

# Serum Cytokine Profile (Interleukin-6) among Women with Pelvic Organ Prolapse

Kenneth C. Ekwedigwe<sup>1</sup>, Ileogben Sunday-Adeoye<sup>1</sup>, Monday O. Eliboh<sup>1</sup>, Maradona E. Isikhuemen<sup>1,2</sup>, Henry Uro-chukwu<sup>1</sup>, Paul Ezeonu<sup>3</sup>

<sup>1</sup>National Obstetric Fistula Centre, <sup>2</sup>Federal Teaching Hospital, Abakaliki, <sup>3</sup>Department of Obstetrics and Gynaecology, University of Benin Teaching Hospital, Benin City, Nigeria

## Abstract

**Background:** Pelvic organ prolapse (POP) is a common gynecological problem, particularly in the grand multipara. There are indications that serum concentration of cytokines is higher in women with POP and even more so when there is evidence of infection. This study assesses the serum cytokine level (interleukin [IL]-6) in women with POP. **Materials and Methods:** This study was conducted among 96 women with POP and a control group of 96 women. A case-control study using a quota system nonprobability sampling technique was done. The serum cytokine level was determined using a commercial standard enzyme-linked immunosorbent assay kit. **Results:** The mean age and parity were  $53.54 \pm 10.1$  years and  $7.04 \pm 2.33$ , respectively. The mean level of serum IL-6 and standard error of mean was  $95.79 \pm 18.6$ ,  $\pm 1.9$  as against  $17.92 \pm 7.62$ ,  $\pm 0.78$  for control and as against  $<20$  pg/ml for the general population. *P* values were 0.00 and 0.08, respectively. The result showed that IL-6 was significantly increased in women with POP. **Conclusion:** This study suggests that cytokine levels were significantly elevated in patients with POP.

**Keywords:** Cytokines, interleukin-6, pelvic organ prolapse

## INTRODUCTION

Pelvic organ prolapse (POP) is the herniation of the pelvic organs from its anatomical confines.<sup>[1]</sup> It is of considerable importance to the practising gynecologist in the middle- and low-income countries.<sup>[2-7]</sup> Poor conduct of labor has been implicated as a risk factor. POP is also common in conditions of chronically raised intra-abdominal pressure, which include chronic obstructive airway diseases, obesity, abdominal tumors, straining during defecation, and heavy physical exertion.<sup>[2-4,8]</sup> It is commonly associated with urinary tract infection (both symptomatic and asymptomatic) due to anatomical and physiological changes.<sup>[1]</sup>

There are indications that serum concentration of cytokines is higher in women with POP and even more so when there is evidence of infection.<sup>[9,10]</sup> Cytokines are proteins and peptides, secreted by cells, which act as humoral regulators to modulate the intercellular actions of the cell.<sup>[9]</sup> These actions include paracrine effects, such as the promotion of maturation of B-cells progenitors by interleukin (IL)-7, and endocrine effects such as acute-phase response during inflammation are produced

by IL-1 and tumor necrotic factor (TNF).<sup>[10]</sup> Cytokines also induce autocrine effects.<sup>[10]</sup>

There are a number of pro- and anti-inflammatory cytokines and chemokines. They are extremely potent, versatile, pluripotent mediators of an immense array of reactions ranging from induction of normal immune responses, rejection of allografts, autoimmune diseases, and hypersensitivity reactions. Cytokines are produced in all tissues of the body, and currently, their study has received much attention from reproductive immunologists.<sup>[11]</sup>

The pro-inflammatory cytokines include IL-6, TNF- $\alpha$ , IL-1, IL-2, IL-8, IL-12, IL-18, IL-16, and IL-15,<sup>[12-18]</sup> while

**Address for correspondence:** Dr. Maradona E. Isikhuemen, Department of Obstetrics and Gynaecology, University of Benin Teaching Hospital, Benin City, Nigeria.  
E-mail: maradona4real2002@yahoo.com

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the anti-inflammatory cytokines include IL-4, IL-5, and IL-10.<sup>[19,20]</sup>

Anti-inflammatory cytokines: IL-10 is a homodimeric protein and anti-inflammatory cytokine with subunits, which have a length of 160 amino acids. IL-10 is secreted by T-lymphocytes and monocytes following cell activation by bacterial lipopolysaccharides. IL-10 downregulates Th1 helper T-cell function by inhibiting cytokine production by the macrophage.<sup>[15]</sup> IL-4 is originally called B-cell-stimulating factor, and it is a protein of 129 amino acids, which is secreted mainly by the Th2-lymphocytes but also by the non-T/non-B-cells of most cell lineage.<sup>[20]</sup>

Studies on POP are available in Nigeria. However, there is a paucity of data if any on cytokine and POP in Nigeria and the world at large. The superiority of cytokines than traditional tests such as histology, microbial culture, and C-reactive protein, as an indicator of the inflammatory and infective process, makes this study necessary.<sup>[21]</sup>

Our aim is to assess serum cytokine (IL-6) levels in women with POP.

