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RESEARCH ARTICLE

A POSSIBLE IMMUNO-MODULATORY ACTION FOR CERVICAL CERCLAGE IN WOMEN AT RISK FOR PRETERM LABOR.

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Abstract

Objectives: To estimate and evaluate changes in cervico-vaginal fluid (CVF) content of interleukin (IL) 6 and 18 and granulocyte macrophage- colony stimulating factor (GM-CSF) in pregnant women with past history of preterm birth (PTB) underwent prophylactic cervical cerclage (CC), or vaginal progesterone (VP) or both.

Patients & Methods: Baseline TVU was performed for estimation of cervical length (CL) and women had CL >25 were considered as Control group and women had CL <25 mm were divided into three groups according to provided prophylactic therapy, A, B and C, respectively. At the 24th week (24-wk) gestational age (GA), CVF samples were obtained for ELISA estimation of levels of IL-6, IL-18 and GM-CSF and repeated with estimation of CL at 30-wk GA. Frequency of PTB and the effect of applied therapeutic lines on CVF levels of studied cytokines were determined.

Results: At 24-wk, 27 women had CL >25 mm and 169 women had CL <25 mm, at 30-wk CL was significantly shorter in study than control women. Seven control and 46 study women had PTB prior to 34 wk with significantly higher frequency in group B than group C. At 24-wk, IL-6 levels were significantly higher, while IL-18 and GM-CSF levels were non-significantly higher in study versus control women. At 30-wk, IL-6 and GM-CSF levels significantly decreased in groups A and C, while increased in group B than their 24-wk levels with significant difference versus other groups. At 30-wk, IL-18 levels decreased significantly than 24-wk levels with non-significant inter-group differences. Duration of pregnancy showed negative significant correlation with CL%, but positive significant correlation with extent of decrease in cytokine levels. Percentage of decrease of studied cytokines and duration of pregnancy showed positive significant correlation with taking CC as line of management. Statistical analyses defined CL% as negative predictor and the extent of decreased IL-6 levels was the only significant positive predictor among studied cytokines.

Conclusion: Disturbed local immune milieu towards increased pro-inflammatory cytokine production plays a role in pathogenesis and acceleration of PTB. CC induced down-regulation of inflammatory cytokines production and allowed prolongation of duration pregnancy; thus CC works beyond its mechanical preventive effect.

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Introduction:-

Preterm birth (PTB) is the leading cause of perinatal morbidity and mortality worldwide, so its prevention is an important health-care priority ⁽¹⁾. Cervical dilation in the second trimester is associated with a greater than 90% rate of spontaneous preterm birth (sPTB) and a poor perinatal prognosis ⁽²⁾. Cervical insufficiency (CI) is characterized by premature, progressive dilation and shortening of the cervix during pregnancy ⁽³⁾. Precocious cervical ripening, defined by cervical shortening on transvaginal ultrasonography (TVU) is another category that may be associated with PTB ⁽⁴⁾.

Such dilemma of PTB, its pathogenesis and sequele still needed to be investigated ⁽⁵⁾. Clinically, once a woman has been identified as being at risk of sPTB due to a short cervical length or CI, a decision regarding prophylactic treatment must be made ⁽⁶⁾.

Cervical cerclage (CC), vaginal progesterone (VP) and pessary used separately showed similar effectiveness as management strategies for women with previous sPTB and short cervix ⁽⁷⁾. In comparative study, CC and VP therapies showed similar effectiveness for women with asymptomatic short cervix but without a history of PTB ⁽¹⁾. Treatment of precocious cervical ripening with cervical pessary plus VP did not reduce the PTB rate compared to pessary alone ⁽⁴⁾. Cerclage, indomethacin, and antibiotics in twin pregnancies with dilated cervix ≥ 1 cm before 24 weeks were associated with significantly prolonged duration of pregnancy, decreased rate of sPTB and improved perinatal outcome compared to expectant management ⁽²⁾.

Cervical cerclage (CC) is a well-known surgical procedure carried out during pregnancy. It involves positioning of a suture around the neck of the cervix, aiming to cervical mechanical support and thereby reduces PTB risk ⁽⁸⁾. However, its effect may extend far from being only mechanical barrier; the current study hypothesized that disturbed local immune milieu may have a role in development of PTB and CC may have an effect on local immune milieu.

