



Evaluation of Serum Interleukin-6 and C-Reactive Protein Levels among Women During Term Labour

Ubongabasi A. James¹, John O. Imaralu² and Ijeoma Esiaba^{1*}

¹*Department of Biochemistry, Babcock University, Ilishan-Remo, Ogun State, Nigeria.*

²*Department of Obstetrics and Gynaecology, Babcock University Teaching Hospital/Babcock University, Ilishan-Remo, Ogun State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors IE and JOI designed the study. Authors IE and UAJ performed the statistical analysis, wrote the protocol and managed the literature searches and the laboratory analyses of the study. Author UAJ wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the serum concentrations of C-reactive protein (CRP) and interleukin 6 (IL-6) as well as level of leukocytosis, as inflammatory contributory factors in women with uncomplicated term pregnancies before, during and after labour.

Study Design: This is a cross-sectional study.

Place and Duration of Study: The study was carried out in the Department of Obstetrics and Gynaecology of Babcock University Teaching Hospital (BUTH) and Department of Biochemistry, Babcock University, Ogun State Nigeria between June 2019 and February 2020.

Methodology: 45 venous blood samples were obtained from 34 selected women and grouped into three; prenatal (≥ 32 weeks, $n = 18$), labour (4 to 6 cm dilation, $n = 12$) and postnatal (≤ 24 hour postpartum, $n = 15$). Sixteen blood samples were also obtained from the umbilical cord. Levels of CRP and IL-6 were determined by Enzyme Linked Immunosorbent Assay (ELISA) techniques and the leukocyte count, by hematologic method. Differences in statistical mean were evaluated by one way analysis of variance (ANOVA) with Bonferroni post hoc comparison. The level of significance was set at $P < 0.05$.

*Corresponding author: E-mail: esiabai@babcock.edu.ng;

Results: Concentration of serum IL-6 was significantly high during labour (1354.79 ± 189.16 pg/mL) compared to the prenatal (14.94 ± 4.86 pg/mL, $P < .001$) and postnatal (13.17 ± 3.06 pg/mL, $P < .001$) periods. The low level of CRP observed during active labour compared to the prenatal and postnatal periods did not reach significant difference. The levels of these inflammatory markers were low in the cord blood. Leukocyte counts ($P = .011$) as well as neutrophils ($P = .014$) and MID cell fractions ($P = .004$) were significantly higher during the postnatal period.

Conclusion: The high levels of serum IL-6 observed in this study supports human term labour as an inflammatory event not associated with increased leukocytosis.

Keywords: C-reactive protein; interleukin-6; inflammation; labour; prenatal; postnatal.

1. INTRODUCTION

The interactions between hormonal and mechanical factors ensures successful pregnancy outcome when inflammation plays a physiological role at the onset of term labour by transforming the quiescent myometrium into a contractile one. But presence of infection, bleeding and excessive uterine stretch will trigger pathological inflammation that may lead to preterm labour [1,2]. Anti-inflammatory mechanisms suppress inflammatory cytokines during pregnancy to maintain cervical competence and preserve pregnancy [3]. This, however, changes at term as pro-inflammatory cytokines induce cervical ripening [4], myometrial contraction and parturition [5]. Inflammatory cytokines have been detected within the uterine tissues [6].

CRP is an acute phase reactant that is deposited at sites of inflammation and tissue damage in physiological and experimental conditions [7]. Increased levels of this reactant is one of the most sensitive acute phase parameters in tissue injury or inflammation [8]. Production of CRP from the liver is in response to released pro-inflammatory cytokines such as interleukin-1 and interleukin-6. In pregnancy, high CRP levels have been associated with maternal infection, preterm delivery [9,10], preeclampsia and premature rupture of membranes.

IL-6 is a multifunctional cytokine that plays important roles in acute and chronic inflammation, and autoimmunity. Infection, injury and stress induce IL-6 production and its elevated expression is associated with several chronic inflammatory and auto-immune diseases [11,12]. High levels of IL-6 have been described in the amniotic fluids of women who go into spontaneous labour [13]. It is suggested to be a predictor of preterm labour between 24 and 36 weeks gestation [14] and is particularly raised in

preterm labour associated with intra-amniotic infection [15]. Cervicovaginal IL-6 is believed to be produced by myometrial macrophages [16]. It can also be produced from other cells such as the myocytes and the blood levels rise quickly during stress [12].

