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CERVICOVAGINAL FLUID CYTOKINES' LEVELS IN PRETERM BIRTH HIGH-RISK WOMEN COULD BE USED AS EARLY PREDICTORS FOR RECURRENT PRETERM BIRTH

By

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ABSTRACT

Background: Differences in cytokine distribution across different biological fluids; maternal plasma, cervicovaginal fluid (CVF), and amniotic fluid are evident throughout normal pregnancy, but profiles of numerous cytokines varied across trimesters in women delivering term versus preterm in both CVF and serum.

Objective: To evaluate cytokine profile in cervicovaginal fluid (CVF) of women had previous preterm birth (PTB) and the effect imposed by cerclage on these levels.

Patients and Methods: Seventy study women with previous PTB and currently had cercical length (CL) of <25 mm and 20 control women with no history of PTB and had CL of >25 mm were studied. At the 24th gestational week (GW; T1), all women had CL estimation and CVF sampling. At time of labor or removal of the suture (T2), study women had CL re-estimation and CVF re-sampling. CVF levels of monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 were ELISA estimated. Shirodkar cervical cerclage (CC) was performed within 4 days after T1 sampling. Outcomes included difference in T1-CVF cytokines' levels between study and control women, effect of cerclage on CL and CVF cytokines' levels and the value of T1-CVF cytokines' levels as predictors of pregnancy duration.

Results: T1-CVF levels were significantly higher in study than control women and to their T2- levels. T1- and T2-CL in study women was significantly shorter than T1-CL in controls with significant difference between T1- and T2-CL. Percentage of T2-CL decrease was negatively correlated with the percentage of decrease of T2-CVF levels. Duration of pregnancy of study women was negatively correlated with percentage of CL shortening, while was positively correlated with percentage of decrease of T2-CVF levels of MCP-1 and TNF-α. Statistical analyses defined high T1-CVF levels of MCP-1, IL-β and TNF-α as significant early predictor for PTB.

Conclusion: High CVF cytokines' levels were associated with increased risk of PTB. Cerclage worked beyond its mechanical action through reduction of CVF cytokines' levels. High CVF cytokines' levels at 24th GW, especially high levels of MCP-1 and TNF-α, may help to predict PTB.

Keywords: Preterm birth, Cervicovaginal fluid, Cytokines, Pregnancy duration

INTRODUCTION

Cervical insufficiency (CI) is defined as premature, progressive dilation and shortening of the cervix during pregnancy *(Monsanto et al., 2017)*. Mid-trimester CI has deleterious maternal and fetal effects secondary to development of intra-amniotic infection/inflammation that occurs in up to 50% of women who developed CI *(M?nckeberg et al., 2019)*. CI if untreated it can lead to prolapse and rupture of amniotic membrane resulting in mid-trimester pregnancy loss or preterm birth (PTB), which leads to severe newborn complications *(Raja et al., 2019)*.

Midtrimester ultrasound is a valuable method for identifying asymptomatic women at risk for spontaneous preterm delivery *(Kiefer et al., 2016)*. Cervical cerclage, vaginal progesterone and pessary were used in multiple studies and showed similar effectiveness for management of women with short cervix or previous PTB *(Alfirevic et al., 2013)*. However, the response to various treatments was variable in the clinical setting *(Kiefer et al., 2016)*.

Multiple previous studies found vaginal progesterone reduced the rate of early PTB in women with short cervix with reduction of rate of neonatal morbidity/ mortality in singleton pregnancies, but failed to prevent PTB in twin pregnancies *(Rode et al., 2011, Serra et al., 2013* and *Schuit et al., 2015)*. The use of a pessary proved to prevent PTB in asymptomatic women with mid-trimester short cervical length (CL); however, the precise mechanisms for this effect remain unclear *(Mendoza Cobaleda et al., 2019)*. Cervical cerclage (CC) involves positioning of a stitch around the cervical neck to give the cervix mechanical support to reduce risk of PTB *(Alfirevic et al., 2017)*.

Differences in cytokine distribution across different biological fluids; maternal plasma, cervicovaginal fluid (CVF), and amniotic fluid are evident throughout normal pregnancy *(Ashford et al., 2018a)*, but profiles of numerous cytokines were varied across trimesters in women delivering term versus preterm in both CVF and serum *(Ashford et al., 2018b)*.

This study aimed to evaluate the cytokine profile in CVF of women with history of previous PTB and the effect imposed by cerclage on these levels.

