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Cervicovaginal Inflammatory Cytokines, Obesity and Inter-Pregnancy Interval Negatively Affect Pregnancy Duration in Pregnant Women at High-Risk for Recurrent Spontaneous Preterm Birth

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Abstract

Objectives: Evaluation of change of cervicovaginal fluid (CVF) cytokines' levels during pregnancy and its relation to incidence of preterm birth (PTB). **Patients & Methods:** Pregnant women with history of PTB and cervical length < 25 mm (Study group) and 42 normal pregnant women (Control group) gave CVF sample (Sample-I) at start of second trimester. Study women undertook McDonald cervical cerclage (CC) and Sample-II of CVF was obtained at time of labor or removal of cerclage stitch. ELISA estimation of CVF tumor necrosis factor-α (TNF-α) and interleukins (IL)-6 and -10 levels. Study outcomes included differences in cytokines' levels between samples and groups. **Results:** Sample-I cytokines' levels were significantly higher in study than control women. Cytokines' levels in Sample-II were significantly higher in control, while were significantly lower in study women compared to Sample-I. Sixteen study women had PTB and had significantly higher CVF levels of IL-10 and TNF-α estimated in both samples than women had no PTB. Pregnancy duration was negatively correlated with maternal body mass index (BMI) and cytokines' levels, while was positively correlated with inter-pregnancy interval (IPI). Cytokines' levels were positively correlated with BMI and negatively correlated with IPI. Short IPI and high TNF-α levels are negative predictors for pregnancy duration. **Conclusion:** High BMI, short IPI and high CVF inflammatory cytokines' levels negatively affect pregnancy duration especially in women with history of recurrent PTB. Early prophylactic CC for women at high-risk of SPTB can modulate local immune disturbance, reduce incidence of SPTB and prolong pregnancy duration.

Keywords

Preterm Birth, Inter-Pregnancy Interval, Cervicovaginal Fluid, Cytokines, Body Mass Index, Cervical Cerclage

1. Introduction

Maternal immune system regulation is crucial for maintenance of a healthy pregnancy and fetal development [1]. These processes depend on the ability of the mother's immune system to adapt in order to tolerate the fetus through the process of immunoregulation [2]. Transition from an anti- to pro-inflammatory state in the mother and at the maternal-fetal interface may underlie the pathophysiology of preterm birth (PTB) [3].

Global prevalence of preterm birth (PTB) ranges between 5% and 18% [4] and spontaneous preterm birth (SPTB) accounts for 35% - 45% of all PTB cases [5]. Preterm infants, defined as infants who were born before <37 gestational weeks (GW), are at increased risk of morbidity and mortality and long-term disabilities [4].

The cervicovaginal (CV) space is metabolically active during pregnancy [6] and proteins in cervicovaginal fluid (CVF) are involved in immune regulation [7]. Thus, CVF is considered as a valuable source of information about female reproductive tract in pregnant and non-pregnant women [8]. Moreover, changes in CVF constituents may be observed early in pregnancy prior to any clinical symptoms [6] and these changes alone or in combination with clinical risk factors may help to identify women most at risk of PTB [7].

Cervical fetal fibronectin, α-fetoprotein, C-reactive protein (CRP) and interleukin 6 (IL-6) and cervical length can have an overall good diagnostic accuracy in identifying pregnancies at risk of SPTB [9]. However, these parameters are not effective for predicting the risk of SPTB in asymptomatic women [10]. One the other hand, the reported association between the changes in the levels of IL-6 and IL-10 early in pregnancy and decreased cervical length, suggests an imbalance of immune regulation that could impact cervical length [11].

1.1. Objectives

The current study aimed to evaluate the change of CVF cytokines' levels during pregnancy and the relation between the extent of this change and incidence of SPTB in women at high-risk for recurrent SPTB in comparison to normal pregnant women.

1.2. Design

Comparative prospective multicenter interventional study.

1.3. Setting

Gynecology & Obstetrics Department at Benha University Hospital in conjunc-

tion with multiple private Obstetric centers and Medical Biochemistry Department, Faculty of Medicine, Benha University.

2. Patients & Methods

All women attending the Antenatal Care Unit for assurance of being pregnant were eligible for evaluation. Clinical evaluation and abdominal ultrasonographic examination was performed and women were asked to attend the clinic at start of the 2nd trimester for re-evaluation, giving CVF samples for lab examinations (Sample 1) and transvaginal ultrasonographic examination for estimation of cervical length (CL) and women with CL < 25 mm were considered at a risk for having PTB [12] and undertake cervical cerclage (CC), irrespective of having history of previous PTB.

Exclusion criteria included multiple pregnancy, fetal or uterine anomalies, history of recurrent early pregnancy loss, cervical surgeries, previous pregnancy-induced complications, manifest diabetes mellitus, autoimmune diseases, current manifest vaginal infection were excluded from the study. Also, women refused to sign the consent for study participation, lost during follow-up or gave birth outside the participating centers were excluded from the study.

