Original Article

Estimation of Inflammatory Cytokines in Maternal Serum for Assessing the Outcome of Threatened Miscarriage

Hakeem A. Lawal[1], Gbolahan O. Obajimi[1], Yusuf O. Bello[2,3], Abdul Fatai Onifade[4], Muideen A. Jimoh[5], Ayodele O. Arowojolu[1], Imran O. Morhason-Bello[1,3]

[1]Department of Obstetrics and Gynaecology, Faculty of Clinical Sciences, College of Medicine/ University College Hospital Ibadan, University of Ibadan, [2]Department of Statistics, Faculty of Science, University of Ibadan, [3]Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, [4]Division of Immunology, Department of Chemical Pathology, Faculty of Basic Medical Sciences, College of Medicine, University College Hospital Ibadan, University of Ibadan, Oyo State, [5]Hamzainab College of Health Technology and Sciences, Osogbo, Osun State, Nigeria

Abstract

Objectives: This study investigates the use of pro- and anti-inflammatory cytokines in predicting the outcome of pregnancy complicated by threatened miscarriage. **Materials and Methods:** Of the 140 eligible pregnant women recruited for the study, maternal serum levels of selected inflammatory cytokines (IL-2, IFNγ, IL-4, and IL-13) for 70 women with threatened miscarriage were analysed for this study. Serum concentrations were measured using the enzyme-linked immunosorbent assay (ELISA) kit. Inevitable miscarriage or ongoing pregnancy was used as the outcome, whereas serum levels of selected inflammatory cytokines, women's sociodemographic characteristics, gynaecologic history, and clinical history were used as the explanatory variables. The Student's *t* test was used to compare the cytokine profiles between women with inevitable miscarriages and women with normal ongoing pregnancy after 13 weeks of gestation. Poisson regression models were performed to investigate the factors associated with inevitable miscarriage. **Results:** The result revealed significantly higher pro-inflammatory cytokines, IL-2 ($P < 0.001$), and IFNγ ($P < 0.001$) in women with a pregnancy that resulted in an inevitable miscarriage than in those that resulted in an ongoing pregnancy. The incidence rate of inevitable miscarriage increased by 16% (IRR = 1.16, 95% CI: 0.58–2.32) for a unit increase in IL-2 and by 25% (IRR = 1.25, 95% CI: 1.09–1.43) when adjusted for sociodemographic characteristics, gynaecology, and clinical history. **Conclusion:** The IL-2 was the best biomarker for predicting the outcome of threatened pregnancy with a sensitivity of 80% and a specificity of 70% at 1.30 pg/mL cut-off point.

Keywords: *Bleeding, cytokine, miscarriage, Nigeria, pregnancy*

Introduction

Threatened miscarriage is the most common complication of ongoing pregnancy and it has been estimated to occur in up to 30% of all pregnancies.[1] Generally, miscarriage is a very devastating occurrence to most couples who are desirous of a positive outcome of pregnancy, especially in cases of recurrent miscarriages, thus making its prevention and management very important.[2] Pregnant women who present with threatened miscarriage are often extremely distressed and providing care can be challenging to the health care professionals because it is difficult to provide definite information on the potential outcome.

The major pro-inflammatory cells include IFNγ, TNF-ß, and IL-2. The anti-inflammatory cells are responsible for inducing antibody production and are commonly found in association with strong humoral immunity. Major anti-inflammatory cytokines include IL-4, IL-5, and IL-13. The pro- and anti-inflammatory cells are known to be equally incompatible with each other as an individual who produces a strong pro-inflammatory response usually tends to have a low anti-inflammatory response and *vice versa*.[3] Evidence also suggests that pro-inflammatory responses are weakened during pregnancy while the anti-inflammatory response is enhanced.[4,5]

Pro-inflammatory cytokines have a lot of deleterious effects on pregnancy and they are known to produce cytotoxic factors that initiate several cell-mediated inflammatory reactions which ultimately culminate in pregnancy losses.[6] This has been corroborated by an earlier study, which showed that injection of pro-inflammatory cytokines such as IL-2 was associated with pregnancy losses.[7] In a previous study,[8] it was reported that there was a significant association between elevated serum levels of

