm pregnancy compared to the term pregnancy. The mean level of IL6 among the preterm pregnancy was 59.57 and in the term pregnancy was 15.85. Similarly, the mean level of IL8 among the preterm pregnant women was 57.15 pg/ml and in term pregnancy was 15.87pg/ml ($p<0.05$). On assessment of ROC curve the predictability of the IL-6 with higher mean with preterm delivery outcome with AUC=0.911, with 95% CI, lower =0.856 and upper =0.967 and for IL-8 was AUC

=0.891, with 95% CI, lower =0.821 and upper =0.961 (p<0.05). Similar to present study, Murtha et al, discovered that increased serum interleukin-6 levels in women who deliver prematurely.

On assessment of the salivary progesterone of the pregnant women it was discovered to be markedly less among the preterm pregnancy compared to term pregnancy. The mean level of salivary progesterone was estimated to be 1421.74 pmol/ml in preterm pregnancy and 3229.17 pmol/ml among the term pregnancy (p<0.05). On assessment of ROC curve for the prediction efficacy of salivary progesterone with lower mean with preterm delivery outcome with AUC=-0.912, with 95%CI, lower =-0.0.85 and upper =-0.92 (p<0.05).

Table 2: Comparison of mean variables between the groups and their significance.

Variable	Preterm	Term	P value
Maternal age (years)	25.41	26.44	0.167
Birth weight	2.5 kg	3.2kg	0.001
Apgar	5,7	7,9	0.001
Perinatal morbidity	24	9	0.001
CRP (mg/dl)	97.65	25.35	0.001
IL6 (pg/ml)	59.57	15.49	0.001
IL8 (pg/ml)	57.15	15.87	0.001
Salivary progesterone	1421.74 pmol/ml	3229.17 pmol/ml	0.001

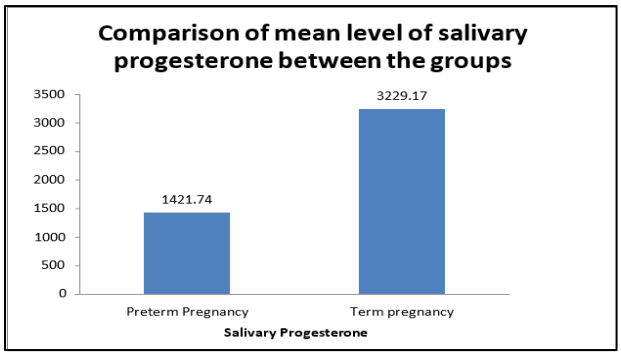

Figure 3: Comparison of mean level of salivary progesterone among the groups.

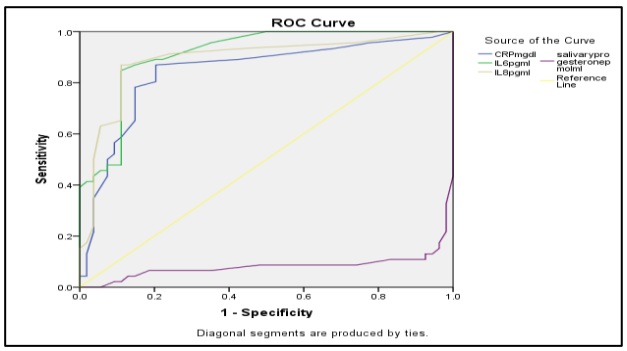

Figure 4: ROC analysis of biochemical parameters among study groups.

On assessment of ROC curve for the prediction efficacy of salivary progesterone with lower mean with preterm delivery outcome with AUC= -0.912, with 95% CI, lower =-0.0.85 and upper =-0.92 (p<0.05).

DISCUSSION

On assessment of the biomarkers to predict the preterm and term pregnancy, we assessed for the blood level of CRP, IL6, IL8, and salivary progesterone among the pregnant women at 20-24 weeks of gestation and followed till the outcome of pregnancy. Among which 46% were underwent preterm delivery and 54% with term pregnancy.

The serum CRP was significantly higher among the preterm pregnancy compared to term pregnancy. The mean CRP in preterm pregnancy was 97.65 and in term pregnancy was 25.35 mg/dl (p<0.05). On assessment of ROC curve the predictability of the CRP with higher mean was with preterm delivery outcome with AUC=0.842, with 95% CI, lower =0.759 and upper =0.925 (p<0.05).

Hvilsom et al done a prospective nested case control study done at Denmark.[7] It consists of 84 women with preterm deliveries(cases) and 400 women with term deliveries (controls) and studied the relationship between the CRP levels in the maternal serum early in the second trimester with that in PTD. Depending on the CRP cut off value 5.6-16.4 mg/l (75-95th percentile) odds ratios between 1.7 and 2 were calculated. The largest value was present at 85th percentile (7.6 mg/l); odds ratio 2.0 [95% confidence interval (CI), 1.2-3.5]. They documented high CRP levels in early second trimester was associated with two-fold risk of preterm pregnancy.[8]

Table 3: Characteristics of CRP in various studies.

	95% confidence interval
Hvislom et al[7]	1.2-3.5
Our study	0.759-0.925

Table 4: Diagnostic accuracy of CRP in various studies.