Inclusion criteria included singleton fetus, attendance to OPC at beginning of the 2nd trimester, absence of exclusion criteria and signing a written consent. Women had previous SPTB were eligible for evaluation and those at high-risk for recurrent PTB were included as Study group and women free of history of SPTB and had CL > 25 mm at the beginning of 2nd trimester as Control group which must include women of cross-matched age, gestational age (GA) and body mass index (BMI) in number equal to women of study group. The study protocol was approved by the Local Ethical Committee and all enrolled women signed written fully informed consent. All women of study and control groups were maintained on vaginal toilet to guard against development of infection and were asked to attend the outpatient clinic biweekly for evaluation for development of PTB, which was defined as any birth before 37 weeks completed weeks of gestation [4] [13]. At time of labor or removal of the CC suture, another CVF samples were obtained (Sample II).

2.1. Methods

2.1.1. CVF Sample Obtaining and Processing

Vaginal speculum was applied and to obtain high vaginal smear of CVF a Dacron swab was placed in posterior vaginal fornix, maintained in situ for 10 seconds to achieve saturation, and then was transferred into 750 ml of standard phosphate-buffered saline solution mixed with freshly prepared protease inhibitor solution. The swab was then removed, placed in a clean tube, vortexed for 10 sec and centrifuged at 2500 g for 10 minutes, at 4˚C and the resulting fluid was collected and added to the fluid in the original tube, well-mixed and centrifuged for a further 10 minutes to remove cell debris. Cell-free supernatants were collected and divided into aliquots and stored at −80˚C until being ELISA assayed.

2.1.2. Investigations

CVF levels of tumor necrosis factor-α (TNF-α), IL-6 and IL-10 were measured using ELISA kits according to the manufacturer's instructions and were read using a 96 well microplate ELISA reader (Dynatech, MR 7000).

1) Human TNF-α was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab179886, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique [14].

2) IL-6 with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46042, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique [15].

3) Serum IL-10 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46034, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique [16] (Poll, 1996).

2.1.3. Cervical Cerclage

McDonald cervical cerclage (CC) was performed using within 4 days after obtaining CVF sample in women with CL < 25 mm. CC was performed using the McDonald procedure with a non-absorbable suture. After performing CC, women were asked to avoid any sexual activity, use of tampons or douching, prolonged standing for >4 h, heavy physical work, lifting heavy weights, straining or any activity that brings on symptoms of pelvic pressure or discomfort.

2.2. Study Outcomes

1) Primary outcome: the difference in cytokines' levels between the two obtained samples.

2) Secondary outcomes included:

a) The difference in cytokines' levels between study and control women;

b) The frequency of PTB among women of both groups;

c) The value of Sample I cytokines' levels for prediction of PTB.

2.3. Statistical Analysis

Obtained data were presented as mean ± SD, numbers and percentages. Results were analyzed using paired t-test, One-way ANOVA Test and Chi-square test (χ^2 test). Possible relationships were investigated using Pearson linear regression analysis. Regression analysis (Stepwise method) was used for stratification of studied parameters as specific predictors. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015; IBM, South Wacker Drive, Chicago, USA) for Windows statistical package. P value < 0.05 was considered statistically significant.

3. Results

During the duration of study since June 2018, 67 pregnant women with history of SPTB were evaluated; 25 women were excluded and 42 women were included as study group (**Figure 1**). Another 42 normally pregnant women free of history

