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To cite this article: Gbemisola Eniola Osanyin & Omololu Adegbola (2016) Maternal serum interleukin 6 levels and fetomaternal outcomes in women with preterm premature rupture of membranes in Lagos, South-western Nigeria, The Journal of Maternal-Fetal & Neonatal Medicine, 29:21, 3506-3510, DOI: [10.3109/14767058.2015.1135123](https://doi.org/10.3109/14767058.2015.1135123)

To link to this article: <http://dx.doi.org/10.3109/14767058.2015.1135123>



Accepted author version posted online: 12 Jan 2016.  
Published online: 10 Feb 2016.



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ORIGINAL ARTICLE

## Maternal serum interleukin 6 levels and fetomaternal outcomes in women with preterm premature rupture of membranes in Lagos, South-western Nigeria

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### Abstract

**Objective:** This study was done to assess the relationship between maternal serum IL-6 levels and fetomaternal outcome following PPRM.

**Methodology:** This was a prospective cohort study comprising 45 cases of PPRM and 45 controls of similar age, parity, and gestational age. Five milliliters of maternal serum was collected after obtaining informed consent. They were followed up till delivery and records of the delivery and neonatal outcomes were obtained. Serum IL-6 levels were determined by standard enzyme-linked immunosorbent assay [ELISA]. PPRM patients were categorized into two groups using a threshold of 14 pg/ml. Chi-square ( $\chi^2$ ) test was used to compare categorical outcomes. *p* values of <0.05 were taken as significant.

**Results:** The mean serum IL-6 level for the women with PPRM was (20.2 ± 11.0 pg/ml), which was significantly greater than for the control subjects (13.9 ± 5.8 pg/ml, *p* < 0.001). Fetomaternal outcomes were all worse in those with IL-6 ≥ 14 pg/ml. Nevertheless, only the difference in early neonatal deaths was statistically significant.

**Conclusion:** Measurement of maternal serum IL-6 can help to indicate hostile intrauterine environments to the fetus as well as identify patients who may benefit from pregnancy prolongation or intervention.

### Keywords

Fetomaternal outcomes, preterm premature rupture of membranes, serum interleukin 6

### History

Received 30 September 2015

Revised 12 December 2015

Accepted 18 December 2015

Published online 8 February 2016

### Introduction

Research has estimated that 10% of all perinatal deaths are directly or indirectly attributable to premature rupture of membranes [PROM] [1], defined as the rupture of the fetal membranes with release of the amniotic fluid more than 1 h prior to the onset of labor [2,3]. PROM can be subdivided into term PROM (rupture of membranes from 37 weeks of gestation and beyond) and preterm PROM (PPROM, i.e. rupture of membranes prior to 37 weeks of gestation) [2,3].

Significant risks to both the fetus and mother following PPRM have been identified over the years. The most significant risks to the fetus after PPRM are complications of prematurity which includes respiratory distress, neonatal infections, intraventricular hemorrhage, and necrotizing enterocolitis. On the other hand, the primary maternal risk with expectant management of preterm PROM is infection which includes chorioamnionitis, endometritis, puerperal sepsis, and maternal death [4–9].

Interleukin 6 [IL6], a polyfunctional cytokine, has been found to be significantly elevated in pregnant women with PPRM who develop infection [10]. It is secreted by tissues in the fetomaternal interface in response to microbial products [11]. In comparison to newborns with intact membranes, high levels of IL6 are found in maternal blood, umbilical cord blood, and in the blood of newborns who develop sepsis [12]. Consequently, elevated maternal serum IL6 concentration has been found to be indicative of an increased risk of intrauterine infection, and it can be used to identify pregnancies where early intervention would be appropriate [12].

So far, maternal serum IL6 levels as an inflammatory parameter following PPRM have not been fully explored in Nigeria. As the management of PPRM in the absence of clinical or laboratory evidence of infection is expectant [13], assaying IL6 levels in maternal serum can be useful for the early identification of asymptomatic uterine infection in women with PPRM as well as facilitating prompt intervention in pregnancies complicated by this event and thereby improving the fetomaternal outcomes. This study was therefore designed to assess the relationship between maternal serum IL6 levels and fetomaternal outcomes following PPRM in Lagos, South-West Nigeria.

