

Cervical Cerclage for Pregnant Women at High-risk of Recurrent Preterm Birth can Act Beyond its Mechanical Action

Original
Article

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ABSTRACT

Aim: Evaluation of predictive value of levels of cervicovaginal fluid (CVF) interleukin (IL)-6 and 8, and matrix metalloproteinases (MMP) estimated at time of performing cervical cerclage (CC) in women with history of spontaneous preterm birth (SPTB) for pregnancy duration.

Materials and Methods: 59 women had history of SPTB (Study group) and 25 women with no history of SPTB (Control group). Two CVF samples were obtained for ELISA estimation of IL-6, IL-8, MMP8 and MMP9 levels at 14-18 gestational weeks (GW), S1 sampling time, and at time of removal of CC suture, S2 sampling time. CC was applied 4-days after S1 sampling.

Results: S1-CVF levels were significantly higher in study versus control women. S2-CVF levels were significantly higher in control, while were significantly lower in study women compared to S1 levels. S2-CVF IL-6 and IL-8 levels were non-significantly higher, while levels of MMP were significantly lower in study than control women. Percentages of change in cytokines' levels showed significant differences between study and control groups. Nineteen study women had PTB at <37 GW, while 40 women had labor at >37 GW. Study women had significantly shorter CL and pregnancy duration compared to control women. Pregnancy duration was negatively correlated with percentage of CL change, while positively correlated with percentage of decrease of cytokines levels.

Conclusion: CC induced significant decrease of CVF cytokines' levels and allowed prolongation of pregnancy duration for >37 GW in 67.8% of studied women at high risk of SPTB

Key Words: Cervicovaginal fluid, inflammatory cytokines, preterm birth, pregnancy duration

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INTRODUCTION

Cervical insufficiency (CI) is a condition of pregnancy which causes the cervix to soften, shorten and dilate between 18 and 22 gestational weeks (GW)^[1] and is an important cause of preterm birth (PTB)^[2]. The World Health Organization and the United Nations consider PTB as a major factor for the global rates of neonatal death and to longer-term health problems for surviving infants^[3].

Etiology of CI and the subsequent spontaneous PTB (SPTB) is multifactorial^[4]. High cytokine concentrations that were detected during pregnancy indicated that pregnancy is associated with an active inflammatory state^[5]. Disturbed plasma inflammatory markers in early pregnancy were associated with decreased cervical length (CL) and suggest an impact of imbalance of immune regulation on CL^[6]. The levels of inflammatory proteins in the cervicovaginal fluid (CVF) were found to be correlated with inflammatory proteins in the amniotic fluid (AF), but not with that in the plasma^[7] and indicates disturbed local immune balance in female genital organs mostly towards the inflammatory side^[8]. The optimum method of collection of CVF in pregnant women for cytokine measurement

is unknown; however, both polyvinyl acetal sponge collection of cervical fluid and menstrual cup collection of CVF are robust for measurement of local cytokines during pregnancy^[9].

Cervical cerclage (CC) is currently one of the primary methods of CI treatment^[10]. Progesterone preparations have different efficacy in prevention of PTB prior to 37 GW in women at risk^[11]. Vaginal progesterone effectively allowed prolonged duration of pregnancy in nullipara women pregnant in singleton fetus with 10-20 mm CL in the mid-trimester^[12], but was not effective in twin pregnancy^[13].

Hypothesis : This study hypothesized that disturbed levels of cytokines in the CVF have a role in the initiation of cervical incompetence (CI) with subsequent spontaneous PTB (SPTB), but cervical cerclage (CC) may act beyond its mechanical role for prolongation of duration of pregnancy through an effect on CVF cytokine milieu.

AIM OF WORK

Estimation of levels of interleukin-6 and 8, and matrix metalloproteinases (MMP) in CVF in women with past history of SPTB in comparison to normal pregnant women

at time of performing CC (S1 sampling time) and removal of CC suture (S2 sampling time).

PATIENTS AND METHODS

This is a prospective comparative interventional study conducted at University Hospital, Benha, Egypt and multiple private obstetrics centers

All pregnant women who presented to the Antenatal Care Unit (ACU) with past history of SPTB were eligible for evaluation for inclusion and exclusion criteria. Inclusion criteria included pregnancy with singleton fetus, past history of SPTB secondary to CI, GA >12 GW and absence of exclusion criteria. Exclusion criteria included manifest diabetes mellitus, multiple pregnancy, and presence of pregnancy-induced complications and/or vaginal infection. Also, women lost during follow-up or gave pregnancy out of participating centers were excluded from the study. The study also included 25 age-matched normal pregnant women free of history and signs of SPTB, and exclusion criteria, and presented at the same GA as control group. The study protocol was approved by the Local Ethical Committee and all enrolled women signed written fully informed consent.