Studies have evaluated CRP and IL-6 levels during preterm labour, preeclampsia, infections, and preterm premature rupture of membranes [17,18]. There are fewer studies on levels of these markers in the serum of women undergoing term uncomplicated labour. Hence the need to evaluate the levels of these parameters in the serum before, during and after labour in this group. The aims of this study are (i) to determine and compare the levels of serum CRP and IL-6 and, blood leukocyte subpopulations in pregnant women at their third trimester (≥ 32 weeks), active phase of labour (4 to 6 cm dilation) and post-natal periods and (ii) to compare the levels of these parameters in the maternal and cord blood samples.

2. METHODOLOGY

2.1 Study Population

Women with singleton pregnancies were recruited for this cross-sectional study from June 2019 to February 2020 at the clinics of the Obstetrics and Gynecology Department of Babcock University Teaching Hospital, Ogun State, Nigeria. They were divided into the following groups based on when they were sampled: Prenatal group comprising pregnant women in the third trimester (≥ 32 weeks gestation, $n = 18$), labour group comprising women in active phase of labour (4 cm to 6 cm cervical dilation, $n = 12$) and postnatal group comprising women within 24 hours of delivery ($n = 15$). Forty-five venous blood samples were drawn from 34 women for the three groups. A fourth group comprised sixteen (16) neonates

(cord blood samples). Pregnant women below 32 weeks gestation, who had any known medical condition [pre-eclampsia, pre-labour rupture of membranes, infection (genitourinary tract, bacterial vaginosis, upper or lower respiratory tract or other infection) or who presented with gestational diabetes or iron deficiency] and were on medications or who delivered by Cesarean section or their neonate had a congenital disorder were excluded from the study. Also, patients with features suggestive of malaria or fever (body temperature $\geq 37.5^{\circ}\text{C}$), placenta praevia or other contraindication to vaginal delivery were excluded from the study. None of the participants in the study was found to have a low lying placenta. A short structured questionnaire was administered to obtain brief demographic and medical information of each participant.

2.2 Laboratory Analysis

Five milliliters (5 mL) of blood samples were collected from maternal veins and neonatal umbilical cord. Three (3) and two (2) milliliters were transferred into plain and EDTA bottles respectively. The samples in the plain bottles were centrifuged for 10 minutes at 3000 rpm while the samples in the EDTA bottles were sent to the hematology laboratory for leukocyte count. Aliquot of serum were stored at -20°C until analysis. The serum was used to assess CRP and IL-6 levels using their respective ELISA assay kits (Calbiotech, Inc. and Elab Science, USA) according to manufacturer's instructions. Counts of leukocytes and its fractions comprising lymphocytes, neutrophils and MID cells (monocytes, basophils and eosinophils) were determined with the hematology analyzer Sysmex XP-300.

2.3 Data Analysis

Data were analyzed using IBM Statistical Package for Social Sciences (SPSS Inc., Chicago, Standard version 23.0) and Microsoft

Excel 2019. One way analysis of variance (ANOVA) with Bonferroni post hoc comparison was used to compare mean and standard deviation between the groups. $P < 0.05$ was considered significant.

3. RESULTS

3.1 Demographic Characteristics of Study Participants

Table 1 shows the summary of clinical and demographic characteristics of study participants. They had unremarkable medical history. They did not smoke or take alcoholic beverages. Most were multigravida, apparently healthy and not on medications.

3.2 Biochemical Parameters

Table 2 shows the biochemical parameters of study participants. Significant difference was observed in the level of CRP between the groups ($F(3,57) = 5.828, P = 0.002$). A post hoc analysis demonstrated no significant difference in CRP levels between the maternal samples although the levels during labour was lower than during the prenatal and postnatal periods. However, the level of CRP in the cord blood was significantly lower than in the maternal postnatal ($P = 0.002$) and prenatal ($P = 0.037$) groups.

IL-6 level was significantly different between the groups ($F(3,57) = 903.721, P < 0.001$). Post hoc analysis demonstrated significantly higher concentration of IL-6 at labour compared to the prenatal and postnatal groups or in the cord blood.