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Address for correspondence:
Dr. Imran O. Morhason-Bello, Department of Obstetrics and Gynaecology, Faculty of Clinical Sciences, College of Medicine, University College Hospital, University of Ibadan, Ibadan, PMB 5116, Oyo State, Nigeria. E-mail: imranmorhasonbello@gmail.com

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some specified pro-inflammatory cytokines (such as IL-2; IFNγ, and TNF-α) and first-trimester pregnancy losses. Furthermore, Jain *et al*.[1] assessed and compared IL-2 and IL-6 cytokines among pregnancies with threatened miscarriage and reported that elevated levels of IL-2 were associated with threatened miscarriage. Thus, it was concluded that IL-2 had a deleterious effect on pregnancy.

Anti-inflammatory cytokines tend to produce humoral immunity that serves as a protective factor for the successful outcome of pregnancy. Jain *et al*.[1] reported that as compared with normal pregnancy, reduced level of T-helper type 2 cells among women with threatened miscarriage has been associated with successful pregnancy outcome. However, Abdullah and Mahdi reported in their study[8] that there was no significant association between anti-inflammatory cytokines and pregnancy losses in the first trimester. This was adduced to the presence of a multiplicity of factors that could be responsible for cytokine production. This study did not control for these factors, which could serve as confounders. Similar to the findings of Abdullah and Mahdi, Vitoratos *et al*.[9] reported that elevated serum levels of IL-1B and TNF-α as well as a non-significant level of IL-6 were associated with threatened miscarriage in the first trimester of pregnancy. They further opined that a distinct immune response was very important in the prediction of an adverse pregnancy outcome following an episode of threatened miscarriage.

Data on cytokine profiles in women with threatened miscarriage is relatively scarce, especially in resource-poor countries such as Nigeria. The knowledge on the immunologic basis of pregnancy is far from complete and a study that can show that pro and anti-inflammatory cytokines could be useful in predicting the outcome of pregnancy complicated by threatened miscarriage will be useful in managing this condition. This study assesses the roles of cytokines in the outcomes of threatened miscarriage in Nigeria.

Subjects and Methods

Study design

This longitudinal study was conducted among women diagnosed with threatened miscarriage and women with normal ongoing pregnancies at two different health facilities in the Ibadan metropolis.

Study setting

The study was conducted in the Department of Obstetrics and Gynaecology of Adeoyo Maternity Hospital (AMH) and University College Hospital (UCH), Ibadan between April 2019 and April 2020. UCH provides tertiary health care to the people of Oyo State and neighbouring states. An average of 870 gynecological patients are reported at the gynecological clinic per annum with about 230 miscarriage-related cases seen annually with threatened miscarriage accounting for 25% of admission into the gynecological wards. AMH is a government-owned secondary health care centre located in the metropolitan city of Ibadan with an antenatal booking rate of about 150–200 pregnant women per week. AMH has an annual delivery rate of about 4800–6000 deliveries per annum with 4.3% of pregnancies ending in threatened miscarriage. The two hospitals have consultant obstetricians and trained midwives who manage women with threatened miscarriages as per the protocol of the hospital, which includes bed rest, investigation, and treatment based on the outcome of the investigation.

Eligibility criteria

Selection of cases: We recruited consenting pregnant women diagnosed with threatened miscarriage between 6 and 10 weeks of gestation.

Selection of controls: These were consenting pregnant women without threatened miscarriage in index pregnancy at 6–10 weeks of gestation.

Exclusion criteria: Pregnant women with a remarkable previous gynaecologic history, known history of a uterine anomaly, previous uterine and cervical surgeries, and significant history of tobacco or alcohol use.

Sampling technique and data collection

Women with threatened miscarriage were recruited consecutively, whereas women in the controls were matched for age and gestational age. The data were collected by trained medical doctors (house physicians) who served as research assistants. Prior to the commencement of this study, the research assistants were trained by the investigators for 2 days on data collection procedures including the use of proforma designed for the study. We obtained written informed consent after providing a detailed explanation of the study procedure including the collection of blood samples and storage for future use.