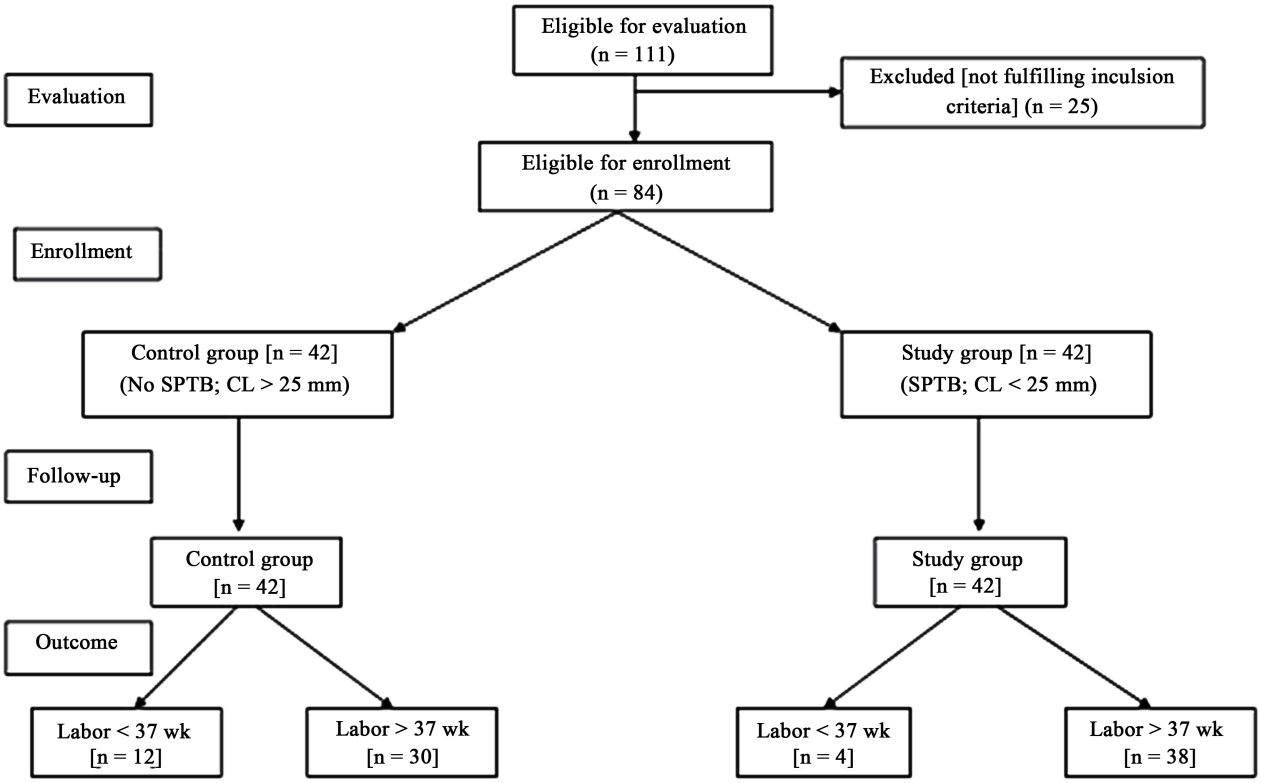

Figure 1. Shows consort flow sheet.

of SPTB and had CL > 25 mm was enrolled as control group. Enrollment data of women of both groups showed non-significant (P > 0.05) differences except for the inter-pregnancy interval that was significantly (P = 0.005) longer in control women than study women (Table 1).

Throughout the study duration, 4 study women (4.8%) had labor at GA < 34 GW, 12 women (14.3%) had labor at GA in range of >34 and <37 GW and the remaining 68 women (80.9%) had labor at GA > 37 GW with significantly higher frequency of women had labor at GA < 37 GW among study women. Mean duration of pregnancy of control women (38.8 ± 1.3 wk) was significantly longer than that of study women (37.2 ± 1.8 wk) (Table 2).

Estimated cytokines' levels in CVF samples obtained from control women were significantly higher in Sample II than in Sample I as it was elevated up to 146%, 120% and 132% for IL-6, TNF-α and IL-10, respectively of its levels in Sample I. On the other hand, estimated IL-6, TNF-α and IL-10 in Sample II of CVF samples obtained from study women were significantly decreased in Sample II than in Sample I down to 82%, 80% and 76%, respectively of its levels estimated in Sample I with subsequent significant difference between the extent of change between both groups. Cytokines' levels estimated in Sample I were significantly higher in women of study group compared to women of control group. On contrary, estimated levels of IL-6 and IL-10 estimated in Sample II were non-significantly higher, while estimated levels of TNF-α were non-significantly lower in control versus study women.

Table 1. Enrolment data of women of both groups.

Group Variables		Control (n = 42)	Study (n = 42)	P value
Maternal age (years)		28 ± 5.1	28.6 ± 1.1	0.396
Weight (kg)		85.9 ± 8.3	87.4 ± 7.5	0.379
Height (cm)		169.8 ± 4.9	170 ± 3.2	0.835
Body mass index (kg/m^2)		29.8 ± 3.1	30.3 ± 3.2	0.372
Obstetric data	Gravidity	2.7 ± 0.5	2.8 ± 0.7	0.373
	Parity	1.4 ± 0.5	1.3 ± 0.7	0.213
	Interpregnancy interval (m)	15 ± 5	11.5 ± 6.1	0.005
	Gestational age at enrolment (wk)	13.8 ± 1.7	13.4 ± 2	0.303
Hemodynamic variables	Systolic blood pressure (mmHg)	113.4 ± 4.9	114.1 ± 3.7	0.485
	Diastolic blood pressure (mmHg)	73.8 ± 7.1	72.6 ± 5.9	0.415
	Heart rate (beats/min)	93.6 ± 8.8	96.8 ± 6.8	0.068
Investigations	Fasting blood glucose (mg/dl)	98.6 ± 7	97.8 ± 8.7	0.661
	Total leucocytic count (10^3/ml)	11.4 ± 1.6	11.2 ± 1.7	0.538
	CRP (ng/ml)	4.59 ± 2.4	5.18 ± 3	0.325

Data are presented as presented as mean ± SD; P indicates significance of difference between both groups; P > 0.05 indicates non-significant difference; P < 0.05 indicates significant difference.

Table 2. Distribution according to duration of pregnancy of women of both groups.

Group Variables			Control (n = 42)	Study (n = 42)
PTB (<37 GW)	<34 GW	Number	0	4 (9.4%)
		Duration	0	32.9 ± 0.14
	>34 - <37 GW	Number	3 (7.1%)	9 (21.4%)*
		Duration	35 ± 0.6	35.75 ± 0.7
	Total	Number	3 (7.1%)	13 (31%)*
		Duration	35 ± 0.6	35 ± 1.5
>37 GW	Number		39 (92.9%)	29 (69%)
	Duration		39.1 ± 0.8	38.4 ± 0.56
Total			38.8 ± 1.3	37.2 ± 1.8*

Data are presented as presented as numbers, percentages, mean ± SD; *indicates significance of difference between both groups at 0.05 level.