## MATERIALS AND METHODS

The study was conducted at the National Obstetric Fistula Centre, Abakaliki, Nigeria. Abakaliki is the state capital of Ebonyi State. It was a case-control intervention study with the aim of assessing IL-6 in patients with POP between June 2014 and July 2015. The study considered only patients with POP, while patients with medical conditions and those who refused to give consent were excluded from the study.

The sample size was calculated by a statistical formula based on the prevalence of 2.1%–3.91%<sup>[3,22-24]</sup> for POP and a confidence level set at 95% with an error margin of 0.05.

Where,

$$N \text{ is the sample size } N = \frac{PQ}{(E/1.96)^2}$$

1.96 is a known constant (standard normal deviation corresponding to 95% confidence level).

P is the maximum known prevalence of the disease = 3.91%

Q is 1 – P (proportion of the persons free from the disease) = 0.962

E is the error margin = 0.05

$$N = \frac{3.91\% (1 - 0.3.91\%)}{(0.05 / 1.96)^2}$$

= 0.0376 / 0.000651 calculated minimum sample size = 57.7.

One hundred ninety-two patients were recruited for the study into two groups. This implies 96 cases and 96 controls.

The patients in the case group were those with POP, while those in the control group were without POP. Both case and control were age and parity matched. Nonprobability quota

sampling method was used. Questionnaires were used to obtain patients' information.

Venous blood of about 3–5 ml was collected from each patient by venepuncture technique and introduced into a sterile container. The blood sample was processed, and the serum was subjected to cytokine determination using standard commercial enzyme-linked immunosorbent assay kits obtained from Abcam, UK, according to the manufacturer's instruction.<sup>[25]</sup>

Collected quantitative data were entered and analyzed using IBM SPSS Statistics for Windows, version 20 (IBM Corp., Armonk, N. Y., USA).

Ethical approval was obtained from the Ethics and Research Committee of the National Obstetric Fistula Centre, Abakaliki, before the commencement of the study. Individual informed consent was attached to each questionnaire and the respondents gave consent before the questionnaires were filled.

## RESULTS

A total of 192 patients participated in this study, 96 cases and 96 controls. The findings of this study are presented as follows:

**Table 1: Sociodemographic variables**

		CASE		CONTROL	
		n=96	Percent	n=96	Percent
Age	20-29	2	2.1	2	2.1
	30-39	8	8.3	8	8.3
	40-49	38	39.6	38	39.6
	50-59	28	29.2	38	29.2
	60-69	18	18.8	18	18.8
Age	70-79	2	2.1	2	2.1
Mean=53.54+10.1 S.E. +1.03 x2=16.035 df=15 p=0.276					
Parity	1	0	0	2	2.1
	2-4	11	11.5	10	10.4
	5-8	53	55.2	52	54.2
	>8	32	33.3	32	33.3
Mean=7.04+2.33 S.E. +0.24 x2=22.874 df=6 p<0.00					
Tribe	IGBO	96	100.0	96	100.00
	CHRISTIANITY	92	95.8	96	100.00
Religion	ATR	4	4.2	0	0.00
	MARRIED	72	75.0	94	97.9
	SINGLE	2	2.1	2	2.1
Marital Status	DIVORCED	2	2.1	0	0.00
	WIDOW	20	20.8	0	0.00
	NO FORMAL EDUCATION	82	85.4	26	27.1
Level of Education	PRIMARY	12	12.5	46	47.9
	SECONDARY	0	0.00	24	25.0
	TERTIARY	2	2.1	0	0.00
	UNEMPLOYED	26	27.1	36	37.5
	UNSKILLED	68	70.8	60	62.5
Occupation	PROFESSIONAL	2	2.1	0	0.00

**Table 2: Mean, Standard Deviation, standard error of mean and p-values of serum cytokine levels in cases of POP compared with control**