Blood samples were collected twice from women with threatened miscarriages. The first blood sample was collected at the point of diagnosis of threatened miscarriage between 6 and 10 weeks of gestational age, whereas the second sample was collected either at the point of inevitable miscarriage (gestational age at which inevitable miscarriage occurred was noted) or when the symptoms of threatened miscarriage have resolved. Each woman with threatened miscarriage was followed up until the 13th week of pregnancy before the second sample was collected. The diagnosis of threatened miscarriage was based on the clinical history of vaginal bleeding with or without abdominal pain and closed internal cervical os at digital examination and confirmed with a pelvic scan. The diagnosis was made by the senior registrar and confirmed by a consultant obstetrician. We also collected blood samples two times from the control population within 24 h of when samples were collected among women with threatened miscarriage.

About 10 mL of venous blood samples were obtained from the patients at the ante-cubital veins after sitting calmly and resting for 5 min. Cytokines and other inflammatory markers are not affected by sample collection, transportation, and processing. Similar to other proteins, they are affected by the storage temperature. Thus, the blood samples were stored in a plain bottle. Thereafter, the collected blood samples were centrifuged within 2 h of collection at 2500 rpm for 5 min. The plasma supernatant was withdrawn and frozen at −20°C until analysis. Consent was obtained to store the remaining samples in the ultralow freezer for future research use.

Sample size determination

The sample size for the study obtained using the formula for sample size estimation for comparison of two means[10] was 63. However, 10% was added to the sample size to allow for non-response. A total sample size of 70 cases and 70 controls were used for the study.

$$n = \frac{r+1}{r}\frac{\sigma^2\left(Z_\beta + Z_{\alpha/2}\right)^2}{d^2}$$

where

r = ratio of controls to cases = 1,

σ = standard deviation of the outcome variable[8] = 8.62,

Z_β = desired power of the study at 80% = 0.84,

$Z_{\alpha/2}$ = level of statistical significance at 5% = 1.96,

Mean serum level of IFNγ among the patients[8] = 19.25 pg/mL,

Mean serum level of IFNγ among the controls[8] = 14.95 pg/mL,

d = difference between the means (effect size) (19.25–14.95)[8] = 4.3,

$$n = \frac{(1+1)}{1}\frac{8.62^2(1.84+0.96)^2}{4.43^2} = 63$$

Study instrument and study procedure

Serum concentrations of the cytokines in theblood samples of the subjects were measured using the commercially available enzyme-linked immunosorbent assay (ELISA) kit, Assay MaxTM Human C-Reactive Protein (CRP), ELISA Kit (Catalog No. EC1001-7, www.assaypro.com). The kit uses a quantitative double-antibody sandwich enzyme-linked immunoassay technique according to the instructions of the manufacturer. It is designed for the detection of various cytokines but is limited to assays of pro-inflammatory cytokines (IL-2 and IFNγ) as well as anti-inflammatory cytokines (IL-4 and IL-13).

Required materials for the procedure such as micro Elisa strip plate, standard concentration, sample diluent, horseradish peroxidase conjugate reagent, wash solution, chromogen solutions A and B, stop solution, closure plate membrane as well as standard micro-plate reader, precision pipettes and disposable pipettes tips, and incubator set at 37°C were made available and checked properly for optimum functionality before the procedure.

About 50 mL of standards and controls were pipetted into the standard and control wells respectively according to the manual's protocol. Thereafter, 10 µL of each of the samples was pipetted into the sample wells diluted with 40 µL of sample diluent. A 100 µL of HPR conjugate reagent was then added into each well, covered with an adhesive strip, and incubated for an hour at 37°C. At the end of 60 min, each well was then aspirated of its contents and washed thoroughly with 400 µL of wash solution five times. Following the last wash, complete removal of the wash solution was ensured by inverting the micro-plate and blotting the remnants against a clean paper towel.

Following the above, 50 µL of chromogen solutions A and B were added sequentially into each well and a characteristic pinkish colour was noticed. The microplate and its contents were gently mixed and incubated for an additional 15 min at 37°C. At the end of the incubation, 50 µL of stop solution was then added into each well and the initial observed pinkish coloration changed into yellow. Finally, the microplate was inserted into the microplate reader at an optical density of 450 nm for 15 min to obtain the results.