PATIENTS AND METHODS

Design: Prospective comparative study.

Setting: Obstetrics & Gynecology Department in association with Clinical Pathology Department, Faculty of Medicine, Al-Azhar University.

Time: Thirty months starting from January 2016 till June 2018.

Patients: The study included women with history of previous PTB and currently were pregnant in singleton fetus, free at time of diagnosis of pregnancy-induced complications and having CL of <25 mm as determined by transvaginal ultrasonography (TVU). Exclusion criteria included multiple pregnancies, fetal anomalies, diabetes mellitus, systemic or local inflammatory disorders and current vaginal infection at time of sample collection. Pregnant women having singleton fetus, had no history of previous PTB, free at time of diagnosis of pregnancy-induced complications, having

CL of >25 mm and free of exclusion criteria were included as control women.

At time of 1st attendance, baseline data included age, number of previous pregnancies and its outcome, blood pressure and random blood glucose level of pregnant women eligible for evaluation and then all women underwent full obstetric history taking and examination including TVU. Women were asked to attend the Antenatal Care Unit (ACU) at the beginning of the 24th gestational week (GW; T1 time) for assurance of inclusion and exclusion criteria especially the presence of cervicovaginal infections, TVU estimation of CL (T1 CL) and women with CL <25 mm were considered at a risk for having PTB *(Owen et al., 2001)*. Samples of cervicovaginal fluid (CVF) were assayed for T1 levels of cytokines and were twenty cross-matched age and body mass index pregnant women, free of previous PTB, fulfilling the inclusion and exclusion criteria for the study group and has T1 CL> 25 mm were included as control group.

All enrolled women were maintained on vaginal toilet to guard against development of infection. Women of the study were asked to attend the ACU biweekly for follow-up for development of PTB, which was defined as any birth before 37 completed weeks of gestation *(Meher and Alfirevic, 2014)*. At time of labor or removal of the suture (T2), all study women undertook estimation of CL (T2 CL) and another CVF sample was obtained to estimate T2 CVF cytokines' levels. For both T2 CL and CVF cytokines' level. The percentage of change between T1 and T2 measures was calculated as T1-T2 measures divided by T1 measures and multiplied by 100 (% of change= ([T1-T2 levels]/[T1 level]) x (100)).

Methods

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^{o}C .

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^{o}C until being ELISA assayed.

Investigations: CVF levels of monocyte chemo attractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 were measured using ELISA kits according to the manufacturer's instructions and were read using a 96 well micro plate ELISA reader (Dynatech, MR 7000):

1. Monocyte chemoattractant protein-1 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab181421, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique *(Deshmane et al., 2009)*.
2. 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 *(Coughlan et al., 2001)*.

3. IL-1β was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46052, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique *(Heng et al., 2014)*.
4. IL-6 using the Eagle Biosciences Human IL-6 ELISA Assay Kit (Eagle Bioscience Inc., USA; Catalogue No IL631-K01) which employs the quantitative sandwich enzyme immunoassay technique *(Gaines-Das and Poole, 1993)*.

Cervical cerclage: Cervical cerclage (CC) was performed using the Shirodkar procedure with a non-absorbable suture within 4 days after T1 CVF sample obtaining. 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:

1. Primary outcome: The difference in T1-CVF cytokines' levels between women with PTB (Study group) and without history of PTB (Control group).

2. Secondary outcomes :

a. The frequency of PTB among study women.

b. The effect of cerclage on CL and CVF cytokines' levels defined as the calculated percentage of change.

c. The value of T1-CVF cytokines' levels as predictors for extent of shortening of CL as measured on T2 time and for duration of gestation.

Statistical analysis:

Obtained data were presented as mean±SD, numbers and percentages. Results were analyzed using paired t-test, One-way ANOVA Test followed by post – HOC test (Bonferroni procedure) and Chi-square test (X2 test). Possible relationships were investigated using Pearson linear regression analysis. Regression analysis (Stepwise method) was used for stratification of T1 estimated cytokines' levels as specific predictors. T1 estimated CVF levels as predictors for duration of pregnancy were evaluated using the 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) for Windows statistical package. P value <0.05 was considered statistically significant.

RESULTS

During 30 month study duration, 70 women at high-risk of PTB and fulfilling the inclusion criteria were enrolled as study group and 20 women free of risk for PTB and were age and BMI-matching women of study group were also enrolled in the study as control group. There were non-significant differences between study and control women regarding inclusion criteria as shown in table 1 apart from the presence of history of previous PTB (Table 1).