IL-10 and TNF-α levels estimated in CVF of Sample I and II were significantly higher in samples obtained from women had pregnancy duration < 37 wk compared to that obtained from women had pregnancy duration > 37 wk. Regarding IL-6, levels estimated in CVF of Sample I were non-significantly higher, while that of Sample II were significantly higher in samples obtained from women had pregnancy duration < 37 wk compared to that obtained from women had pregnancy duration > 37 wk. The percentage of change in cytokines' levels was sig-

nificantly higher in women had pregnancy duration > 37 wk compared to women had pregnancy duration < 37 wk (Table 3).

Pregnancy duration was negatively correlated with maternal BMI and CVF cytokines' levels, while was positively correlated with gravidity, parity and IPI. CVF cytokines' levels were positively correlated with maternal BMI and negatively correlated with IPI that was negatively correlated with BMI (Table 4). Regression analysis defined short IPI ($\beta = -0.179$, P = 0.048) and high CVF levels of TNF-α ($\beta = -0.554$, P = 0.0007) as negative predictors for short pregnancy duration.

Table 3. Cytokines' levels estimated in CVF samples from women of both groups.

Variable	Sample	Control	Study		
			Total	<37 GW	>37 GW
IL-6 (ng/ml)	Sample 1	4.97 ± 1.68	10.37 ± 2.2	10.95 ± 2.93	10.1 ± 1.8
	P value	P1 < 0.0001		P3 = 0.256	
	Sample 2	7.2 ± 2.6	6.71 ± 1.7	8.72 ± 0.9	5.82 ± 1.1
	P value	P1 = 0.326		P3 = 0.0001	
		P2 < 0.0001	P2 < 0.0001	P2 = 0.007	P2 < 0.0001
	Change in relation to S1 level	Increase by 140% (IQR: 131 - 151)	Decrease by 32.6% (IQR: 19 - 46)	Decrease by 16% (IQR: 7 - 26)	Decrease by 37.2% (IQR: 32 - 50)
	P value	P1 < 0.0001		P3 = 0.0001	
TNF-α (ng/ml)	Sample 1	0.82 ± 0.19	1.44 ± 0.5	2.08 ± 0.3	1.15 ± 0.22
	P value	P1 < 0.0001		P1 < 0.0001	
	Sample 2	0.98 ± 0.26	1.11 ± 0.45	1.68 ± 0.29	0.86 ± 0.23
	P value	P1 = 0.117		P1 < 0.0001	
		P2 = 0.0008	P2 = 0.001	P2 = 0.001	P2 < 0.0001
	Change in relation to S1 level	Increase by 114% (IQR: 110 - 129)	Decrease by 19% (IQR: 17 - 31)	Decrease by 18% (IQR: 14 - 21)	Decrease by 21% (IQR: 17 - 32)
	P value	P1 < 0.0001		P1 = 0.041	
IL-10 (ng/ml)	Sample 1	0.96 ± 0.25	1.98 ± 0.6	2.63 ± 0.34	1.69 ± 0.43
	P value	P1 < 0.0001		P1 < 0.0001	
	Sample 2	1.25 ± 0.33	1.13 ± 0.6	1.96 ± 0.32	0.75 ± 0.15
	P value	P1 = 0.255		P1 < 0.0001	
		P2 < 0.0001	P2 < 0.0001	P2 < 0.0001	P2 < 0.0001
	Change in relation to S1 level	Increase by 129% (IQR: 115 - 141)	Decrease by 44% (IQR: 35 - 57)	Decrease by 31% (IQR: 16 - 35)	Decrease by 51% (IQR: 42 - 65)
	P value	P1 < 0.0001		P1 < 0.0001	

Data are presented as presented as mean ± SD; P1 indicates significance of difference between both groups; P2 indicates significance of difference Samples I & II of the same group; P > 0.05 indicates non-significant difference; P < 0.05 indicates significant difference.

Table 4. Pearson's correlation between pregnancy duration and other variables collected in women of both groups.

Variables		Pregnancy duration	BMI	Inter-pregnancy interval
Maternal age (y)	r	0.145	−0.214	0.073
	P	0.189	0.050	0.510
BMI (kg/m^2)	r	−0.223		−0.245
	P	0.041		0.028
Gravidity	r	0.214	−0.103	−0.097
	P	0.050	0.350	0.379
Parity	r	0.231	−0.092	0.097
	P	0.034	0.406	0.379
Inter-pregnancy interval (m)	r	0.307	−0.245	
	P	0.005	0.028	
Sample I IL-6 levels (ng/ml)	r	−0.456	0.285	−0.273
	P	0.0003	0.008	0.012
Sample I TNF-*α* levels (ng/ml)	r	−0.569	0.303	−0.355
	P	0.0009	0.005	0.001
Sample I IL-10 levels (ng/ml)	r	−0.446	0.247	−0.464
	P	0.0002	0.023	0.000

BMI: Body mass index; r = Pearson's correlation coefficient; IL-: Interleukin; TNF-*α*: Tumor necrosis factor-*α*; P > 0.05 indicates non-significant value; P < 0.05 indicates significant value.