	IL-6 case results	IL-6 control results
Mean	95.79	17.92
Std. Error of Mean	1.90	0.78
Std. Deviation	18.62	7.62
Range	78	32
Minimum value (pg/ml)	62	6
Maximum value (pg/ml)	140	38
Cut of value for normal population is <20pg/ml <sup>13,14</sup>		

**Table 3: Serum Interleukin 6 levels**

	IL-6 (pg/ml) case		IL-6 (pg/ml) control	
	n=96	percent	n=96	Percent
0-9	0	0	8	8.3
10-19	0	0	54	56.3
20-29	0	0	22	22.9
30-39	0	0	12	12.5
40-49	0	0	0	0
50-59	0	0	0	0
60-69	8	8.3	0	0
70-79	14	14.6	0	0
80-89	16	16.7	0	0
90-99	22	22.9	0	0
100-109	10	10.4	0	0
110-119	16	16.7	0	0
120-129	2	2.1	0	0
130-139	6	6.3	0	0
140-149	2	2.1	0	0

Cut of value for normal population is <20pg/ml<sup>13,14</sup>

The mean age and parity, as shown in Table 1, were 53.54 ± 10.1 yearas and 7.04 ± 2.33, respectively. All women were Ibo and the majority were Christians. Majority of the women in both the groups were married.

The mean serum IL-6 level and standard error of mean were 95.79 ± 18.6, ±1.9 for cases and 17.92 ± 7.62, ±0.78 for the control, respectively. This is shown in Table 2. The minimum and maximum values of IL-6 for the case were 62 pg/ml and 140 pg/ml, respectively, while those of control were 6 pg/ml and 38 pg/ml, respectively. The cutoff value for the normal population was <20 pg/ml.

From this study, majority of the respondents used as control (64.6%) had a normal level of IL-6, while all the patients (100%) used as the case had markedly elevated levels of IL-6, as shown in Table 3.

The standard IL-6 curve was drawn as above and used to find the appropriate concentrations of IL-6 from the [Figure 1] optical density as measured with spectrophotometry.

From this study, IL-6 values progressively increased as the age of the respondents increased, as shown in Table 4. The

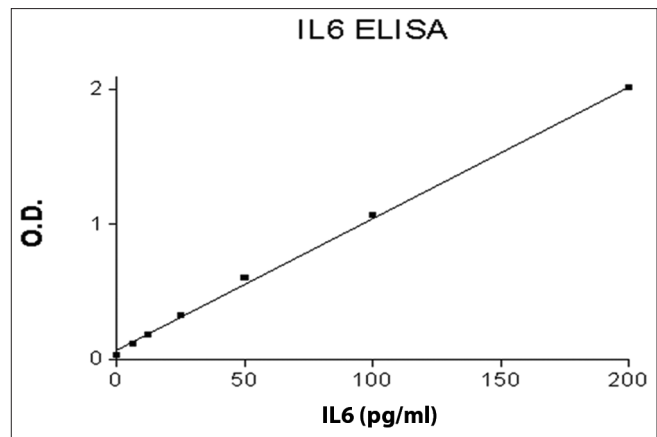


Figure 1: IL-6 Standard curve ranging from 6.25 to 200

association between the ages of the patients and IL-6 values was statistically significant ( $P = 0.004$ ).

## DISCUSSION

The age group 40–49 years and 50–59 years constituted the greatest percentage of the patients (25%) and (33.3%), respectively, with POP, and the mean age was 52.79 years with a standard deviation of 10.84 years. This is similar to the studies done in Lagos,<sup>[2]</sup> Enugu,<sup>[3]</sup> India,<sup>[4]</sup> Ibadan,<sup>[6]</sup> and Nnewi.<sup>[26]</sup> This could be due to a reduction in the production of female hormones, especially estrogen, during the perimenopausal and menopausal age range and the subsequent effect of these reduced hormones in the pelvic supporting structures,<sup>[27]</sup> second, most women in this age range are multipara and its attendant effect on the ligaments and other pelvic supporting organs.<sup>[2,9,28]</sup> Majority of the women were grand multiparous (that is 5 or more deliveries), with the highest value being para 6 (18.8%). The mean parity was 6.74, with a standard deviation of 2.27. This is also similar to studies done in Lagos,<sup>[2]</sup> Enugu,<sup>[3]</sup> India,<sup>[4]</sup> Ibadan,<sup>[6]</sup> Nnewi,<sup>[26]</sup> and Benin<sup>[29]</sup> where the majority of the women were grand multiparous, and the mean parities were 6 and above. The explanations are that pregnancy increases the intra-abdominal pressure and puts a lot of tension on the pelvic ligaments, fascia, muscles, and other supporting structures which tend to support pregnancy. Bearing down in the second stage of labor further puts even much more tension on these pelvic supporting structures, thereby predisposing these women to POP.<sup>[1]</sup> Increased number of pregnancies will cause repetition of these events; thus, the higher the number of pregnancies, the higher the risk of developing POP.<sup>[1,5]</sup> Majority of the women were married (78.1%). This is because, in the study area, a lot of emphasis and attachment are given to marriage before getting pregnant. Majority of the respondents were uneducated (84.4%). This is similar to the a study done in Enugu, South East Nigeria<sup>[3]</sup> where only 2% of the respondents had a tertiary level of education.