Outcome variable

The outcome of threatened miscarriage could result in the resolution of the presenting symptoms and subsequent progression of pregnancy to up and beyond 13 weeks' gestation or progression to inevitable miscarriage.

Explanatory variables

The main explanatory variable was the inflammatory cytokines, the pro-inflammatory cytokines (IFNγ, IL-2), and the anti-inflammatory cytokines (IL-4, IL-13). Other explanatory variables considered include the sociodemographic characteristics such as age, occupation, husband's occupation, religion, education, tribe, and family setting; obstetric history such as gravidity, number of conceptions before 28 weeks, number of children alive, gestational age (GA) at recruitment, and GA at follow-up; clinical history such as bleeding per vagina, lower abdominal or back pain, drainage of fluid per vagina, overt smell and discharge per vagina, history of fever, previous history of threatened miscarriage, previous history of infertility, chronic medical condition, pelvic ultrasound scan done, ultrasound findings, and history of previous ectopic preterm delivery.

Statistical analysis

The descriptive statistics of sociodemographic characteristics, gynaecologic, and clinical (GC) history

Table 1: Descriptive statistics of the threatened pregnancy outcome

Variables	Pregnancy outcome		*P* value
	Aborted	Ongoing	
	n (%)	*n* (%)	
Baseline cytokine profiles			
IL-2 mean (SD)	1.78 (0.63)	1.10(0.52)	**<0.001**
IFNγ mean (SD)	257.45 (63.70)	197.24(52.37)	**<0.001**
IL-4 mean (SD)	10.34 (3.55)	7.95 (2.86)	**0.003**
IL-13 mean (SD)	733.35(58.09)	920.43(182.8)	**<.001**
Sociodemographic			
Age			0.883
<25	0 (0)	1 (2.5)	
25–29	10 (34.5)	11 (27.5)	
30–34	14 (48.3)	21 (52.5)	
35–39	4 (13.8)	5 (12.5)	
40–44	1 (3.4)	2 (5.0)	
Age mean (SD)	31.66 (0.726)	31.78 (0.675)	
Occupation			0.096
Student	7 (23.3)	4 (10.0)	
Unemployed	6 (20.0)	3 (7.5)	
Artisan/Trader	11 (36.7)	17 (42.5)	
Professional	6 (20.0)	16 (40.0)	
Husband occupation			0.113
Student	2 (6.9)	0 (0)	
Unemployed	2 (6.9)	0 (0)	
Artisan/Trader	12 (41.4)	21 (52.5)	
Professional	13 (44.8)	19 (47.5)	
Religion			0.445
Christianity	20 (66.7)	30 (75.0)	
Islam	10 (33.3)	10 (25.0)	
Education			0.608
Primary	0 (0)	1 (2.5)	
Secondary	7 (23.3)	10 (25.0)	
Tertiary	20 (66.7)	22 (55.0)	
Postgraduate	3 (10.0)	7 (17.5)	
Ethnicity			0.844
Yoruba	26 (86.7)	34 (85.0)	
Igbo/Hausa	4 (13.3)	6 (15.0)	
Family setting			0.125
Monogamous	30 (100.0)	37 (92.5)	
Polygamous	0 (0.0)	3 (7.5)	
Gynaecologic history			
Gravidity			0.251
One	9 (30.0)	18 (45.0)	
Two	11 (36.7)	8 (20.0)	
>Two	10 (33.3)	14 (35.0)	
Number of conception before 28 weeks			0.574
One	25 (83.3)	34 (85.0)	
Two	4 (13.3)	3 (7.5)	
Three	1 (3.3)	3n(7.5)	
Number of children alive			0.400
One	25 (83.3)	30 (75.0)	
More than one	5 (16.7)	10 (25)	
GA at recruitment (weeks)			0.745
6–6 + 6	2 (6.7)	2 (5.0)	
7–7 + 6	2 (6.7)	6 (15.0)	
8–8 + 6	11 (36.7)	13 (32.5)	
9–10 + 6	15 (50.0)	19 (47.5)	
GA at follow-up (weeks)			**<0.001**
7–9 + 6	0 (0.0)	25 (62.5)	
10 + 1–13	30 (100)	15 (627.5)	
Clinical history			
Bleeding per vagina			-
Yes	30 (100)	40 (100)	
No	0 (0.0)	0 (0.0)	
Lower abdominal or back pain			1.000
Yes	9 (30.0)	13 (32.5)	
No	21 (70.0)	27 (67.5)	
Drainage of fluid per vagina			0.429
Yes	1 (3.3)	0 (0.0)	
No	29 (96.7)	40 (100)	
Overt smell and discharge per vagina			0.307
Yes	3 (10.0)	1 (2.5)	
No	27 (90.0)	39 (97.5)	
History of fever			0.066
Yes	6 (20.0)	2 (5.0)	
No	24 (80.0)	38 (95.0)	
Previous history of threatened miscarriage			0.067
Yes	12 (40.0)	8 (20.0)	
No	18 (60.0)	32 (80.0)	
Previous history of infertility/subfertility			0.646
Yes	3 (10.0)	2 (5.0)	
No	27 (90.0)	38 (95.0)	
Chronic medical conditions like diabetesand hypertension			0.087
Yes	7 (23.3)	3 (7.5)	
No	23 (76.7)	37 (92.5)	
Pelvic ultrasound scan done?			–
Yes	30 (100.0)	40 (100.0)	
No	0 (0.0)	0 (0.0)	