Table (1): Enrolment data of women included in both groups

Variables \ Groups		Control (n=20)	Study (n=70)	P value
Age (years)		28.5±5	28.9±3	> 0.05
BMI data	Body weight (kg)	85.5±8.3	87.4±7	> 0.05
	Body height (cm)	170.7±3.7	169.9±3.1	> 0.05
	BMI (kg/m^2)	29.4±3.2	30.3±2.9	> 0.05
Family history of PTB	Yes	3 (15%)	27 (41.4%)	0.048
	No	17 (85%)	43 (58.6%)	
Blood pressure measures (mmHg)	Systolic	112.8±5	114.1±3.6	> 0.05
	Diastolic	73.3±8.1	72.3±4.3	> 0.05
Obstetric history	Gravidity	2.5±0.5	2.7±0.7	> 0.05
	Parity	1.4±0.5	1.5±0.9	> 0.05
	Previous PTB	0	70	> 0.05
Fasting blood glucose (mg/dl)		86.7±9.3	89.9±10.2	> 0.05

Data were presented as mean±SD, numbers & percentages; BMI: Body mass index; PTB: Preterm birth

Mean T1-CVF cytokines' levels were significantly higher in women of study group than in women of control group, while mean T2-CVF cytokines' levels were significantly lower in study women compared to their corresponding T-1 levels. On comparison to T1 control levels, mean T2-CVF levels of MCP-1 were non-significantly higher than T1 levels, while mean T2-CVF of TNF-α, IL-1β and IL-6 were still significantly higher than T1-levels of control women. The percentages of decreased T2 cytokines' levels were 57±19.7%, 36.9±3.8%, 20.1±8% and 38.9±10%, for MCP-1, TNF-α, IL-1β and IL-6, respectively (Table 2).

Table (2): Mean levels of cytokines estimated in CVF of women included in both groups

Parameters \ Groups		Control (n=20)	Study (n=70)		
			T1 levels	T2 levels	% of change
MCP-1 (g/ml)	Level	9.97±3.6	28±14.4	10.2±4.1	57±19.4
	P1		<0.001	> 0.05	
	P2			<0.001	
TNF-α (ng/ml)	Level	0.811±0.24	1.51±0.47	0.947±0.288	36.9±3.8
	P1		<0.001	0.047	
	P2			<0.001	
IL-1β (ng/ml)	Level	6.25±1.85	16±6.2	12.64±4.7	20.1±8
	P1		<0.001	<0.001	
	P2			<0.001	
IL-6 (ng/ml)	Level	4.9±1.5	9.7±2.8	6±2.1	38.9±10
	P1		<0.001	0.036	
	P2			<0.001	

Data were presented as mean±SD; T1: at 24th GW; T2: at time of spontaneous labor; MCP-1: Monocyte chemoattractant protein-1, TNF-α: Tumor necrosis factor-α, IL: Interleukin; P1 indicates significance versus control levels; P2 indicates significance versus T1 levels.

Mean T1-CL (23.6±2.1 mm) and T2-CL (18.9±1.7 mm) in study women was significantly shorter compared to T1-CL of control women (26.8±1.6 mm) with significantly shorter T2-CL than the corresponding T1-CL in study women. The percentage of T2-CL decrease in relation to T1-CL was negatively correlated with the percentage of decrease of T2-CVF levels of studied cytokines in relation to T1-CVF levels (Table 3).

Throughout duration of pregnancy, 13 study women (18.6%) had PTB (<37GW) for a mean duration of pregnancy of 35±0.8 GW, while the remaining 57 women (81.4%) had mean duration of pregnancy of 38.2±1.3 GW.

There were negatively significant correlations between duration of pregnancy and the percentage of CL shortening, while the correlations were positively significant between duration of pregnancy and the percentage of decrease of T2-CVF levels of MCP-1 and TNF-α in relation to T1 levels. However, duration of pregnancy showed positive non-significant correlation with percentage of decrease of T2-CVF levels of IL-6 and IL-1β (Table 3).