Most of the patients were unskilled laborers (67.7%), and as much as (24%) of them were unemployed. A previous study in Ibadan has reported this disease to be predominant in a

**Table 4: Association between Serum Interleukin-6 and age of patients.**

AGE	IL-6pg/ml					TOTAL (%)
	20-39 (%)	40-59 (%)	60-79 (%)	80-99 (%)	>99(%)	
20-29	0(0.0)	2(100)	0(0.0)	0(0.0)	0(0.0)	2(2.1)
30-39	1(7.7)	7(53.8)	0(0.0)	5(38.5)	0(0.0)	8(8.3)
40-49	0(0.0)	4(16.7)	2(8.3)	6(25.0)	12(50.0)	38(39.6)
50-59	1(3.1)	3(9.4)	8(25)	4(12.5)	16(50)	28(29.2)
60-69	0(0.0)	2(10)	2(10)	8(40)	8(40)	18(18.8)
70-79	0(0.0)	0(0.0)	0(0.0)	1(33.3)	2(66.7)	2(2.1)
$\chi^2 = 46.754$ , $df=24$ , $p=0.004$ TOTAL	2(2.1)	18(18.8)	12(12.5)	26(27.1)	38(39.5)	96(100)

low-income setting.<sup>[6]</sup> This may be the reason why they could not assess health care early enough for prompt management of their ailment.

The level of IL-6 was  $95.8 \pm 18.7$  pg/ml. This value is very high compared to the cutoff value of  $<20$  pg/ml for the normal population.<sup>[9,10]</sup> In a study among children with UTI and asymptomatic bacteriuria conducted in Sweden, the serum IL-6 level was 30 pg/ml, while the urinary IL-6 was 39 pg/ml.<sup>[9]</sup> These values were high though lower than the value from this study. This could be due to other comorbidities in our environment, such as increased incidence of pelvic inflammatory diseases among our women; others include sexually transmitted infections, endometritis, and pelvic tumors. A study done in Turkey on serum, and urinary IL-6 level on patients undergoing short-wave lithotripsy found no statistically significant difference between the serum and urinary level, pre- and post-procedure,<sup>[10]</sup> while a study done in Sweden on cytokine levels in elderly patients with acute cystitis and asymptomatic bacteriuria showed elevated IL-6 levels among patients with asymptomatic bacteriuria as well as acute cystitis.<sup>[11]</sup> It is worthy of note that these studies were carried out in conditions other than POP.

From this study, IL-6 values progressively increased as the age of the respondents increased; it also increased with increasing parity, with more than 55% of patients who were para 7 and above having extreme values of IL-6. This could be explained by a wide spread cellular presence of IL-6 in the epithelial cells and the effect of aging on these tissues.<sup>[10]</sup> Extreme values of IL-6 level were found among patients without formal education (41.9%) compared to those with part and full primary education (22.2%) and (33.3%), respectively. High values were also found among patients with a middle level of occupation (50%).<sup>[10]</sup> These could be explained because ignorance and poverty are both end-products of no education and unemployment and both are risk factors for infectious diseases; consequently, IL-6 being a pro-inflammatory and regulatory hormone is elevated.<sup>[11]</sup>

## CONCLUSION

The average level of IL-6 in POP patients from this study was rather very high compare to the cutoff value of  $<20$  pg/ml for the general population.

This study was very revealing and was able to show that majority of the patients with POP had elevated pro-inflammatory cytokines (IL-6).

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## Conflicts of interest

There are no conflicts of interest.

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