Table 1: continued

Variables	Pregnancy outcome		*P* value
	Aborted	Ongoing	
	n (%)	*n* (%)	
History of previous ectopic preterm delivery, molar pregnancy, or foetal anomaly?			0.429
Yes	1 (3.3)	0 (0.0)	
No	29 (96.7)	40 (100.0)	

Table 2: Correlation between maternal serum level of inflammatory cytokines and the gestational age of threatened miscarriage

	Gestational age	
	Rho	*P* value
IL-2	0.10	0.40
IFNγ first sample	0.10	0.40
IL-4 first sample	0.10	0.42
IL-13 first sample	0.10	0.42

were computed for each of the threatened miscarriage outcomes (inevitable miscarriage and ongoing pregnancy) after 13 weeks of gestation. Chi-square test of association was used to examine the association between the outcomes and the demographic characteristics; clinical history and gynaecologic history. Independent Student's *t* test was used to compare the cytokine profiles in women with inevitable miscarriages and women with normal ongoing pregnancy after 13 weeks of gestation. Furthermore, four different Poisson regression models were obtained to investigate the factors associated with inevitable miscarriage. The first model explored the relationship between each of the cytokine profiles and the threatened miscarriage outcomes while the other models were adjusted for demographic characteristics, clinical history, and gynaecologic history respectively. The level of statistical significance was set at 5%.

Results

Table 1 shows the descriptive statistics of the participants with threatened pregnancy together with their sociodemographic characteristics and GC history. Of the 70 women with threatened miscarriages, 30 (42.86%) pregnancies resulted in inevitable miscarriage whereas 40 (57.14%) continued with the pregnancy. The average age of women with inevitable miscarriages and those with ongoing pregnancies was obtained to be approximately the same (32 years). The majority of the women with inevitable miscarriages (36.7%) and women with ongoing pregnancies (42.5%) were traders or artisans. No inevitable miscarriage was recorded for pregnancy from a polygamous family and the highest percentage (66.7%) of the participants with inevitable miscarriage had tertiary education.

About 83% of the participants had at least one conception before 28 weeks and at least one child alive. While all the women with threatened miscarriage had vagina bleeding, the majority of the participants who had inevitable miscarriages did not experience lower abdominal or back pain; drainage of fluid per vaginal; overt smell and discharge per vagina; history of fever, history of infertility and chronic medical conditions such as diabetes and hypertension.

The chi-square test of association between the pregnancy outcomes and the variables of interest revealed no significant association between the pregnancy outcomes and sociodemographic characteristics; GC history except for the GA at follow-up ($P < 0.001$).