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## Methodology

### Study setting

This prospective cohort study was carried out in three government owned hospitals in Lagos state, South-West Nigeria. Lagos University Teaching Hospital (LUTH) is a tertiary hospital owned by the Federal Government of Nigeria and serves as a referral center for both public and private hospitals in the Lagos metropolis, Nigeria. The Lagos Island Maternity Hospital and Gbaja Maternal and Child Health Care Center are secondary health care facilities owned by the Lagos State Government.

The study population consisted of consenting pregnant women whose gestational ages were between 28 and 36 weeks with a clinical diagnosis of preterm premature rupture of membranes (study group) or with intact membranes (control group). Women with PPRM were however excluded if they have antenatal history of polyhydramnios, multiple gestation, cervical incompetencies, congenitally malformed fetus, sexual intercourse in the preceding 24h, or have had a digital examination done.

Women with PPRM were diagnosed using the screening guidelines of the Royal College of Obstetricians and Gynecologists (RCOG) which are maternal history and observation of fluid pooling in the posterior vaginal fornix, or leaking from the cervical os on sterile speculum examination [14] and a positive nitrazine paper test (colour changes from yellow to blue-black) [15].

A minimum sample size of 45 subjects was calculated for each group using the sample size formula for two independent means [16], which incorporates the standard deviation for interleukin 6 in healthy pregnant women without preterm premature rupture of membranes (PPROM), the value was obtained from Gulati et al.'s study as 2.7 pg/mL [17]. A total sample size of 90 was utilized for both groups. All consenting consecutive women with PPRM served as the case while the controls were consenting women with similar gestational ages without PPRM.

### Study instrument and data collection

Using a structured proforma, information was obtained on the socio-demographic characteristics, parity, estimated gestational age (EGA) – this was calculated from the first day of the last menstrual period or from an early ultrasound scan, past medical history as well as previous and present obstetric history. All the participants were then followed up till delivery and fetal outcomes such as the birth weight, special care baby unit (SCBU) admission, neonatal sepsis, and the existence of neonatal morbidity and/or mortality were also noted. Outcomes of babies admitted to the SCBU were also determined to know those babies who eventually were discharged home or those suffered an early neonatal death (ENND).

A sterile speculum examination was performed on all women who presented to the labor ward with complaints of drainage of fluid per vaginam and any pooling of liquor in the posterior fornix of the vagina was noted. To further confirm the diagnosis, samples of the pooled amniotic fluid in the vagina were collected and tested using nitrazine paper.

The nitrazine paper became blue-black upon application of amniotic fluid.

About 5 ml of venous blood was then collected into a plain bottle and transferred from the point of collection (the labor ward or the lying-in ward) to the Central Research Laboratory of the College of Medicine, University of Lagos, where it was centrifuged for 10 min at 3200 revolutions per minute (rpm) using an Eppendorf® centrifuge. The resulting supernatant (serum) was transferred into clean cryogenic tubes using a Pasteur pipette in 0.5 mL aliquots and stored in cryogenic boxes and placed in the freezer at a temperature of  $-20^{\circ}\text{C}$ . This was done on consecutive patients with PPRM meeting the selection criteria and selected control subjects until the minimum sample size was achieved.

Interleukin 6 levels in the maternal sera were then assayed after separating the PROM samples from the non-PROM samples. Each sample in the two separate groups was then assayed at the same time enzymatically with the enzyme-linked immunosorbent assay (ELISA) method, using reagents obtained from the manufacturer (Anogen Inc. Ontario, Canada). The minimum detectable dose of IL6 using the Anogen kit (Anogen Inc. Ontario, Canada) was 2 pg/ml as specified by the manufacturer.