3.3 Leukocyte Count

Figs. 1-4 show the counts of leukocytes and the lymphocyte, neutrophil and MID cell fractions. Significantly higher counts of leukocytes ($F(3,57) = 4.117, P = .011$), neutrophils ($F(3,57) = 3.866, P = .014$) and MID cells ($F(3,57) = 5.033, P = .004$) were observed during the postnatal period.

Table 1. Demographic characteristics of the study participants

Particulars	Value
Maternal Age (years, n = 29)	30.17+2.28
Weight before pregnancy (kg, n = 29)	70.39+17.08
Weight during pregnancy (Kg, n = 29)	79.69+17.78
Systolic Blood pressure (mmHg, n = 29)	117.10+10.16
Diastolic Blood pressure (mmHg, n = 29)	74.59+8.22
Birthweight (kg, n = 16)	3.29+0.26
Gestation age at prenatal sampling (weeks, n = 18)	34.94+2.18
Gestation age at Delivery (weeks, n = 16)	38.98+1.79

* Results are expressed as Mean+Standard Deviation

Table 2. Biochemical parameters

	Prenatal	Labour	Postnatal	Cord blood	P value
CRP (ug/mL)	5.42+3.63	3.11+4.42	6.93+4.98	1.71+2.54	.002
IL-6 (pg/mL)	14.94+4.86	1354.79+189.16	13.17+3.06	20.87+8.43	<.001

Results are expressed as Mean+ Standard Deviation. CRP – C-reactive protein; IL-6 – Interleukin-6

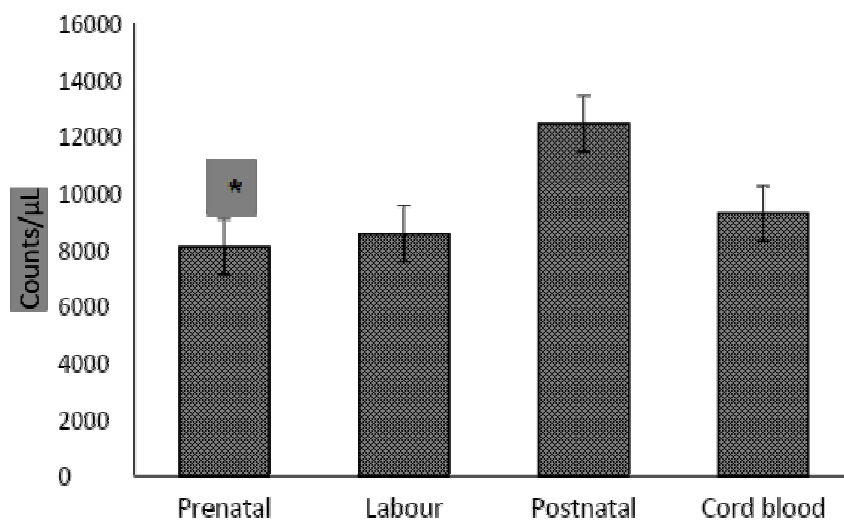


Fig. 1. Leukocyte count in study participants

* Postnatal: significant from prenatal, * $P < 0.05$; Plots are Mean \pm S.E.M = Mean values \pm Standard error of means