Table 1 also shows the comparison between cytokines profile levels between the inevitable miscarriage and the ongoing pregnancy. Regarding the pro-inflammatory cytokines, the mean values of pro-inflammatory cytokines, IL-2($P < 0.001$) and IFNγ ($P < 0.001$) were significantly higher in threatened miscarriages that resulted in an inevitable miscarriage than those that resulted in an ongoing pregnancy. Similarly, there were significant differences in the mean values of the anti-inflammatory cytokines, IL-4 ($P = 0.003$) and IL-13 ($P < 0.001$) between threatened miscarriages that resulted in an inevitable miscarriage and those that resulted in ongoing pregnancy.

Table 2 shows the relationship between the maternal serum level of selected inflammatory cytokines and the gestational age of threatened abortion using Pearson correlation. A weak positive correlation existed between gestational age and each inflammatory cytokine.

Table 3 shows the incidence rate ratio of pregnancy resulting in inevitable miscarriage obtained using the Poisson regression with robust variance. Model 1 shows the effect of each baseline cytokine profile while models 2 to 4 provide the rate ratio after adjusting for sociodemographic characteristics, gynaecology, and clinical history respectively. For every unit increase in IL-2 profile, the rate of inevitable miscarriage increased by 31% (IRR = 1.31, 95% CI: 0.98–1.76). There was a 5% increase in the rate of inevitable miscarriage for every unit increase in the IL-4 profile (IRR = 1.05, 95% CI: 1.00–1.09). Both the IFNγ and IL-13 profiles showed no significant change in the rate of inevitable miscarriage (IRR = 1.00, 95% CI: 0.99–1.01) and (IRR = 1.00, 95% CI: 1.00–1.003) respectively. The behaviour of the IFNγ and IL-13 cytokine profiles remained the same when adjusted for sociodemographic characteristics, gynaecology, and clinical history. The rate of inevitable miscarriage increased by 16% (IRR = 1.16, 95% CI: 0.58–2.32) for a unit increase in IL-2 and by 25% (IRR = 1.25, 95% CI: 1.09–1.43) when

Table 3: Poisson regression of threatened pregnancy outcome with adjusted variables

Variables	Model 1	Model 2	Model 3	Model 4
Baseline cytokine profiles				
IL-2	1.31 (0.98–1.76)	1.36 (0.93–2.00)	1.29 (0.78–2.15)	1.16 (0.58–2.32)
IFNγ	1.00 (0.99–1.01)	1.00 (0.99–1.01)	1.00 (0.997–1.008)	1.00 (1.00–1.007)
IL-4	1.05 (1.00–1.09)	1.05 (0.99–1.12)	1.10 (1.02–1.18)	1.25 (1.09–1.43)
IL-13	1.00 (1.00–1.003)	1.00 (1.00–1.004)	1.00 (1.00–1.004)	1.00 (1.00–1.005)
Sociodemographic				
Age				
Less than or equal to 30		1	1	1
Greater than 30		0.65 (0.36–1.18)	0.76 (0.36–1.61)	0.97 (0.46–2.04)
Occupation				
Unemployed		1	1	1
Artisan/Trader		0.68 (0.34–1.35)	0.46 (0.26–0.83)	0.57 (0.26–1.23)
Professional		0.52 (0.23–1.19)	0.36 (0.15–0.90)	0.38 (0.14–1.01)
Husband occupation				
Unemployed		1	1	1
Artisan/trader		0.55 (0.11–2.85)	0.35 (0.11–1.10)	0.12 (0.01–1.48)
Professional		0.76 (0.13–4.54)	0.44 (0.12–1.60)	0.10 (0.01–1.90)
Religion				
Christianity		1	1	1
Islam		1.48 (0.88–2.46)	1.60 (0.98–2.59)	2.37 (1.17–4.80)
Education				
Secondary and below		1	1	1
Tertiary		1.58 (0.48–5.17)	1.30 (0.52–3.21)	2.64 (1.00–6.98)
Tribe				
Yoruba		1	1	1
Igbo/Hausa		0.80 (0.32–2.01)	0.81 (0.33–2.00)	1.53 (0.51–4.62)
Family setting				
Monogamous		1	1	1
Polygamous		0	0	0
Gynaecologic history				
Gravidity				
One			1	1
Two			0.91 (0.41–2.05)	0.99 (0.43–2.29)
>Two			0.88 (0.31–2.47)	0.49 (0.16–1.56)
Number of conception before 28 weeks				
One			1	1
Two			1.87 (0.64–5.42)	2.08 (0.48–9.15)
Three			3.54 (0.56–22.36)	4.54 (0.62–33.35)
Number of children alive				
One			1	1
More than one			0.53 (0.16–1.71)	1.14 (0.28–4.71)
GA at recruitment (weeks)				
6–6 + 6			1	1
7–7 + 6			0.17 (0.04–0.70)	0.02 (0.002–0.26)
8–8 + 6			0.84 (0.32–2.21)	0.89 (0.17–4.61)
9–10 + 6			0.54 (0.21–1.40)	0.54 (0.13–2.24)
Clinical history				
Lower abdominal or back pain				
No				1
Yes				1.20 (0.47–3.08)
Drainage of fluid per vagina				
No				1
Yes				0.05 (0.002–1.01)