	Sensitivity	Specificity
Ghezzi et al	80%	69%
Our study	69%	85%

In concordance, Lohsoonthorn et al, reported that increased CRP levels were related with high risk of delivery at a gestational age <34 weeks (i.e., very PTD).[9] They also discovered that high levels of CRP were not linked with PPROM.

Two nested case-control studies on a total of 201 women who underwent spontaneous labour at <37 weeks and 517 controls delivered at term reported that serum CRP levels at 5-19 weeks' of gestation was more in the mothers who underwent preterm delivery.[8,10]

Halder et al considered the predictive significance of CRP in spontaneous PTD and reported that high levels of CRP in early pregnancy in the absence of any medical/surgical or obstetric problem were linked with approximately two-fold high chances of preterm delivery. It also documented that such patients are more prone to have new born outcomes such as premature birth, decreased birth weight, septicaemia, birth asphyxia, and others.[11]

The serum IL6 and IL8 were also significantly higher among the preterm pregnancy compared to the term pregnancy. The mean level of IL6 among the preterm pregnancy was 59.57 and in the term pregnancy was 15.85. Similarly, the mean level of IL8 among the preterm pregnant women was 57.15 pg/ml and in term pregnancy was 15.87 pg/ml ($p<0.05$). On assessment of ROC curve the predictability of the IL-6 with higher mean with preterm delivery outcome with AUC=0.911, with 95% CI, lower =0.856 and upper =0.967 and for IL8 was AUC=0.891, with 95% CI, lower =0.821 and upper =0.961 ($p<0.05$). Similar to present study, Murtha et al, discovered that increased serum interleukin-6 levels in women who deliver prematurely.[12]

Kawilarang et al, considered that mean values of IL8 in the preterm delivery was 34.98±34.79 and for the normal term pregnancy was 11.41±3.32, with t=3.81 and p=0.001 with t-independent test shows marked variation ($p<0.05$).[13]

A similar finding was achieved in another study conducted by Nakamura in Japan, which demonstrated an increase in IL8 value in preterm labour in contrast to normal term pregnancy.[14]

It was also demonstrated in research by Tanaka et al and Novak et al.[15,16] Both studies found that preterm labour was associated with greater levels of IL8 than normal preterm pregnancy. Many recently published studies were in agreement with the outcomes of our study as they support that the role of interleukin-8 and interleukin-6 in prediction of preterm labour.

On assessment of the salivary progesterone of the pregnant women it was discovered to be markedly less among the preterm pregnancy compared to term pregnancy. The mean level of salivary progesterone was estimated to be 1421.74 pmol/ml in preterm pregnancy and 3229.17 pmol/ml among the term pregnancy ($p<0.05$). On assessment of ROC curve for the prediction efficacy of Salivary progesterone with lower mean with preterm delivery outcome with AUC=-0.912, with 95% CI, lower =-0.0.85 and upper =-0.92. ($p<0.05$).

In similar to present study, Priya et al, documented the average level of salivary progesterone was markedly decreased in all women who delivered preterm with gestational age lower than completed 37 weeks of gestation compared to term pregnancy group ($p<0.05$). The permitted cut-off value for salivary progesterone was 2575 pg/ml. More than 80% of women delivered prematurely before 34 weeks of gestation with progesterone levels less than 2575 pg/ml, with sensitivity, specificity, negative and positive predictive values of 83%, 86%, 95% and 60%, respectively.[17] Lachelin et al documented that the evaluation of salivary progesterone helps to predict early preterm labour and in deciding which women will benefit from supplementation of progesterone. They found that women delivering lower than 34 weeks had the saliva progesterone significantly lower compared to the delivery of gestational age of 34-37 weeks.[18]

There were some limitations of the study. Sample size was small. The study has been done in a single centre. The study was carried out in a tertiary care hospital, so hospital bias cannot be ruled out.

CONCLUSION

The present study documented the significant higher efficacy of IL6, IL8, CRP and salivary progesterone in predicting the preterm pregnancy. We documented a higher mean of the IL6, IL8, CRP among the preterm pregnancy compared to term pregnancy outcome. Similarly, the progesterone level in the saliva was markedly lower among the pregnancy with preterm delivery compared to term delivery outcome. The fetal outcome among the preterm delivery was significantly with morbidity and mortality compared to term delivery. The ROC curve showed the estimation of IL6, IL8, CRP and salivary progesterone at 20-24 weeks of gestation can predict the outcome of preterm pregnancy.

ACKNOWLEDGMENTS

I wish to express my deep sense of gratitude to my beloved teacher. Dr. Nafeesa Farheen S. K. for her valuable guidance and cooperation. I am thankful to Dr. V. Sitalakshmi, MD, Professor and HOD and Dr. Ramamani Professor, department of obstetrics and gynecology, Narayana Medical College and Hospital, Nellore for their support.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Jyothi VJ, Farheen SKN, Deepthi M, Swapna L. Evaluation of screening efficacy of IL6, IL8, CRP and salivary progesterone in predicting preterm pregnancy. Int J Reprod Contracept Obstet Gynecol 2023;12:940-5.