4. Discussion

The obtained results showed a negative impact of high maternal body mass index (BMI) and short inter-pregnancy interval (IPI) on duration of pregnancy and this impact was more manifest in women at high risk of preterm birth. In support of these findings, statistical analyses showed a negative significant correlation between BMI and both of IPI and pregnancy duration. Similarly, Tsur *et al.* [17], (2017) reported that adjusted relative risks for PTB for pregnant obese of grades I, II and III are 1.1, 1.15 and 1.26, respectively. Recently, Ratnasiri *et al.* [18] found women with class III obesity have a higher risk for PTB by 33% than women with a normal BMI and Liu *et al.* [19] found maternal obesity is significantly associated with the risk of PTB and this risk increases with maternal aging.

Moreover, in line with the impact of IPI on duration of pregnancy, Zhang *et al.* [20] reported that adjusted relative risk for PTB was 2.04 for IPI < 6 m and Palmsten *et al.* [21] reported a higher risk of PTB after IVF and non-IVF deliveries of 22.2% and 6.4%, respectively, after IPI of 12 - <24 m with increasing risk with decreasing IPI. Thereafter, Haight *et al.* [22] reported that in comparison to IPI of 18 - 23 months, adjusted risk ratios for PTB for IPI < 6, 6 - 11, and 12 - 17 months were 1.62, 1.16 and 1.03, respectively. Also, Ahrens *et al.* [23] docu-

mented that IPI < 6 m since last live birth is associated with increased risks for PTB.

For evaluation of pregnancy-associated inflammatory response, the current study estimated cytokines' levels in CVF samples for being simple to obtain, can provide largest sample volume and having the modest concentrations of cytokines that are independent on sample weight and protein concentration [24]. In support of the use of CVF samples to reflect the intra-amniotic status and progress of pregnancy, multiple previous studies assured its advantages and higher concentration of cytokines [24] and correlation with intra-amniotic cytokines than serum [25].

Levels of cytokines estimated in CVF sample obtained at time of labor (Sample II) of control women were significantly higher than that estimated in Sample I (at the beginning of the 2nd trimester). These findings indicated a possible role for inflammatory cytokines for initiation and progression of the process of labor. In line with these findings, Ashford *et al.* [26] detected variations of cytokine profiles across trimesters in women delivering term and Buxton *et al.* [27] reported high concentrations of CVF cytokines among term pregnancies. Also, Buxton *et al.* [27] and Stokkeland *et al.* [28] suggested that these high concentrations indicated that normal pregnancy is associated with an active inflammatory state.

Women at high risk of PTB (Study group) had significantly higher cytokines levels in their Sample I compared to women of control group. Moreover, high CVF cytokines' levels in Sample I showed negative significant correlation with pregnancy duration; a finding that suggests a role of disturbed local inflammatory cytokines in initiation of PTB. In support of this assumption, Regression analyses defined high Sample I cytokines' levels as positive significant predictor for short pregnancy duration and this risk was magnified in women had short IPI especially in women with high BMI. In line with the diagnostic value of estimation of CVF cytokines' levels, Jung *et al.* [29] reported that in women with PTB, CVF IL-6 and IL-8 and cervical length are the most important parameters that could predict spontaneous PTB within 7 days of sampling. Also, Yavari *et al.* [30] found no relationship between serum IL-6 and both of CVF IL-6 and preterm labor, while CVF values of IL-6 were correlated with PTB and concluded that maternal CVF not serum IL-6 is suitable as biomarker for predicting PTB. Thereafter, Yoo *et al.* [7] documented that estimated values of biomarkers in CVF could be non-invasive predictors of spontaneous PTB at <32 weeks in women with cervical insufficiency or short cervix and this may be improved by the combined use of clinical and laboratory data. Moreover, Ashford *et al.* [26] detected significantly higher values of CVF values of IL-6, IL-8, IL-10, TNF-α, and CRP in women who delivered preterm than the full-term women. Recently, Ronzoni *et al.* [31] documented that higher maternal anti-inflammatory cytokines increased latency until delivery in women had premature preterm rupture of membranes which causes one-third of PTB and this effect is likely due to counterbalancing of proinflammatory load.

Cervical cerclage provided both mechanical and immunological protection against PTB as evidenced by the reduction of the incidence of labor before 37 GW despite the fact that all women of study group were at high risk and had cervical length of <25 mm at the beginning of the 2nd trimester of pregnancy and by the significantly lower CVF cytokines' levels in Sample II compared to Sample I of women of Study group.

In line with these findings, Wang *et al.* [32] documented that CC for women with an asymptomatic short cervix and prior PTB history had more benefits in maternal and neonatal outcomes than vaginal progesterone therapy and Kiefer *et al.* [33] detected beneficial effect of CC for mid-trimester short cervix for prevention of PTB especially in patients with high inflammation. Thereafter, Monsanto *et al.* [34] found CC led to a significant decline in CVF pro-inflammatory cytokines levels and suggested that cerclage may help reduce local inflammation. Enakpene *et al.* [35] found that in comparison to vaginal progesterone alone for treatment of women with extremely shortened cervix, addition of CC significantly decreased overall spontaneous PTB rates, prolonged pregnancy latency by 2-fold, and decreased the overall neonatal complications. Also, Conde-Agudelo *et al.* [36] reported that cerclage significantly decreased the risk of PTB, perinatal morbidity/mortality and birth weight < 1500 g in comparison to no cerclage.