Table 3: Continued

Variables	Model 1	Model 2	Model 3	Model 4
Overt smell and discharge per vagina				
No				1
Yes				0.17 (0.04–0.71)
History of fever				
No				1
Yes				1.30 (0.34–4.97)
Previous history of threatened miscarriage				
No				1
Yes				2.09 (0.71–6.14)
Previous history of infertility/ subfertility				
No				1
Yes				0.36 (0.09–1.50)
Chronic medical conditions like diabetes and hypertension				
No				1
Yes				1.34 (0.31–5.72)
History of previous ectopic preterm delivery, molar pregnancy, or foetal anomaly?				
No				1
Yes				8.56 (1.28–57.20)

adjusted for sociodemographic characteristics, gynaecology, and clinical history.

Receiver operating characteristics curve of cytokines and risk of inevitable miscarriage

Figure 1 depicts the receiver operating characteristic curve for IL-2, IFNγ, IL-4, and IL-13 in predicting the risk of inevitable miscarriage. Area under curve for IL-2 was 0.83 (95% CI: 0.72–0.93), IFNγ was 0.78 (95% CI: 0.66–0.89), IL-4 was 0.70 (95% CI: 0.58–0.83) and IL-13 was 0.81 (95% CI: 0.70–0.93) among the pregnant women. IL-2 was observed to be the best biomarker that predicts the risk of inevitable miscarriage, with the area under the curve of 0.83((95% CI: 0.72–0.93). A cut-off point of 1.30 pg/mL for IL-2 gave a sensitivity of 80% and a specificity of 70%.

Discussion

The study estimated the maternal serum levels of inflammatory cytokines that can predict the risks of inevitable miscarriage. This study revealed IL-2 to be the best biomarker for predicting the outcome of a threatened miscarriage with a cut-off point of 1.30 pg/mL. Elevated levels of IL-2 had been reported in spontaneous miscarriage leading to the conclusion that successful pregnancy is associated with significantly lower concentrations of IL-2 receptor. Gucer *et al*.[11] in their study reported an increase in IL-2 receptor levels in threatened miscarriage with bad outcomes compared to normal pregnancy. IL-2 has a deleterious effect on pregnancy which induces several cytotoxic and inflammatory reactions via cell-mediated immunity against conceptus and has been reported to have an abortogenic effect.[3,5,12]

We assessed the relationship between maternal serum level of inflammatory cytokines and gestational age of threatened miscarriage. Findings showed a weak positive correlation between gestational age and the cytokines profiles although not statistically significant. Our finding was similar to a study by Maduka and Uzoho[13] that showed no association between the gestational age and cytokine biomakers. The findings from our study should be interpreted with caution because the women were not followed up until delivery. A longer duration before conducting the follow-up would have given a better and more reassuring result and causal relationship.