Table (3): Pearson's correlation between percentage of decrease T2 CL and CVF levels of studied cytokines and duration of pregnancy

		Percentage of decrease of CL		Duration of pregnancy	
		"r"	p	"r"	p
Percentage of decrease of CL				-0.317	0.008
Percentage of decrease of T2 CVF levels of	IL-6	-0.289	0.015	0.198	0.101
	TNF-α	-0.327	0.006	0.275	0.021
	IL-1β	-0.317	0.008	0.208	0.084
	MCP-1	-0.492	0.0006	0.381	0.001

Regression analysis of high T1-CVF cytokines' levels as predictors for short pregnancy duration excluded high T1-CVF level of IL-6 and TNF-α as predictors, but defined high T1-CVF levels of MCP-1 (β=-0.486, <0.001) and IL-β (β=-0.345, p=0.001) as significant early predictors for short duration of pregnancy and development of PTB. On other hand, ROC curve analysis defined high T1-CVF level of MCP-1 and TNF-α as the significant (p=0.006 & 0.004) early

predictor for PTB with AUC= 0.872 (95% CI: 0.744-1.001) and 0.885 (95% CI: 0.728-1.041), respectively.

DISCUSSION

Women had history of previous PTB were at high-risk for redeveloping PTB, throughout follow-up during the current study, PTB occurred by a rate of 18.6%, among studied high-risk women, which is higher than that reported by *Blencowe et al. (2013)* who reported that worldwide an estimated 11.1% of all live births in 2010 were born preterm, with rates increasing in most countries with reliable trend data. Also, the reported PTB rate was higher than that reported by *Hernandez-Andrade et al. (2014)* who found the spontaneous PTB at < 37 GW among women with internal cervical os low strain values on US elastography was 11%. On the other hand, the reported figure for PTB among studied high-risk women was in accordance with *Chan et al. (2015)* who reported PTB in 17.4% of women who had history-indicated cerclage, but was lower than that reported by *Sun et al. (2016)* and *Jung et al. (2016)* who reported spontaneous PTB rate of 35.6% and 49%, respectively, among women who had significantly more advanced cervical dilatation at presentation

For estimation of cytokines' concentrations, the current study used cervicovaginal fluid (CVF) sample that was collected as high as possible from the vagina; depending on previous findings that levels of proinflammatory cytokines were elevated in CVF not in serum in patients with cervical insufficiency than in control women *(Monsanto et al., 2017)*. Also, *Short et al. (2018)* detected highest concentrations of IL-1β, IL-8 and IL-6 in ranking order of cervical fluid> CVF > plasma, and documented that CVF collection was simpler, provided the largest volume of sample with the potential for undiluted usage, and allowed for self-insertion.

Mean T1-CVF cytokines' levels were significantly higher in study than in control women; this finding spots light on a possible role for disturbed local cytokine milieu for development of PTB. In support of this assumption, there was negative significant correlation between duration of pregnancy and T1-CVF cytokines' levels and such correlation was highly significant with T1-CVF levels of TNF-α and MCP-1.

In line with these findings and assumption, *Monsanto et al. (2017)* reported that women with cervical insufficiency have higher CVF levels of proinflammatory cytokines in comparison to normal women. Also, *Ashford et al. (2018b)* found CVF values of IL-6, 8 and 10, TNF-α, and CRP was significantly higher in those who delivered preterm than those who delivered full-term. Moreover, *Caritis et al. (2018)* detected significantly elevated baseline cervical fluid concentration of IL-6 and 10, TNF-α among women whose earliest prior delivery occurred between 16 and 23 GW compared to those with earliest delivery occurred between 32 and 36 GW and these levels were unaffected by 17-hydroxyprogesterone caproate. Recently, *Buxton et al. (2019)* detected high concentrations of CVF cytokines measured in a monthly sample collected from the 2nd-9th month of term pregnancies, but these levels were largely stable over time and documented that this

finding indicated that pregnancy is associated with an active inflammatory state.

At time of labor or removal of stitch (T2), mean CVF levels were significantly lower than T1-CVF levels, but were non-significantly higher compared to T1-levels of control women. Similarly, *Monsanto et al. (2017)* detected significant decline in CVF proinflammatory cytokines after cerclage and suggested that cerclage may help reduce local inflammation in CI. Also, *M?nckeberg et al. (2019)* found patients free of intra-amniotic infection/inflammation who underwent cerclage had longer admission-to-delivery interval, higher gestational age at delivery and had higher neonatal survival rate than those with intra-amniotic infection/inflammation.