At time of presentation demographic and clinical data of enrolled women were obtained and at time of performing the CC, all women were re-evaluated and CVF samples were obtained. Then, all women undertook transvaginal ultrasonography (TVU) for estimation of CL; women had CL <25 mm were considered at high-risk for SPTB^[14] and underwent Shirodkar's cervical cerclage within 4 days after CVF sampling (S1 sampling time) and were considered as Study group. All enrolled women were maintained on vaginal toilet to guard against development of infection. Women of the study group were asked to attend the ACU biweekly for follow-up for development of SPTB, which was defined as any birth before 37 weeks completed weeks of gestation^[15]. At time of labor or removal of the suture, women of both groups undertook CVF sampling (S2 sampling time) for re-estimation of CVF cytokines' levels and the percentage of change in CVF cytokines' levels was calculated as the percentage of difference between levels estimated in both samples multiplied by 100 (% of change= $[(S1-S2 \text{ levels})/(S1 \text{ level})] \times (100)$).

CVF sample obtaining and processing :

Vaginal speculum was placed and the cervix visualized. An ectocervical sample was collected by sweeping the cervix 360° and maintained in situ for 30 seconds to maximize saturation. On removal of the cervical swab, a Dacron swab was swept 360° in vaginal vault and posterior vaginal fornix, and maintained in situ for 10 seconds to achieve saturation. Then, swabs were 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^[16].

Investigations

CVF levels of IL-6 and -8 and matrix metalloproteinases (MMP) were measured using ELISA kits according to the manufacturer's instructions and were read using a 96 well microplate ELISA reader (Dynatech, MR 7000). Human IL-6 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue No IL631-K01; Eagle Bioscience Inc., USA) which employs the quantitative sandwich enzyme immunoassay technique^[17]. Human IL-8 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46032, abcam Inc., Cambridge, USA) which employs the quantitative sandwich enzyme immunoassay technique^[18]. Human Matrix metalloproteins 8^[19] and 9^[20] was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab181421 and ab100610, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique.

Cervical cerclage

Cervical cerclage was performed using the Shirodkar procedure with a non-absorbable suture at GA of 14-18 GW and within 4 days after S1 sampling time. 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.

Study Outcomes : Primary outcome includes the effect of pregnancy on the CVF cytokines' levels. Secondary outcomes included the effect of cerclage on CL and CVF cytokines' levels in women of study group as well as the value of early estimation of CVF cytokines' levels as predictors for pregnancy duration.

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 (X^2 test). Possible relationships were investigated using Pearson linear regression analysis. Predictability of S1-CVF cytokines' levels for pregnancy duration was evaluated by Regression analysis (Stepwise method) and receiver operating characteristic (ROC) curve analysis, judged by the area under the curve (AUC) that was compared versus null hypothesis that AUC=0.5. 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.

Results

Seventy-five women with past history of SPTB were eligible for evaluation; 16 women were excluded for not

fulfilling inclusion criteria and 59 were included in the study as study group. Twenty-five women with no history of SPTB were also enrolled as control group (Figure 1).