5. Conclusion

High body mass index, short inter-pregnancy interval and high CVF inflammatory cytokines' levels affect the duration of pregnancy. Pregnant women with history of recurrent SPTB are vulnerable to be seriously affected by this triad. Early prophylactic cervical cerclage for women at high-risk of SPTB can modulate local immune disturbance, so in addition to its mechanical impact can reduce the incidence of SPTB and prolong the duration of pregnancy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Aung, M.T., Ferguson, K.K., Cantonwine, D.E., Bakulski, K.M., Mukherjee, B., Loch-Caruso, R., McElrath, T.F. and Meeker, J.D. (2019) Associations between Maternal Plasma Measurements of Inflammatory Markers and Urinary Levels of Phenols and Parabens during Pregnancy: A Repeated Measures Study. *Science of The Total Environment*, **650**, 1131-1140. https://doi.org/10.1016/j.scitotenv.2018.08.356

[2] Azizieh, F.Y. and Raghupathy, R. (2017) IL-10 and Pregnancy Complications. *Clinical and Experimental Obstetrics and Gynecology*, **44**, 252-258.

[3] Arenas-Hernandez, M., Romero, R., St Louis, D., Hassan, S.S., Kaye, E.B. and Gomez-Lopez, N. (2016) An Imbalance between Innate and Adaptive Immune Cells at the Maternal-Fetal Interface Occurs Prior to Endotoxin-Induced Preterm Birth. *Cellular & Molecular Immunology*, **13**, 462-473. https://doi.org/10.1038/cmi.2015.22

[4] Pandey, M., Chauhan, M. and Awasthi, S. (2017) Interplay of Cytokines in Preterm Birth. *Indian Journal of Medical Research*, **146**, 316-327.

[5] Cecati, M., Sartini, D., Campagna, R., Biagini, A., Ciavattini, A., Emanuelli, M. and Giannubilo, S.R. (2017) Molecular Analysis of Endometrial Inflammation in Preterm Birth. *Cellular and Molecular Biology*, **63**, 51-57. https://doi.org/10.14715/cmb/2017.63.3.10

[6] Ghartey, J., Bastek, J.A., Brown, A.G., Anglim, L. and Elovitz, M.A. (2015) Women with Preterm Birth Have a Distinct Cervicovaginal Metabolome. *American Journal of Obstetrics & Gynecology*, **212**, 776.e1-776.e12. https://doi.org/10.1016/j.ajog.2015.03.052

[7] Yoo, H.N., Park, K.H., Jung, E.Y., Kim, Y.M., Kook, S.Y. and Jeon, S.J. (2017) Non-Invasive Prediction of Preterm Birth in Women with Cervical Insufficiency or an Asymptomatic Short Cervix (≤25 mm) by Measurement of Biomarkers in the Cervicovaginal Fluid. *PLoS ONE*, **12**, e0180878. https://doi.org/10.1371/journal.pone.0180878

[8] Starodubtseva, N.L., Brzhozovskiy, A.G., Bugrova, A.E., Kononikhin, A.S., Indeykina, M.I., Gusakov, K.I., Chagovets, V.V., Nazarova, N.M., Frankevich, V.E., Sukhikh, G.T. and Nikolaev, E.N. (2019) Label-Free Cervicovaginal Fluid Proteome Profiling Reflects the Cervix Neoplastic Transformation. *Journal of Mass Spectrometry*, **54**, 693-703. https://doi.org/10.1002/jms.4374

[9] Lucaroni, F., Morciano, L., Rizzo, G., D'Antonio, F., Buonuomo, E., Palombi, L. and Arduini, D. (2018) Biomarkers for Predicting Spontaneous Preterm Birth: An Umbrella Systematic Review. *The Journal of Maternal-Fetal & Neonatal Medicine*, **31**, 726-734. https://doi.org/10.1080/14767058.2017.1297404

[10] Huang, L., Hou, Q., Huang, Y., Ye, J., Huang, S., Tian, J., Tang, R., Liu, C., Long, Y., Qin, X., Weng, X., Huang, Y., Li, M., Yang, X. and Mo, Z. (2019) Serum Multiple Cytokines for the Prediction of Spontaneous Preterm Birth in Asymptomatic Women: A Nested Case-Control Study. *Cytokine*, **117**, 91-97. https://doi.org/10.1016/j.cyto.2019.02.007

[11] Venkatesh, K.K., Cantonwine, D.E., Ferguson, K., Arjona, M., Meeker, J.D. and McElrath, T.F. (2016) Inflammatory and Oxidative Stress Markers Associated with Decreased Cervical Length in Pregnancy. *American Journal of Reproductive Immunology*, **76**, 376-382. https://doi.org/10.1111/aji.12545

[12] Owen, J., Yost, N., Berghella, V., Thom, E., Swain, M., Dildy, G.A., Miodovnik, M., Langer, O., Sibai, B. and McNellis, D. (2001) National Institute of Child Health and Human Development, Maternal-Fetal Medicine Units Network: Midtrimester Endovaginal Sonography in Women at High Risk for Spontaneous Preterm Birth. *The Journal of the American Medical Association*, **286**, 1340-1348.