The pro and anti-inflammatory cytokines were investigated between the participants with inevitable miscarriage and those with ongoing pregnancy. The IL-2 and IFNγ were significantly higher among women with inevitable miscarriages than those with ongoing pregnancies. Similarly, IL-4, an anti-inflammatory cytokine was significantly higher in the samples of women who had threatened miscarriage but resulted in inevitable miscarriage. Regarding IL-13, we observed an inverse response where participants with higher values had threatened miscarriage but progressed with their pregnancy when compared with IL-4. However, previous studies in the literature on anti-inflammatory cytokines showed that this cytokine supports pregnancy.[1]

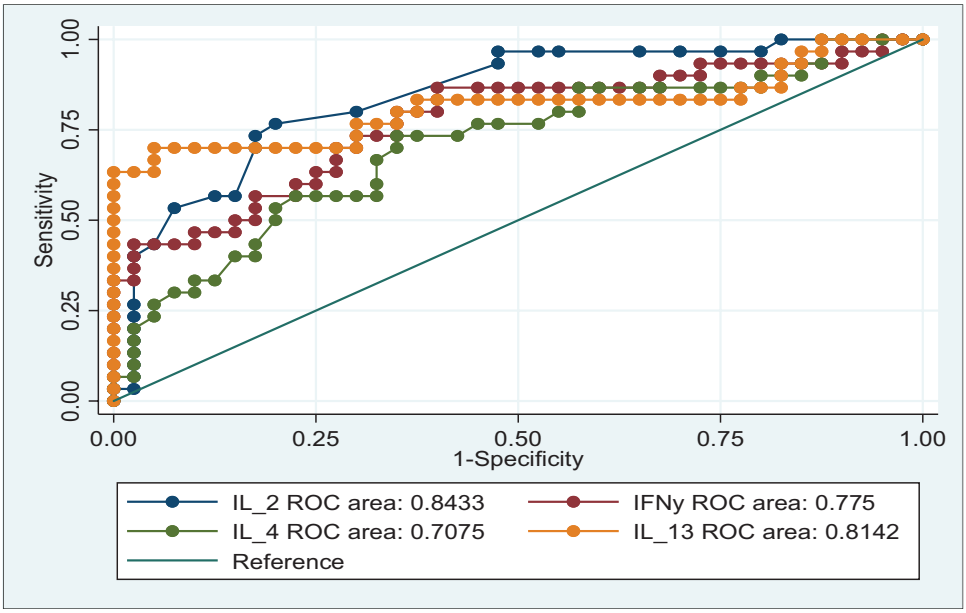

Figure 1: ROC for selected cytokine profiles in predicting inevitable miscarriage

The probable reason for this could be due to recurrent miscarriages. Recurrent spontaneous miscarriage is associated with a higher concentration of Th1 cytokines in the first trimester.[12] Another reason could be the production of IL-4 from other biologic activities other than in pregnancy like allergies.[14]

To our knowledge, this is probably the first study in Nigeria that explored the effect of pre- and anti-inflammatory cytokines in predicting the outcomes of threatened pregnancy. The participants were recruited from two major tertiary healthcare centres that have a common protocol for diagnosing and treating threatened miscarriages. Both facilities also have a basic laboratory that can provide results to make diagnoses of common medical conditions that are associated with threatened miscarriage. However, the follow-up sample was collected at a short interval of time. We collected samples at the end of the first trimester (13th week of gestation). Other possible causes of spontaneous miscarriage such as genetic factors were not considered in the study. The effects of inflammatory markers on pregnancy from conception to delivery should be examined in further research.

It is evident from our study that baseline pro-inflammatory cytokines (IL-2 and IFNγ) are significantly higher among women with threatened miscarriages that resulted into inevitable miscarriages than those that resulted into ongoing pregnancy. Similarly, the baseline anti-inflammatory cytokine (IL-4) is significantly higher among women with inevitable miscarriages than those with ongoing pregnancy while IL-13 is higher among women who had threatened miscarriage but continued with their pregnancy after the first trimester. The IL-2 is the best biomarker in predicting the outcome of a threatened pregnancy with a sensitivity of 80% and a specificity of 70% at 1.30 pg/mL cut-off point. Also, there was a weak positive correlation between the women's gestational age and the cytokine profiles. We recommend more studies with larger sample sizes and longer follow-ups to further understand the biological association of these cytokines with pregnancy outcomes.

Ethical consideration

Ethical approval was obtained from the UI/UCH Institute for Advanced Medical Research and Training Review Committee with reference number UI/EC/19/0029. Informed consent was taken from participants to ensure voluntary participation. The participants also consented to use their data for publication. All information obtained in the study was given code numbers and no name was recorded.

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Conflicts of interest

There are no conflicts of interest.

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