Objectives:-

Estimation and evaluation of changes in cervico-vaginal fluid (CVF) content of interleukin (IL)-6 and -18 and granulocyte macrophage-colony stimulating factor (GM-CSF) in pregnant women with past history of PTB underwent prophylactic management.

Setting:-

Tertiary referral hospital, KSA

Design:-

Prospective comparative double-blinded study

Patients & Methods:-

The study protocol was approved by the Local Ethical Committee. All pregnant women with previous history of PTB and attended the antenatal care (ANC) unit at the hospital since Jan 2015 till Oct 2017 were eligible for evaluation. Inclusion criteria included singleton fetus, free at time of diagnosis of pregnancy-induced complications, had BMI < 35 kg/m², attended all follow-up visits, and gave birth at hospital. Women missing any of inclusion criteria or lost during follow-up or had vaginal infections were excluded from the study. All women were asked to attend the ANC unit at the 16th wk to determine the baseline data and to attend biweekly thereafter for follow-up. Baseline data included age, number of previous pregnancies and its outcome, blood pressure and random blood glucose level.

Study Protocol:-

1. At time of enrolment, baseline TVU was performed for estimation of cervical length (CL) and women with CL < 25 mm were considered at a risk for having sPTB ⁽⁹⁾ and were included as Study group, while those had CL > 25 were considered as Control group.
2. Women included in study group were randomly, using sealed envelopes prepared by a blinded assistant and contained cards carrying the subgroup label, group A included women received transvaginal CC only, group B included women received vaginal progesterone and combination group C included women received transvaginal CC and VP. Control women followed expectant management.

3. At the 24th week (24-wk) gestational age (GA), cervico-vaginal fluid (CVF) sample was obtained for laboratory workup.
4. At 30-wk GA, CL estimation and CVF samples were repeated.

Methods:-

1. CVF sampling and processing: A vaginal speculum was applied and Dacron swab was placed in the posterior vaginal fornix to obtain high vaginal smear of CVF, the swab was maintained in situ for 10 seconds to achieve saturation, then 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 seconds 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 (110 ml) and stored at -80°C until being ELISA assayed.
2. Cervical cerclage (CC) was performed using the Shirodkar procedure with a non-absorbable suture within 4 days after enrolment of women of groups A and C. After conduction of CC procedure 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. As long as no membrane rupture, hemorrhage or labor, the cerclage suture was removed at 37 weeks' gestation.
3. Progesterone regimen was prescribed for women of groups B and C as VP (Crinone 200 mg, Akorn Inc., Millbrook, USA) to be inserted at night.
4. Laboratory Parameters: CVF levels of IL-18, IL-6 and Granulocyte-macrophage colony-stimulating factor (GM-CSF) 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. IL-18 was measured with Human IL-18 ELISA kit from Quantikine, R&D Systems Inc., Minneapolis, MN, USA ⁽¹⁰⁾.
2. IL-6 ⁽¹¹⁾ levels were measured with an ELISA kit from Pelikine™ Inc., Concord, USA.
3. GM-CSF ⁽¹²⁾ levels were measured with Human GM-CSF ELISA Kit from Thermo Scientific™ Inc., Waltham, Massachusetts, USA.

Study Outcomes:-

1. Primary outcome: the frequency of PTB among studied groups
2. Secondary outcome: the effect of applied therapeutic lines on CVF levels of studied cytokines.

Statistical analysis:-

Obtained data were presented as mean±SD, numbers and percentages. Results were analyzed using paired t-test and Chi-square test (X^2 test). Possible relationships were investigated using Spearman's linear regression. Sensitivity & specificity of estimated parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

Results:-

The study included 268 pregnant women with previous history of PTB; 72 women were excluded and 196 women were included in the study. There were non-significant ($p>0.05$) difference between enrolled women as regards demographic and clinical data (Table 1).