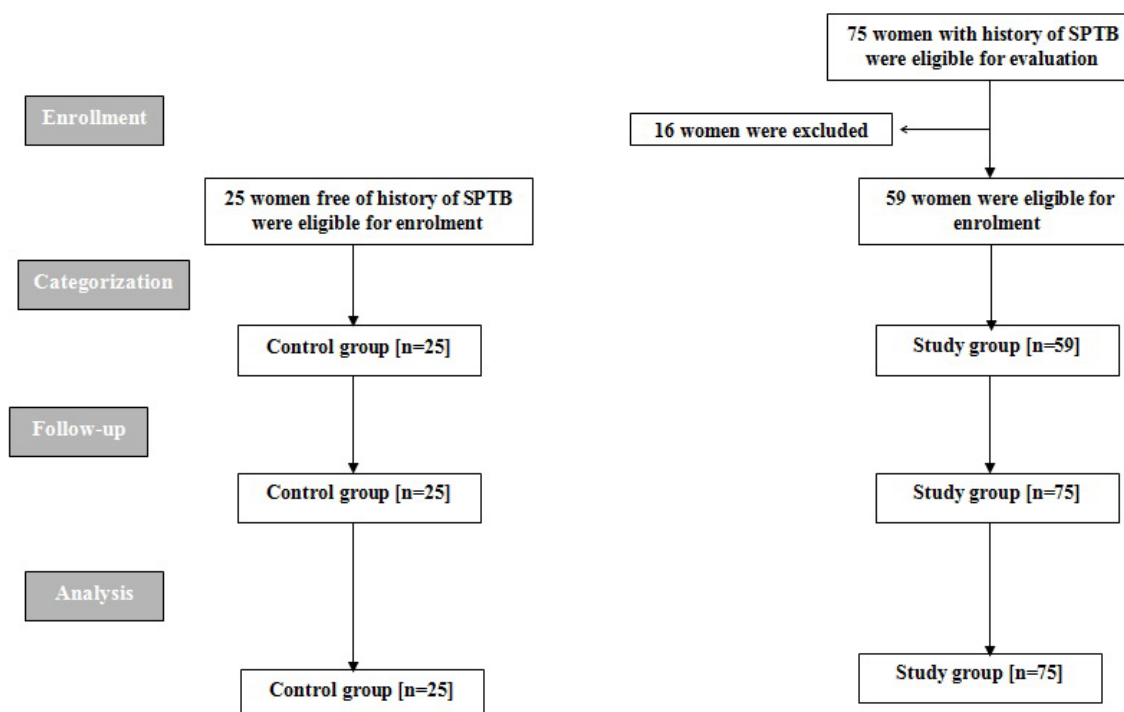


Fig.: Consort Flow sheet

Women's enrollment data showed non-significant ($p>0.05$) differences between both groups apart from the number of women had prior early pregnancy loss that was

significantly ($p=0.02$) higher in women of study group compared to women of control group (Table 1).

Table 1: Enrolment data of women of both groups

Data	Group	Control (n=25)	Study (n=59)	P value
Maternal age (years)		28.5±5.5	28.2±2.7	0.673
Gestational age at time of enrolment (wks)		16.4±2.1	16.2±2.3	0.713
Body weight (Kg)		86±8.5	87±7.1	0.578
Body height (cm)		170±4.6	169.8±3.1	0.831
Body mass index (Kg/m ²)		29.8±3.1	30.2±3	0.578
Obstetric history	Gravidity	2.4±0.6	2.5±0.7	0.533
	Parity	1.4±0.6	1.3±0.9	0.578
	Prior early pregnancy loss	3 (12%)	7 (22%)	0.020
General exam	Systolic blood pressure (mmHg)	113.2±4.8	114.2±3.7	0.278
	Diastolic blood pressure (mmHg)	73.8±8	72.4±4.6	0.324
	Heart rate (beat/min)	94.72±5.7	93.7±4	0.351
	Body temperature (°C)	36.5±0.4	36.6±0.3	0.195
Lab findings	Total leucocytic count (x10 ³ /mm ³)	11.43±1.08	11.21±1.76	0.587
	C-reactive protein (mg/L)	4.65±2.4	5.12±3	0.487
	Fasting blood glucose (mg/dl)	93±11	97.3±9.3	0.071

Data are presented as mean±SD, numbers, percentages; p value indicates significance of difference between both groups; $p < 0.05$ indicates significant difference; $p > 0.05$ indicates non-significant difference

The mean level of S1-CVF cytokines was significantly higher in women of study group in comparison to women of control group. At time of labor, all women of control group had significantly higher S2-CVF cytokines' levels

in comparison to their S1-CVF levels. On the other hand, in women of study group, S2-CVF cytokines' levels were significantly lower compared to their corresponding S1-CVF levels. Moreover, mean S2-CVF levels of IL-6 and IL-8 were non-significantly higher, while mean levels of MMP were significantly lower in studied women compared to control women. Subsequently, the percentages of change in CVF cytokines' levels showed significant differences between both groups (Table 2).