### Data analysis

Data were managed using the Epi Info 7 Statistical Package (Centers for Disease Control and Prevention (CDC), Atlanta, GA) for analysis by the Center for Disease Control and Prevention (CDC). Frequencies and proportions were used to summarize categorical variables of interest while quantitative variables were summarized using means and standard deviations. Inferential statistics was carried out using the independent sample *t* test for continuous variables and the Chi-square ( $\chi^2$ ) test for categorical variables or as appropriate. Fisher's exact test was used. *p* values of  $<0.05$  were taken as the level of significance.

### Ethical consideration

Ethical approval for the study was obtained from Health Research and Ethics Committee of Lagos University Teaching Hospital while approval was also obtained from the management of the hospitals where the study was conducted. In addition, the purpose of the study was explained to the subjects and written consent obtained prior to data and sample collection. The laboratory tests were carried out at no cost to the participants and the information obtained from the subjects was kept strictly confidential.

## Results

### Socio-demographic and obstetrics characteristics

A total of 90 participants were included in the study. Forty-five were in the study group, while 45 were in the control group. There was no significant difference in the mean age of the study and control groups ( $p=0.220$ ). There was however a significant difference in the booking status ( $p<0.001$ ), the mean gestational age at delivery ( $p<0.05$ ), and the mean IL-6 level ( $p<0.001$ ) between the study and control groups (Table 1).

Table 1. Socio-demographic and obstetrics characteristics of respondents.

	Group		p value
	PPROM (n = 45)	Control (n = 45)	
Mean ( $\pm$ SD) age in years	29.86 $\pm$ 6.21	31.44 $\pm$ 5.93	0.220
Parity			
0	14 (31.12)	23 (51.11)	0.088
1	11 (24.44)	12 (26.67)	
2	10 (22.22)	7 (15.56)	
3–5	10 (22.22)	3 (6.67)	
Mean ( $\pm$ SD) parity	1.49 $\pm$ 1.42	1.44 $\pm$ 1.25	0.875
Booking Status			
Booked	15 (33.33)	45 (100.00)	<0.001
Unbooked	30 (66.67)	0 (0.00)	
Mean gestational age at delivery (weeks)	32.00 $\pm$ 4.00 (range 28–36)	39.22 $\pm$ 1.13 (range 38–40)	0.003
Mean gestational age at presentation and sample collection (weeks)	31.08 $\pm$ 2.26 (range 28–35)	32.00 $\pm$ 1.20 (range 28–36)	
Mean ( $\pm$ SD) Interleukin 6 level (pg/ml)	20.17 $\pm$ 11.0 (range 8.36–41.32)	13.88 $\pm$ 5.80 (range 2.7–22.3)	<0.001

SD, standard deviation.

Table 2. Correlation between PROM group and control and gestational age.

PROM		CONTROL	
Gestational age (weeks)	Mean IL6 level	Gestational age (weeks)	Mean IL6 level
28	31.10 $\pm$ 7.25	28	17.21 $\pm$ 5.45
29	27.25 $\pm$ 7.71	29	12.25 $\pm$ 6.44
30	26.24 $\pm$ 9.90	30	18.25 $\pm$ 2.69
31	25.12 $\pm$ 7.66	31	13.09 $\pm$ 2.69
32	12.72 $\pm$ 0.00	32	12.95 $\pm$ 0.00
33	16.02 $\pm$ 3.39	33	15.97 $\pm$ 4.53
34	12.96 $\pm$ 3.60	34	9.94 $\pm$ 5.61
35	7.00 $\pm$ 4.85	35	9.53 $\pm$ 4.09
36	5.00 $\pm$ 2.98	36	4.21 $\pm$ 1.01
Correlation coefficient= 0.60 p < 0.000001		Correlation coefficient= 0.91 p=0.000092	

Table 3. Comparison of fetal outcomes of patients with PPRM having IL6 below and at/above 14 pg/ml.