[13] Meher, S. and Alfirevic, Z. (2014) Choice of Primary Outcomes in Randomised Trials and Systematic Reviews Evaluating Interventions for Preterm Birth Prevention: A Systematic Review. *BJOG: An International Journal of Obstetrics & Gynaecology*, **121**, 1188-1194. https://doi.org/10.1111/1471-0528.12593

[14] Coughlan, M.T., Oliva, K., Georgiou, H.M., Permezel, J.M.H. and Rice, G.E. (2001) Glucose-Induced Release of Tumor Necrosis Factor-Alpha from Human Placental and Adipose Tissues in Gestational Diabetes Mellitus. *Diabetic Medicine*, **18**, 921-927. https://doi.org/10.1046/j.1464-5491.2001.00614.x

[15] Gaines-Das, R.E. and Poole, S. (1993) The International Standard for Interleukin-6—Evaluation in an International Collaborative Study. *Journal of Immunological Methods*, **160**, 147-153. https://doi.org/10.1016/0022-1759(93)90172-4

[16] Poll, T. (1996) IL-10 Release during Endotoxaemia in Chimpanzees: Role of Platelet-Activating Factor and IL-6. *Scandinavian Journal of Immunology*, **43**, 122-125. https://doi.org/10.1046/j.1365-3083.1996.d01-12.x

[17] Tsur, A., Mayo, J.A., Wong, R.J., Shaw, G.M., Stevenson, D.K. and Gould, J.B. (2017) "The Obesity Paradox": A Reconsideration of Obesity and the Risk of Preterm Birth. *Journal of Perinatology*, **37**, 1088-1092. https://doi.org/10.1038/jp.2017.104

[18] Ratnasiri, A.W.G., Lee, H.C., Lakshminrusimha, S., Parry, S.S., Arief, V.N., DeLacy, I.H., Yang, J.S., DiLibero, R.J., Logan, J. and Basford, K.E. (2019) Trends in Maternal Prepregnancy Body Mass Index (BMI) and Its Association with Birth and Maternal Outcomes in California, 2007-2016: A Retrospective Cohort Study. *PLoS ONE*, **14**, e0222458. https://doi.org/10.1371/journal.pone.0222458

[19] Liu, L., Ma, Y., Wang, N., Lin, W., Liu, Y. and Wen, D. (2019) Maternal Body Mass Index and Risk of Neonatal Adverse Outcomes in China: A Systematic Review and Meta-Analysis. *BMC Pregnancy Childbirth*, **19**, 105. https://doi.org/10.1186/s12884-019-2249-z

[20] Zhang, L., Shen, S., He, J., Chan, F., Lu, J., Li, W., Wang, P., Lam, K.B.H., Mol, B.W.J., Yeung, S.L.A., Xia, H., Schooling, C.M. and Qiu, X. (2018) Effect of Interpregnancy Interval on Adverse Perinatal Outcomes in Southern China: A Retrospective Cohort Study, 2000-2015. *Paediatric and Perinatal Epidemiology*, **32**, 131-140. https://doi.org/10.1111/ppe.12432

[21] Palmsten, K., Homer, M.V., Zhang, Y., Crawford, S., Kirby, R.S., Copeland, G., Chambers, C.D., Kissin, D.M. and Su, H.I. (2018) States Monitoring Assisted Reproductive Technology (SMART) Collaborative: *In Vitro* Fertilization, Interpregnancy Interval, and Risk of Adverse Perinatal Outcomes. *Fertility and Sterility*, **109**, 840-848.e1. https://doi.org/10.1016/j.fertnstert.2018.01.019

[22] Haight, S.C., Hogue, C.J., Raskind-Hood, C.L. and Ahrens, K.A. (2019) Short Interpregnancy Intervals and Adverse Pregnancy Outcomes by Maternal Age in the United States. *Annals of Epidemiology*, **31**, 38-44. https://doi.org/10.1016/j.annepidem.2018.12.002

[23] Ahrens, K.A., Nelson, H., Stidd, R.L., Moskosky, S. and Hutcheon, J.A. (2019) Short Interpregnancy Intervals and Adverse Perinatal Outcomes in High-Resource Settings: An Updated Systematic Review. *Paediatric and Perinatal Epidemiology*, **33**, O25-O47. https://doi.org/10.1111/ppe.12503

[24] Short, C.S., Quinlan, R., Bennett, P., Shattock, R.J. and Taylor, G.P. (2018) Optimising the Collection of Female Genital Tract Fluid for Cytokine Analysis in Pregnant Women. *Journal of Immunological Methods*, **458**, 15-20. https://doi.org/10.1016/j.jim.2018.03.014