Table 2: Levels of studied cytokines estimated in S1 and S2 samples obtained from women of both group

Variables	Group	Control			Study		
		S1 sample	S2 sample	% of change	S1 sample	S2 sample	% of change
IL-6 (ng/ml)	Concentration	5.12±1.68	6.92±1.88	37.46±9.8	10.62±2.4	7.21±2.05	-32.64±8.53
	P1		0.0008			<0.0001	
	P2				<0.0001	0.544	<0.0001
IL-8 (ng/ml)	Concentration	7.78±2.04	10.9±2.6	42.3±15.5	15.25±6	11.12±3.6	-25.3±6.9
	P1		<0.0001			<0.0001	
	P2				<0.0001	0.777	<0.0001
MMP9 (ng/ml)	Concentration	39.9±12.1	52.2±19.9	39.8±16.9	54.8±17.8	40.6±16.5	-22.2±7.6
	P1		<0.0001			<0.0001	
	P2				0.0003	0.0008	<0.0001
MMP8 (ng/ml)	Concentration	63.5±18.6	92.4±33.7	46.5±26	104.1±23.2	78.4±20.6	-24.8±9.2
	P1		0.0001			<0.0001	
	P2				<0.0001	0.014	<0.0001

P1: indicates the significance of difference in concentration of CVF cytokines in S1 and S2 samples; P2: indicates the significance of difference between estimated CVF cytokines' concentrations in control and study groups

Nineteen women of study group had PTD after a mean duration of pregnancy of 35.3±0.9 wk, while 40 women of study group had onset of labor that required cerclage suture removal after a mean duration of pregnancy 38.5±0.7 wk. Mean duration of pregnancy of study women was 37.5±1.7 wk with significantly ($p=0.0003$) shorter pregnancy duration compared to control women (39.1±0.7 wk). All women of study group showed shortening of the CL at S2-sampling time compared to S1-sampling time with a mean decrease of CL by 20.7±4.9%. Women who had PTD had significantly ($p=0.017$) higher percentage of decrease of CL (22.9±4.4%) compared to percentage of decrease in women who had labor after 37 GW (19.6±4.9%).

Pregnancy duration of study women showed negative significant correlation ($r=-0.340$, $p=0.008$) with the percentage of change in CL, while showed positive significant correlation with the percentage of change of CVF levels of IL-6 ($r=0.473$, $p=0.0004$), IL-8 ($r=0.396$, $p=0.002$), MMP8 ($r=0.382$, $p=0.003$) and MMP9 ($r=0.347$, $p=0.007$) between S1 and S2 samples.

Regression analysis for S1-CVF cytokines' levels defined elevated levels of IL-8 ($\beta=-0.386$, $p=0.0003$), IL-6 ($\beta=-0.362$, $p=0.0008$), MMP8 ($\beta=-0.284$, $p=0.003$) and MMP9 ($\beta=-0.244$, $p=0.007$) as predictors for short duration of pregnancy. ROC curve analysis defined elevated S1-CVF levels of MMP9 as a sensitive predictor for short duration of pregnancy (Figure 2).

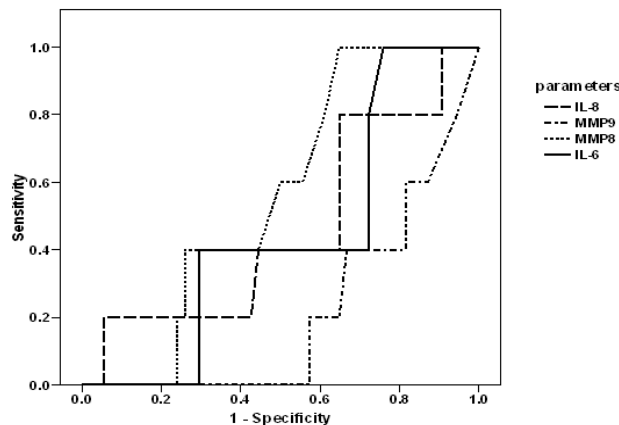


Fig. 2: ROC curve analysis of lab parameters as predictors for short duration of pregnancy

DISCUSSION

The obtained results showed a significant increase of S2-CVF cytokines' levels in control women compared to S1-CVF levels, this finding indicates that pregnancy induces an inflammatory status and its progress is associated with shift of local cytokine milieu towards the inflammatory arm. Moreover, the increased levels at time of labor indicated that the process of delivery itself requires or amplify such inflammatory status.