Table 1:- Demographic and clinical data of studied women

Data		Control group (n=27)	Group A (n=72)	Group B (n=40)	Group C (n=57)
Age (years)		26.5±3.8	27.3±4.3	26.9±3.6	27±2.8
Body weight (kg)		82.4±10.5	83.1±8.3	81.1±8.9	79.4±9.5
Body height (cm)		165.4±3.8	165.7±3.5	166.1±3.3	167±2.4
Body mass index (kg/m ²)		30.2±3.9	30.3±3.2	29.4±3.4	28.5±3.6
Obstetric history	Gravidity	3.3±1.4	2.8±1.2	3.1±1	3±1.3
	Parity	2.3±1.2	2.5±1.6	2.4±1.1	2.1±1.5
Blood pressure	Systolic	117.4±5	118.1±4.5	119.1±2.6	118.7±3.9

(mmHg)	Diastolic	82±4.8	83.4±3	84.2±2.5	83.1±3.9
Fasting blood glucose (mg/dl)		126.5±25.1	125.9±18.8	124.4±19.5	125.4±20.8

Data are presented as mean±SD

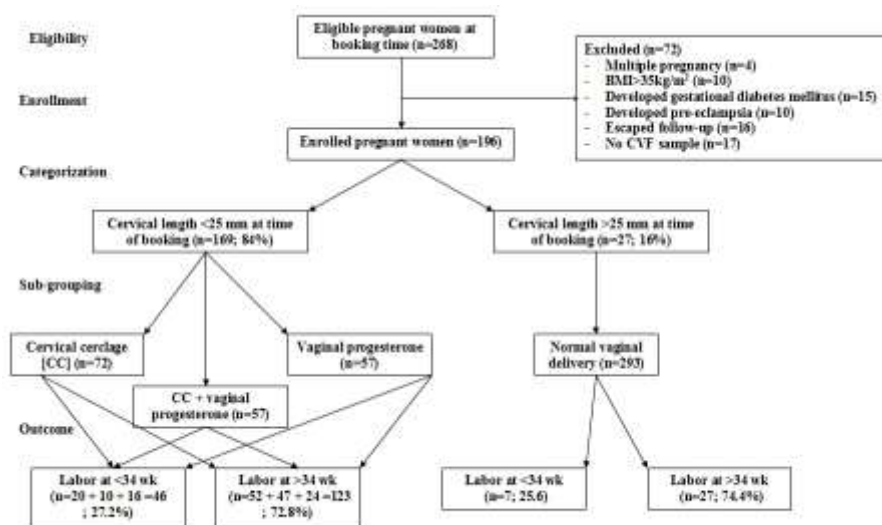


Fig 1: Study Flow Chart

At 24-wk GA, TVU defined 27 women had CL>25 mm and 169 women had CL<25 mm with non-significant ($p>0.05$) difference between study subgroups. Mean CL estimated at 30-wk GA was significantly shorter in study compared to control women, with non-significant ($p>0.05$) difference between study subgroups. At the 30th wk GA, the percentage of CL change (CL%) showed non-significant ($p>0.05$) difference between control and study women, as a total and subgroups (Fig 2).

Fifty-three women had PTB prior to 34 wk GA; 7 controls and 46 study women with non-significantly ($p>0.05$) higher frequency among control than study women. Women of group B showed significantly ($p=0.049$) higher frequency of PTB than women of group C with non-significantly ($p>0.05$) higher frequency among women of group A versus group C.

Estimated IL-6 levels in 24-wk CVF samples was significantly ($p=0.001$) higher in study than control women with non-significant ($p>0.05$) difference between women of study subgroups compared to control women and to each other. IL-6 levels in 30-wk samples were decreased significantly in women of groups A ($p=0.002$) and C ($p=0.043$) compared to their 24-wk levels with non-significantly ($p>0.05$) lower levels compared to 30-wk levels in control women. On contrary, women of group B showed significantly higher IL-6 levels compared both to their 24-wk levels and to 30-wk levels of control women and women of groups A and C. Interestingly, IL-6 levels in 30-wk CVF samples of control women were non-significantly ($p>0.05$) higher compared to their levels estimated in 24-wk samples.