[25] Lee, S.M., Park, K.H., Jung, E.Y., Kook, S.Y., Park, H. and Jeon, S.J. (2018) Inflammatory Proteins in Maternal Plasma, Cervicovaginal and Amniotic Fluids as Predictors of Intra-Amniotic Infection in Preterm Premature Rupture of Membranes. *PLoS ONE*, **13**, e0200311. https://doi.org/10.1371/journal.pone.0200311

[26] Ashford, K., Chavan, N.R., Wiggins, A.T., Sayre, M.M., McCubbin, A., Critchfield, A.S. and O'Brien, J. (2018) Comparison of Serum and Cervical Cytokine Levels throughout Pregnancy between Preterm and Term Births. *American Journal of Perinatology Reports*, **8**, e113-e120. https://doi.org/10.1055/s-0038-1656534

[27] Buxton, M.A., Meraz-Cruz, N., Sánchez, B.N., Foxman, B., Gronlund, C.J., Beltran-Montoya, J., Castillo-Castrejon, M., O'Neill, M.S. and Vadillo-Ortega, F. (2019) Repeated Measures of Cervicovaginal Cytokines during Healthy Pregnancy: Understanding "Normal" Inflammation to Inform Future Screening. *American Journal of Perinatology*. https://doi.org/10.1055/s-0039-1685491

[28] Stokkeland, L.M.T., Giskeødegård, G.F., Stridsklev, S., Ryan, L., Steinkjer, B., Tangerås, L.H., Vanky, E. and Iversen, A.C. (2019) Serum Cytokine Patterns in First Half of Pregnancy. *Cytokine*, **119**, 188-196. https://doi.org/10.1016/j.cyto.2019.03.013

[29] Jung, E.Y., Park, K.H., Lee, S.Y., Ryu, A., Joo, J.K. and Park, J.W. (2016) Predicting Outcomes of Emergency Cerclage in Women with Cervical Insufficiency Using Inflammatory Markers in Maternal Blood and Amniotic Fluid. *International Journal of Gynecology & Obstetrics*, **132**, 165-169. https://doi.org/10.1016/j.ijgo.2015.07.011

[30] Yavari Kia, P., Baradaran, B., Shahnazi, M., Asghari Jafarabadi, M., Khaze, V. and Pourasad Shahrak, S. (2016) Maternal Serum and Cervicovaginal IL-6 in Patients with Symptoms of Preterm Labor. *Iranian Journal of Immunology*, **13**, 229-236.

[31] Ronzoni, S., Steckle, V., D'Souza, R., Murphy, K.E., Lye, S. and Shynlova, O. (2019) Cytokine Changes in Maternal Peripheral Blood Correlate with Time-to-Delivery in Pregnancies Complicated by Premature Prelabor Rupture of the Membranes. *Reproductive Sciences*, **26**, 1266-1276. https://doi.org/10.1177/1933719118815590

[32] Wang, S.W., Ma, L.L., Huang, S., Liang, L. and Zhang, J.R. (2016) Role of Cervical Cerclage and Vaginal Progesterone in the Treatment of Cervical Incompetence with/without Preterm Birth History. *Chinese Medical Journal*, **129**, 2670-2675. https://doi.org/10.4103/0366-6999.193451

[33] Kiefer, D.G., Peltier, M.R., Keeler, S.M., Rust, O., Ananth, C.V., Vintzileos, A.M. and Hanna, N. (2016) Efficacy of Midtrimester Short Cervix Interventions Is Conditional on Intraamniotic Inflammation. *American Journal of Obstetrics & Gynecology*, **214**, 276.e1-276.e6. https://doi.org/10.1016/j.ajog.2015.09.006

[34] Monsanto, S.P., Daher, S., Ono, E., Pendeloski, K.P.T., Trainá, É., Mattar, R., Tayade, C. (2017) Cervical Cerclage Placement Decreases Local Levels of Proinflammatory Cytokines in Patients with Cervical Insufficiency. *American Journal of Obstetrics & Gynecology*, **217**, 455.e1-455.e8. https://doi.org/10.1016/j.ajog.2017.06.024

[35] Enakpene, C.A., DiGiovanni, L., Jones, T.N., Marshalla, M., Mastrogiannis, D. and Della Torre, M. (2018) Cervical Cerclage for Singleton Pregnant Patients on Vaginal Progesterone with Progressive Cervical Shortening. *American Journal of Obstetrics & Gynecology*, **219**, 397.e1-397.e10. https://doi.org/10.1016/j.ajog.2018.06.020

[36] Conde-Agudelo, A., Romero, R., Da Fonseca, E., O'Brien, J.M., Cetingoz, E., Creasy, G.W., Hassan, S.S., Erez, O., Pacora, P. and Nicolaides, K.H. (2018) Vaginal Progesterone Is as Effective as Cervical Cerclage to Prevent Preterm Birth in Women with a Singleton Gestation, Previous Spontaneous Preterm Birth, and a Short Cervix: Updated Indirect Comparison Meta-Analysis. *American Journal of Obstetrics & Gynecology*, **219**, 10-25. https://doi.org/10.1016/j.ajog.2018.03.028