Fetal outcomes	IL6 level		p value
	< 14 pg/ml (n = 14)	$\geq$ 14 pg/ml (n = 31)	
Mean ( $\pm$ SD) birth weight in kg	1.94 $\pm$ 0.56	2.05 $\pm$ 0.68	0.584
SCBU admission			
Admitted	11 (78.57)	25 (80.65)	1.000
Not admitted	3 (21.43)	6 (19.35)	
Neonatal sepsis			
Present	7 (50.00)	20 (64.52)	0.554
Absent	7 (50.00)	11 (35.48)	
Early neonatal death (ENND)			
Present	0 (0.00)	10 (32.26)	0.019
Absent	14 (100.00)	21 (67.74)	
Stillbirth			
Present	0 (21.43)	5 (16.12)	0.069
Absent	14 (78.57)	26 (83.87)	

SD, standard deviation; SCBU, special care baby unit.

### Fetal outcomes and maternal interleukin 6 level

The mean IL6 in the study group was 20.17  $\pm$  11 pg/ml while that of the controls was 13.88  $\pm$  5.80 pg/ml. Table 2 highlights the mean IL6 value with respect to gestational age in weeks in both the PPRM and non-PPROM. There is a positive correlation between gestational age and IL6 values especially in the PPRM group ( $p < 0.000001$ ). Further analysis of the PROM group shows that the IL6 levels were far higher for those who ruptured in early pregnancy ( $< 32$  weeks) ( $p = 0.0001$ ).

Studies done by Gulati et al. [17] and Murtha et al. [19] proposed the use of maternal serum IL 6 levels of 8 pg/ml as a threshold (cut-off) value with women having IL 6 levels at or above this threshold being at an increased risk of adverse fetomaternal outcomes. In this study, however, this threshold could not be used as 42 (93%) of the PPRM cases and as many as 37 (82%) of the control subjects had IL6 values greater than 8 pg/ml. Therefore, for the purpose of this study, a higher threshold (cut-off value) of 14.0 pg/ml was used (being just above the mean value of 13.9 pg/ml obtained for the control group).

The sub-group analysis within the PPRM group showed that babies born to women with IL-6 levels  $\geq 14$  pg/ml were significantly more likely to experience early neonatal deaths compared with babies born to respondents with IL-6 levels below the threshold ( $p < 0.05$ ). Maternal IL-6 levels were not significantly associated with neonatal sepsis, SCBU admission, perinatal mortality, or the mean birth weight of babies born to mothers with PPRM. However, the trend in the result showed that neonatal sepsis, SCBU admission, and perinatal mortality was worse in babies born to mothers with IL-6 levels  $\geq 14$  pg/ml (Table 3).

### Maternal outcomes and maternal interleukin 6 level

Although maternal IL-6 levels was not statistically associated with the individual and combined maternal outcomes among the women with PPRM, the sub-analysis at this level showed consistently that the maternal outcomes were mostly worse in the PPRM cases that had IL-6 levels at or above the threshold used in this study compared with those with IL-6 levels below the threshold (Table 4).

Table 4. Comparison of maternal and combined outcomes of patients with PPRM having IL6 below and at/above 14 pg/ml.

Outcomes	IL6 level		p value
	Below 14 pg/ml (n = 14)	14 pg/ml or Above (n = 31)	
<i>Maternal outcomes</i>			
Fever			
Present	9 (64.29)	25 (80.65)	0.419
Absent	5 (35.71)	6 (19.35)	
Puerperal sepsis			
Present	1 (7.14)	3 (9.68)	1.000
Absent	13 (92.86)	28 (90.32)	
Mode of delivery			
Caesarean section	9 (64.29)	25 (80.65)	0.419
Vaginal delivery	5 (35.71)	6 (19.35)	
<i>Combined outcome</i>			
Infectious morbidity (FSL, PPS, or NNS)			
Present	9 (64.29)	22 (70.97)	0.920
Absent	5 (35.71)	9 (29.03%)	

FSL, foul-smelling liquor; PPS, puerperal sepsis; NNS: neonatal sepsis.