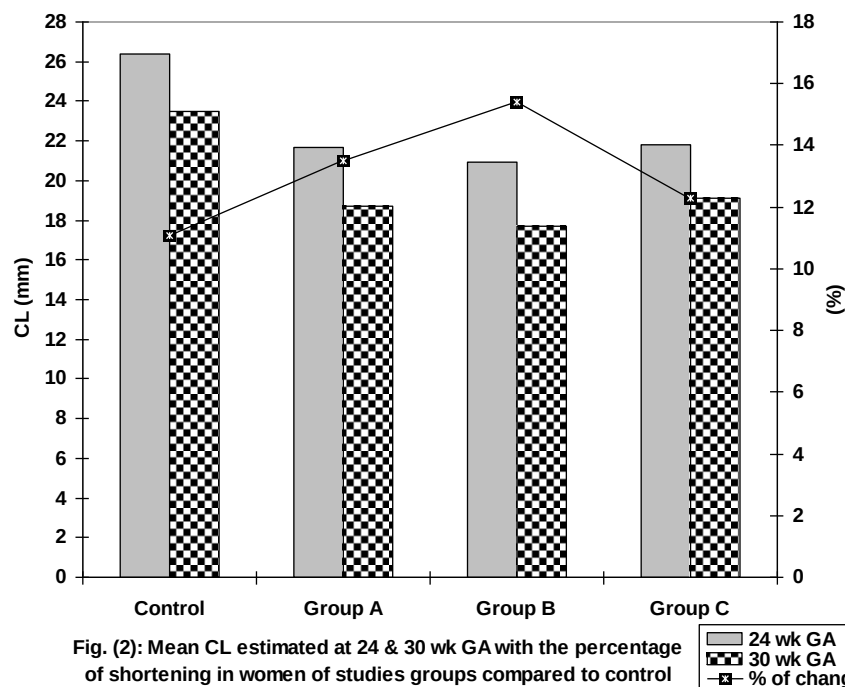


Fig. (2): Mean CL estimated at 24 & 30 wk GA with the percentage of shortening in women of studies groups compared to control

Estimated IL-18 levels in 24-wk CVF samples were non-significantly ($p>0.05$) higher in study than control women with non-significant ($p>0.05$) difference between women of study subgroups and control women and to each other. IL-18 levels in 30-wk samples decreased significantly in women of study subgroups compared to their 24-wk levels with non-significant ($p>0.05$) inter-group differences.

As regards GM-CSF, estimated 24-wk levels were non-significantly ($p>0.05$) higher in study than control women with non-significant ($p>0.05$) difference between women of study subgroups compared to control women and to each other. GM-CSF levels in 30-wk samples were significantly lower in study women compared to their 24-wk levels and to 30-wk levels in control women. Moreover, 30-wk levels were significantly ($p=0.013$) higher in women of group B compared to those of group A, but were non-significantly ($p>0.05$) higher than women of group C, despite of the significantly ($p=0.001$) lower percentage of decrease compared to groups A and C (Table 2).

Table 2:- Mean estimated levels of parameters studied in CVF at 24th and 30th wk GA

Parameter	Time	Control group (n=27)	Study group			
			Total (n=169)	Group A (n=72)	Group B (n=40)	Group C (n=57)
IL-6 (ng/ml)	24 th wk	9.78±3.46	13.56±4.98*	14.1±4.9	14.33±6.73	12.33±5.1
	30 th wk	11.48±3.4	12.7±5.81	11.38±4.58	18±5.61#*†‡	10.39±5.05
	% of change	16.1±7.8	-13.1±22.8	-24.5±14	20.5±9.9	-22.4±13.5
IL-18 (ng/ml)	24 th wk	0.55±0.2	0.6±0.26	0.59±0.24	0.67±0.28	0.58±0.29
	30 th wk	0.63±0.23#	0.54±0.24	0.54±0.22#	0.59±0.25#	0.52±0.25#
	% of change	11.8±4.1	-11.7±4.34	-11±4.14	-13.8±5	-11.2±3.7
GM-CSF (ng/ml)	24 th wk	7.67±1.66	7.93±2.1	7.75±2.15	7.9±2.2	8.19±1.93
	30 th wk	10±1.79#	5.95±1.97*	5.54±2.2#*	6.7±2.2#*†	5.93±1.7#*
	% of change	23.9±5.94	-37.8±20.7	-45.3±21.9	-19.3±11.7†‡	-41.2±16.1