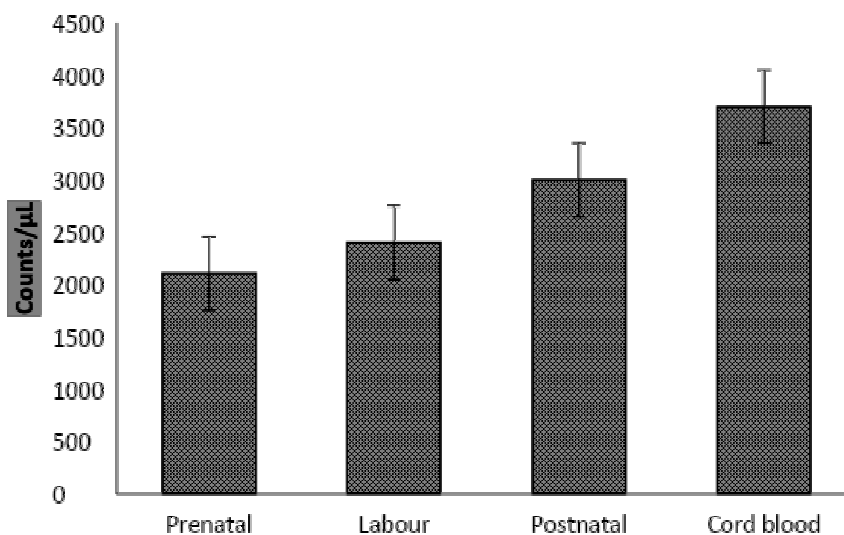


Fig. 2. Lymphocyte counts in study participants

Plots are Mean \pm S.E.M = Mean values \pm Standard error of means

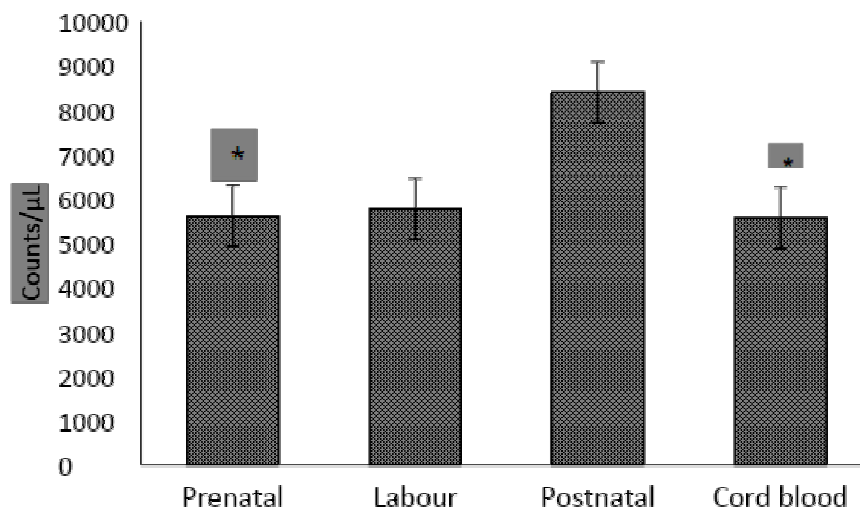


Fig. 3. Neutrophil counts in study participants

*Postnatal: significant from prenatal and cord blood, * P < 0.05; Plots are Mean ± S.E.M = Mean values ± Standard error of means

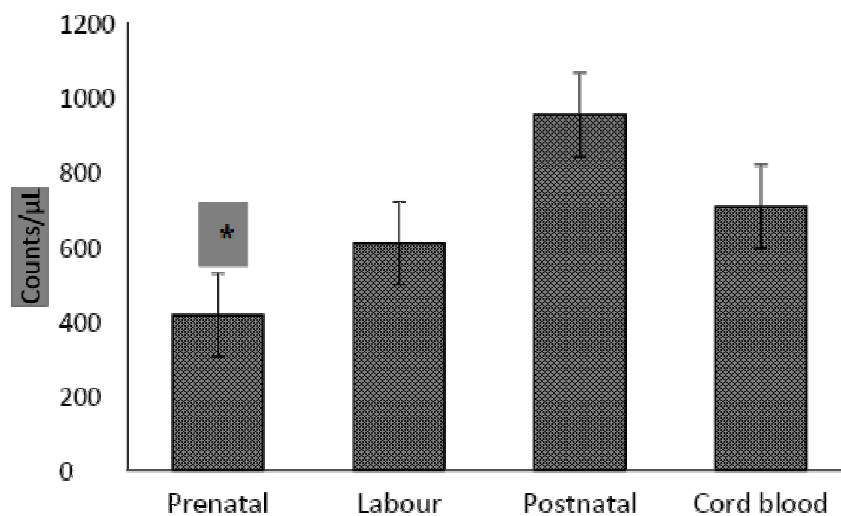


Fig. 4. MID cells (monocytes, eosinophils and basophils) counts in study participants

* Postnatal: significant from prenatal, * P < 0.05; Plots are Mean ± S.E.M = Mean values ± Standard error of means

4. DISCUSSION

Here we present a description of the changes in the concentration of CRP and IL-6 as well as leukocyte counts during pregnancy (third trimester), labour and delivery. We observed higher levels of CRP in the serum of pregnant women at the prenatal and postnatal periods

(Table 2). This shows that acute phase response is associated with not only infection or preterm birth but also with normal pregnancy and delivery. The acute phase response after delivery possibly protects the mother from the effects of injury associated with labour and these increased levels may be associated with host defense mechanisms [8].