## Discussion

This prospective cohort study conducted in Lagos South Western Nigeria found that the mean gestational age at delivery was significantly lower in the women with PPRM compared with women without PPRM. This is most likely as a result of the early commencement of the process of delivery in the women with PPRM in comparison with those women without due to the loss of the protective membranes. The mean gestational age at delivery of 32 weeks in women with PPRM reported in this study is similar to that of Noor et al. [18] who reported a mean gestational age at delivery of 33 weeks. This is not surprising as evidence has shown that PPRM is associated with preterm delivery as a result of multiple aetiopathogenesis [1,5,15].

Studies done by Murtha et al. [19] and Gulati et al. [17] proposed the use of maternal serum IL 6 levels of 8 pg/ml as a threshold (cut-off) value with women having IL 6 levels at or above this threshold being at an increased risk of adverse fetomaternal outcomes. In this study, however, this threshold could not be used as 42 (93%) of the PPRM cases and as many as 37 (82%) of the control subjects had IL6 values greater than 8 pg/ml. First, applying this threshold would have meant that only three women in the PPRM group and eight women in the control group would have IL6 values less than 8 pg/ml. Second, a sub-analysis of the PPRM group would have meant comparing the outcomes of 42 patients with those of only 3. Therefore, for the purpose of this study, a higher threshold (cut-off value) of 14.0 pg/ml was used (being just above the mean value of 13.9 pg/ml obtained for the control group).

Furthermore, the finding from this study is in agreement with the findings from another study among Caucasians [17] which also reported that the mean serum IL 6 levels in patients with PPRM was significantly higher than in healthy women with intact membranes. It was also discovered in this study that the earlier the rupture of membranes the higher the IL6 levels (as shown in Table 2).

The finding from this study therefore further corroborates findings from other studies which have suggested that IL6 can actually be used as a predictor of asymptomatic infection in PPRM patients. In assessing the fetomaternal outcomes at different concentrations of IL6 in the women with PPRM, this study found early neonatal death as the only outcome significantly associated with IL6 values >14 pg/ml. Fetomaternal outcomes (neonatal sepsis, SCBU admission, perinatal mortality, maternal fever, puerperal sepsis, and infectious morbidity), although worse in the women with PPRM as compared with the women without PPRM, was found not to be statistically significant. This finding is difficult to explain in light of the significant early neonatal deaths recorded within the PPRM group. Perhaps increasing the sample size may have proven otherwise. This finding may, however, also suggest that the babies born to the women with PPRM are usually very ill compared with those in the control group and as such require intensive SCBU care which may not readily be available in this country [20].

Previously documented surveys among Caucasians [10,17,21] with different IL6 cut-offs, which were highly sensitive and specific, have reported a significant association between all the fetomaternal outcomes measured in this study and high levels of IL6. This observed difference in significance level in these Caucasian studies could actually be attributed to the different maternal serum IL6 cut-off levels used in their environments. Several studies involving IL6 have shown that higher levels of IL6 have been seen in African American women compared with their Caucasian and Asian counterparts [22–24]. The findings from this study emphasizes the need for more research in this part of the world to determine an applicable threshold for maternal serum IL6 which can be used as a reference value for immediate intervention in pregnancies complicated by this event and thereby improving the fetomaternal outcomes.

It is noteworthy that this study was conducted on women of African descent, and as such, the threshold value of maternal serum IL6 differs from other studies conducted on Caucasian women. More work is needed to clearly define the role of maternal serum IL6 in the management of PPRM and determine an appropriate threshold value for prediction of significant adverse fetomaternal outcomes in this environment.

## Conclusion

Measurement of maternal serum IL-6 can help to indicate hostile intrauterine environments to the fetus as well as identify patients who may benefit from pregnancy prolongation or intervention. However, due to the small sample size of this study, multicentre studies may be necessary for ideal determination of serum IL6 levels appropriate for this environment and population.

## Acknowledgements

The authors would like to acknowledge Drs O. Obilade, O. Akinyemi, and V. Oladotun, for their assistance and support in the initial drafting of this manuscript and data analysis.

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