Data are presented as mean±SD; CC: Cervical cerclage; CC+P: Cervical cerclage & progesterone; *: significant difference versus control group; †: significant difference versus CC group; ‡: significant difference versus CC+P group; #: significant difference versus levels estimated at the 24th wk GA

Duration of pregnancy showed negative significant correlation with the percentage of change of CL ($r=-0.719$, $p<0.001$), but was significantly and positively correlated with the percentage of decrease of IL-6, IL-18 and MG-

CSF levels in CVF and with considering cervical cerclage as a line of management. The percentage of decrease of IL-6, IL-18 and MG-CSF levels in CVF showed positive significant correlation with taking cervical cerclage as line of management (Table 3).

Verification of evaluated parameters as predictors for prolongation of duration of pregnancy using Regression analysis defined the extent of CL shortening as a negative predictor and the extent of decreased MG-CSF level in CVF as a positive predictor for prolonged duration of pregnancy beyond 34 wk GA. Among the studied laboratory parameters, the extent of decreased IL-6 levels in CVF was the only significant positive predictor for prolonged duration of pregnancy (Table 4). ROC curve analysis defined the percentage of decrease in CL as a significant sensitive predictor, while the minimal decrease in CVF level of IL-18 as significant specific predictor for PTB (Table 5, Fig 3).

Table 3:- Correlation coefficient between the percentage of change of CL and estimated levels of parameters studied in CVF with duration of pregnancy and CC as line of management

Parameters		CL	IL-6	IL-18	MG-CSF	CC
Duration of pregnancy (wks)	"r"	-0.719	0.297	0.176	0.262	0.194
	p	<0.001	<0.001	0.014	<0.001	0.006
Cervical Cerclage as line of management	"r"	0.039	0.851	0.392	0.686	
	p	>0.05	<0.001	<0.001	<0.001	

CL: Cervical length; IL: Interleukin; MG-CSF: Granulocyte-macrophage colony-stimulating factor; CC: cervical cerclage; "r": Pearson's correlation coefficient

Table (4):- Regression analysis for the percentage of change of CL and estimated levels of parameters studied in CVF with duration of pregnancy

Parameters			β	t	p
All parameters	Model 1	Percentage of decreased CL	0.703	14.704	<0.001
		Percentage of decreased MG-CSF CVF levels	0.211	4.421	<0.001
	Model 2	Percentage of decreased CL	0.719	14.39	<0.001
Laboratory parameters		Percentage of decreased IL-6 CVF levels	0.297	4.330	<0.001

CL: Cervical length; IL: Interleukin; MG-CSF: Granulocyte-macrophage colony-stimulating factor; CC: cervical cerclage; "r": Pearson's correlation coefficient

Table 5:- ROC curve analysis of percentage of change of evaluated parameters as predictors for pregnancy duration of >35 wk

Parameters	AUC	SE	p	95% CI
Decreased CL	0.279	0.073	0.008	0.136-0.427
Decreased IL-6 levels in CVF	0.477	0.085	0.781	0.311-0.643
Decreased IL-18 levels in CVF	0.619	0.069	0.044	0.484-0.754
Decreased MG-CSF levels in CVF	0.395	0.098	0.153	0.203-0.587

CL: Cervical length; IL: Interleukin; MG-CSF: Granulocyte-macrophage colony-stimulating factor; CC: cervical cerclage; "r": Pearson's correlation coefficient