CRP promotes clearance of dead cells and enhances bacterial phagocytosis, indicating its important role in host defense, prevention of infection and tissue repair [19]. These mechanisms help protect the mother from the impact of injuries she may have incurred during labour and delivery. We observed low CRP levels during labour (Table 2). High levels and significant association between CRP concentrations and preterm delivery have been observed by other researchers [9,10,14,17]. Lebdowic et al reports low CRP levels during labour and high levels up to 48 hours postpartum, while Cicarelli et al., [20] reported rise in CRP levels after delivery reaching a peak at 24 hours and significantly clearing by 60 hours. Our finding of low CRP levels during active labour, which increased shortly after delivery supports these findings.

Increased cytokine concentrations have been reported during spontaneous term labour [21,22]. Fu et al., [23] observed higher serum IL-6 concentration in pregnant women compared to non-pregnant women. Gillespie et al. [24] documented increased IL-6 concentration in late pregnancy which reached peak levels during labour. Ferguson et al. [25] also observed a similar trajectory for IL-6 increasing from 20 weeks gestation to the end of pregnancy. We did not monitor the levels of IL-6 throughout pregnancy but around labour. Nevertheless, we observed that IL-6 levels were increased in labour (Table 2) compared to the weeks prior labour or the hours following delivery. This observation supports the findings of Osman *et al.* [6] that IL-6 induces cervical ripening i.e. IL-6 softens and expands the cervix during labour in preparation for delivery. Amniotic fluid IL-6 have been observed to increase with cervical ripening and dilation [26,27] and reaching a peak at the time of maximal cervical dilation or delivery [28]. So IL-6 which is widely expressed in the female reproductive tract and gestational tissues contributes to the normal physiology of labour [4,5]. It is suspected to upregulate the expression and responsiveness of oxytocin receptors on myometrial cells as well as the secretion of oxytocin from such cells [1].

High levels of acute phase proteins, including CRP, have been reported in cord blood as a marker of early onset sepsis due to intrauterine infections [29] and in small-for-gestation age neonates [30]. In this study we report low levels of CRP and IL-6 in the cord blood thereby supporting a lack of placental transfer during delivery and thus, any increase in the newborn

could be a sign of trauma or infection as the fetus is capable of producing CRP on their own [29]. Cicarelli et al., [20] reports lower CRP levels in the cord blood compared to maternal blood.

The sources of cytokines effecting cervical ripening are majorly from placenta and myometrial membranes [6,31]. This may explain the lower counts of circulating leukocytes we observed during labour compared to the postnatal period as the myometrial macrophages may be responsible for the production of the IL-6 observed during labour [16]. Immune cells (neutrophils and macrophages) are reported to infiltrate the uterine tissue at or around the time of parturition [6]. Leukocyte counts tend to increase slightly during late stage pregnancy due to delayed neutrophil apoptosis [32,33]. Macrophages are believed to contribute to labour as their depletion in experimental pregnant mice prevented lipopolysaccharide induced preterm labour [1]. Arbib et al., [34] describes physiological increase in aseptic leukocytosis during labour but especially immediately following delivery. Inflammatory immune cells (neutrophils, monocytes and macrophages) have been associated with inflammation in the preterm labour with or without infection. The concentration of cytokines in such women correlated with the extent of leukocyte infiltration as detected in extra-placental tissues in preterm deliveries [17]. We did not observe any significant correlations between the leukocyte parameters and CRP and IL-6 concentrations. This suggests that circulating leukocytes may not be involved in the increased levels of IL-6 observed during labour in this study.

5. CONCLUSION

Low levels of CRP observed in the cord blood suggests that maternal CRP does not cross the placental barrier. The high IL-6 levels observed during labour reflects their expression in the female reproductive tract and role in ripening of the cervix during labour. This finding supports the theory of labour as an inflammatory process. Elevated levels of inflammatory cells (neutrophils and MID) after delivery suggests they are needed for tissue repair and recovery during this period. It also implies that the inflammation (high IL-6 levels) observed during labor was not induced by these immune cells. We recommend that further research be carried out to distinguish the physiologically increased levels of CRP, IL-6 and leukocytes associated with normal pregnancy and delivery from the pathological increase that are observed in infections and

morbidities encountered during pregnancy and parturition.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Babcock University Health Research and Ethics Committee (BUHREC), and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Only participants who gave written consent were recruited for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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