These findings go in hand with Basu *et al.*^[21] who experimentally found villous MMP-9 protein increased progressively with increased gestational age and a positive correlation between villous tumor necrosis factor- α (TNF- α) and MMP-9 in 2nd trimester placenta of normal gestation, so attributed successful pregnancy outcome to such relations. Recently, Buxton *et al.*^[5] detected progressively increasing concentrations of cytokines in CVF samples collected monthly from two-to-nine months of gestation among term pregnancies and documented that pregnancy is associated with an active inflammatory state.

Mean S1-CVF cytokines' levels were significantly higher in women who were at high risk of SPTB (Study group) compared to women of control group who had full term delivery. These findings spots light of a possible etiological role of disturbed local cytokines' milieu for development of CI with subsequent SPTB. In line with these findings, Monsanto *et al.*^[22] detected higher levels of IL-1 β , -6 and 12, monocyte chemoattractant protein-1 and TNF- α in CVF of women had CI compared to control pregnant women and suggested that CI may be associated with or develops secondary to dysregulation of the local immune environment. Also, Ashford *et al.*^[23] found the CVF values of IL-6, 8 and 10, TNF- α , and CRP and serum MMP-8 were significantly higher in women who had preterm birth (PTB) than the full-term women.

In support of the etiopathological role of inflammatory cytokines in development of PTB, As Sayaril *et al.*^[24] reported higher expression of genes for IL1 α , IL-1 β , IL-6, and TNF- α in plasma of PTB patients than in control pregnant women. Recently, Song *et al.*^[25] detected positive correlations between amniotic fluid (AF) hypoxia-inducible factor 1 α (HIF1 α) and exosomal HIF1 α with AF levels of IL-1 α , IL-6, and TNF- α in CI patients with physical examination-indicated cerclage and suggested an interaction between AF HIF1 α and exosomes and inflammatory cytokines that may induce or contribute the inflammatory cascade in complicated pregnancies.

Cervical cerclage (CC) induced significant reduction of S2-CVF cytokines' levels compared to S1-CVF levels and the percentage of decrease correlated positively with the duration of pregnancy with significantly lower percentage of decrease in women had PTB (<37 GW) compared to women had pregnancy duration of >37 GW. These findings are consistent with that of Monsanto *et al.*^[22] who found cerclage intervention led to a significant decline in CVF proinflammatory cytokines levels and suggested that

cerclage may help reduce local inflammation in women had CI.

In support of the efficacy of CC as a preventive therapeutic modality for PTB, Wang *et al.*^[26] found CC is more beneficial for better pregnancy outcome than vaginal progesterone therapy for women with an asymptomatic short cervix and prior PTB history. Also, Sinkey *et al.*^[27] found that in women with CI combined CC and intramuscular progesterone resulted in PTB prevention similar to cerclage alone. Moreover, Caritis *et al.*^[28] reported significantly elevated levels of IL-6 and 10, and TNF- α in cervical fluid of women had their prior PTB between 16 and 23 weeks than those had prior PTB between 32 and 36 wks and treatment with 17-hydroxyprogesterone caproate had no significant impact on these cytokines.

Regression analysis defined high S1-CVF levels of IL-6 and IL-8 as the most significant predictors, while ROC curve analysis defined high S1-CVF level of MMP8 as the significant sensitive predictor for short duration of pregnancy. Similarly, Ashford *et al.*^[23] documented that the levels of IL-1 α , IL-6, TNF- α can be used as predicative biomarkers for PTB. Also, Park *et al.*^[29] reported that plasma and AF IL-6 levels had an overall diagnostic performance to predict imminent PTB and Garry *et al.*^[30] reported increased presence of IL-1 α , IL-1 β , IL-2 and IL-13 in vaginal washings of women at risk for PTB. Moreover, Liu *et al.*^[31] found high AF TNF- α and IL-8 levels before CC affect pregnancy outcome in women with CI and levels higher than 3.58 ng/ml and 0.105 ng/ml of IL-8 and TNF- α , respectively could predict perinatal death of the infants.

CONCLUSION

Pregnancy induces an inflammatory state that shifts towards the inflammatory arm with the progress of pregnancy. CI and short CL with subsequent PTB are associated with early higher levels of inflammatory cytokines in CVF. Cervical cerclage induced significant decrease of CVF cytokines' levels, allowed prolongation of pregnancy duration for more than 37 GW in 67.8% of studied women at high risk of SPTB.

CONFLICT OF INTERESTS

There are no conflict of interests.

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