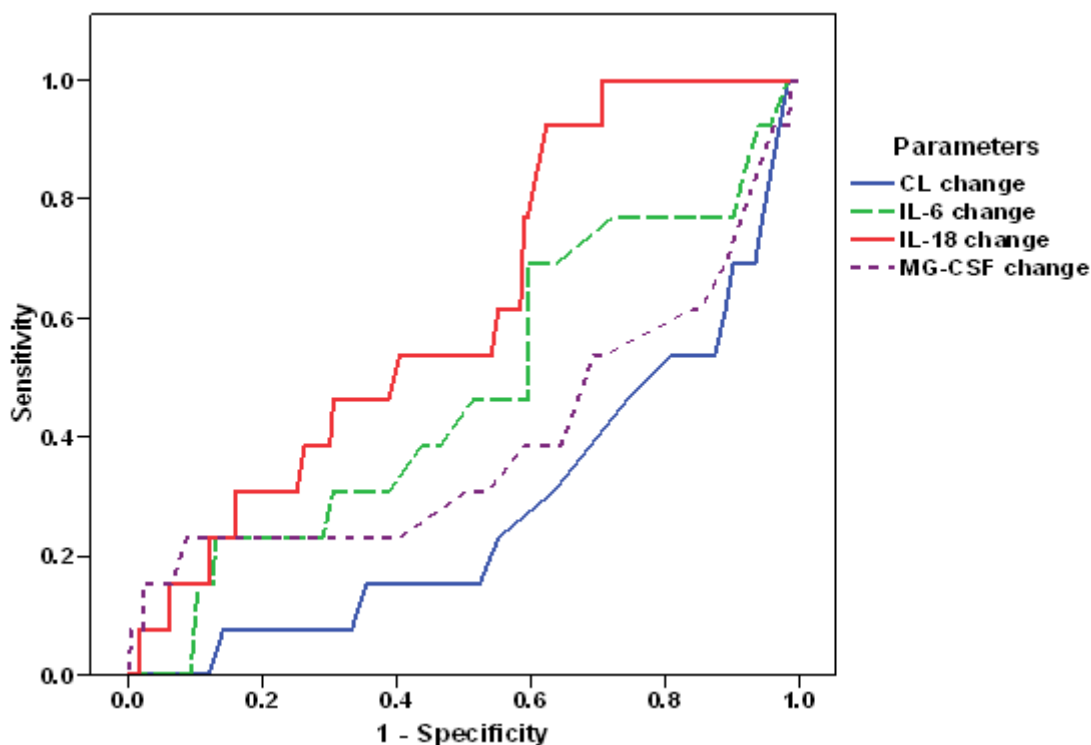


Fig 3:- ROC curve analysis of percentage of change of evaluated parameters as predictors for pregnancy duration of >35 wk

Discussion:-

The results of the current study could allow fulfillment of its target as it yielded multiple positive findings; firstly, the reliance on follow-up measurements of cervical length (CL) of women with history of PTB is insufficient to guard against its recurrence as evidenced by the reported frequency of PTB (25.6%) among women had baseline CL of >25 mm. In line with these data, **Jung et al.**⁽¹³⁾ reported a spontaneous PTB (sPTB) rate of 49% among women had significantly more advanced cervical dilatation at presentation. Furthermore, **Melle et al.**⁽¹⁴⁾ documented that the position of cerclage determined by TVU in immediate postoperative is not predictive of PTB risk.

Secondly, cervical cerclage (CC) did well than vaginal progesterone (VP) for prevention of PTB and prolongation of duration of pregnancy as manifested by the significantly higher frequency of PTB among women received VP than those had CC alone or with VP. Similarly, **Wang et al.**⁽¹⁾ reported that CC showed more benefits for both maternal and neonatal outcomes than VP for women with asymptomatic short cervix and prior PTB history. Also, **Ciavattini et al.**⁽¹⁵⁾ found CC is a valid therapeutic option in presence of cervical insufficiency (CI) when signs of premature labor or infection are not present, with lower expectations of a dilatation >5 cm. Recently, in 2018, **Sinkey et al.**⁽¹⁶⁾, documented that combination of CC with intramuscular progesterone resulted in similar PTB prevention to CC alone.

Thirdly, the detected high 30-wk cervico-vaginal fluid (CVF) levels of IL-6 and 18 with high levels of GM-CSF than 24-wk levels estimated in women of control and VP alone groups points to a possible role for disturbed local immunity in pathogenesis of PTB. In support of such assumption, the reported high frequency of PTB women maintained on VP only and the unpredictable frequency of PTB in control women with CL>25 mm.

These results and suggestions go in hand with **Monsanto et al.**⁽³⁾ who found CI patients had elevated levels of proinflammatory cytokines in CVF than normal women; suggesting dysregulation of local immune environment. Also, **Jung et al.**⁽¹³⁾ who detected significantly higher amniotic fluid (AF) mean neutrophil-lymphocyte ratios (NLR) and IL-6 and IL-8 levels in women had sPTB at <32 wks than those had sPTB at >32 wks. Moreover, **Son et al.**⁽¹⁷⁾ assayed AF samples of women with CI for 15 cytokines and detected significantly increased levels of most of assayed cytokines compared to control women. Recently, in 2018, **Song et al.**⁽¹⁸⁾ reported significant differences in inflammatory cytokines between women with indicated cerclage and control women.

Fourthly, the reported significantly lower CVF levels of studied cytokines at 30-wk than 24-wk, points to a down-regulating effect of CC on local production of inflammatory cytokines. In line with this finding, **Monsanto et al.**⁽³⁾ found cerclage intervention led to a significant decline in CVF pro-inflammatory cytokines content, suggesting that cerclage may help reduce local inflammation in CI and **Kiefer et al.**⁽¹⁹⁾ also reported that cerclage placement for mid-trimester short cervix for PTB prevention appears beneficial in patients with high inflammation and.

As a support for a possible role for CC for reducing levels of pro-inflammatory cytokines, duration of pregnancy showed positive significant correlation with the percentage of decrease of IL-6, IL-18 and MG-CSF levels in CVF and with considering CC as a line of management. Moreover, the percentage of decrease of cytokine levels in CVF showed positive significant correlation with taking CC as line of management.

Similarly, **Jung et al.**⁽¹³⁾ in a multivariable analysis, found high NLR and amniotic fluid IL-8 levels showed a significant correlation with the occurrence of sPTD at <32 wk. Also, **Bernabeu et al.**⁽²⁰⁾ assured that in women at risk for immature or PTB, cerclage for CI demonstrates good perinatal prognosis without increasing maternal morbidity in either singleton or twin pregnancies. Also, **Ito et al.**⁽²¹⁾ reported that rescue CC contributes to prolonged pregnancy and promote neonate survival and was associated with a higher likelihood of reaching at least 28 weeks gestation before delivery in women with bulging fetal membranes. Recently, **Song et al.**⁽¹⁸⁾ reported that when cerclage was indicated on physical examination, AF levels of IL-1 α , IL-6 and TNF- α showed significantly negative association with cerclage-to-delivery interval.

Fifthly, statistical analyses defined the extent of CL shortening as a negative predictor and the extent of decreased MG-CSF level in CVF as a positive predictor for prolonged duration of pregnancy beyond 34 wk GA, but among the studied laboratory parameters, the extent of decreased IL-6 levels in CVF was the only significant positive predictor for prolonged duration of pregnancy.

These findings are in line with **Son et al.**⁽¹⁷⁾ who found the levels of IL-1 β , 6, 7, 15 and 17 α , tumor necrosis factor- α (TNF- α) and Macrophage inflammatory proteins (MIP)-1 α and β were higher in patients with a very PTB than in those with a late PTB, but IL-1 β , 6, 7, 17 α and TNF- α were independently associated with a very PTB. Moreover, **Ito et al.**⁽²¹⁾ found protruding membranes; elevated AF neutrophil elastase levels and peripheral C-reactive protein levels were associated with a significantly reduced likelihood of reaching 28 wk GA before delivery. Also, **Diago Almela et al.**⁽²²⁾ reported that AF IL-6 levels had the highest diagnostic accuracy for good prognosis of rescue cerclage in cases of bulging membranes.

In support of the selection of IL-6 estimation as specific predictor for prolonged duration of pregnancy, **Diago Almela et al.**⁽²²⁾ documented that rescue cerclage and low IL-6 were the best predictors of good outcome of women with bulging membranes and **Jung et al.**⁽²³⁾ found AF IL-6 level at cutoff point of ≥ 7.6 ng/ml could accurately diagnose intra-amniotic inflammation in women with CI or a short cervix.

Conclusion:-

Disturbed local immune milieu towards increased pro-inflammatory cytokine production plays a role in pathogenesis and acceleration of PTB. Cervical cerclage induced down-regulation of inflammatory cytokines production and allowed prolongation of duration pregnancy; thus CC works beyond its mechanical preventive effect.

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