These results illustrate the beneficial effect of cerclage on local immune milieu and indicated an effect of cerclage more than its well-known mechanical effect as a preventive measure against PTB. This effect was manifested as decreased percentage of CL shortening and its positive significant correlation with the percentage of decrease in T2-CVF levels of examined cytokines. In accordance with these data, *Steenhaut et al. (2019)* detected significantly thicker chorion overlying the cervix in women had cerclage and significantly higher expression of 15-hydroxyprostaglandin dehydrogenase and toll-like receptor-2 expression with significantly decreased senescence than in chorion located far from the cervix, and attributed prevention of triggering of parturition to these membrane changes.

Duration of pregnancy showed positive significant correlation with the percentage of decrease of T2-CVF levels of MCP-1 and TNF-α and Regression analysis of high T1-CVF cytokines' levels defined high T1-CVF levels of MCP-1 and IL-β as significant early predictors for short pregnancy duration. ROC curve analysis defined high T1-CVF level of MCP-1 and TNF-α as the significant early predictor for PTB. In line with the obtained data, *Pandey et al. (2017)* found increased maternal levels of IL-1β and IL-6 and low levels of IL-10 were associated with PTB. *Sayaril et al. (2018)* found the levels of IL-1α, IL-6 and TNF-α can be used as predicative markers for PTB. *Lappas (2018)* reported that deficiency of mothers against decapentaplegic homolog (SMADs) in myometrial cells significantly increased IL-1β, 1A, 6 and 8, MCP-1, adhesion molecules and COX-2 mRNA expression with subsequent decreased PGF2α release.

CONCLUSION

Preterm birth in women who had history of previous PTB occurs frequently. High CVF cytokines' levels are associated with increased risk of PTB. Cerclage works beyond its mechanical action through reduction of CVF cytokines' levels. High CVF levels at 24th GW is predictive of PTB, especially high levels of MCP-1 and TNF-α. Wider scale studies are mandatory to define certain cutoff points for these cytokines.

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إستخدام قياس مستوى مادة السيتوكين فى سوائل المهبل وعنق الرحم فى السيدات الأكثر عرضه لحدوث ولادة مبكرة كوسيلة تنبؤية للولادة المبكرة المتكررة

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خلفيــة البحــث: إخـتلاف مسـتوى السـيتوكين فـى السـوائل الحيويـة سـواء كانـت بلازمـا الـدم أو السـائل المهبلـى أو السـائل الأمنيوسـى يمكـن ملاحظتـه بوضـوح طـوال فتـرة الحمـل ولكـن مسـتوى السـيتوكينات كانـت تختلـف أثنـاء فتـرات الحمـل المختلفـة عنـد مقارنـة السـيدات اللائـى يلـدن فـى الشـهر التاسـع واللائـى يلـدن ولاده مبكـره وذلـك عنـد قياسـهم فـى سـوائل المهبل وعنق الرحم أو عند قياسهم فى الدم.

الهــدف مــن البحــث: تقيـيم مـواد السـيتوكين فـى سـوائل المهبـل وعنـق الـرحم فـى السـيدات اللاتـى كـان لـديهن تـاريخ مرضـى للـولادة المبكـرة وتـأثير ربـط عنـق الـرحم علـى مسـتوى هذه المواد.

المرضــى وطــرق البحــث: أجريـت هـذه الدراسـة علـى (70) سـيدة اللائـى كـان لـديهن تـاريخ مرضـى بـولادة مبكـرة؛ وكـذلك كـان طـول عنـق الـرحم لـديهن أقـل مـن 25 مـم، وكـذلك أجريـت علـى (20) سـيدة أخـرى اللائـى لـم يكـن لـديهن ولادات مبكـرة سـابقاً وكـان طـول عنـق الـرحم لـديهن أكثـر مـن 25 مـم. وقـد أجـرى لكـل السـيدات تقيـيم دراسـة أولـي عـن طريـق قيـاس طـول عنـق الـرحم بالسـونار وكـذلك عينـة مـن سـوائل المهبـل وعنـق الـرحم عنـد الأسـبوع 24 مـن الحمـل. وقـد أجـرى لمجموعـة البحـث عمليـة ربـط لعنـق الـرحم بإسـتعمال طريقـة شـيرودكرز فـى الأسـبوع 24 مـن الحمـل فـى خـلال 4 أيـام بعـد أخـذ عينـة سـوائل المهبـل وعنـق الـرحم. وكـذلك تـم عمـل تقيـيم الدراسـة الثـانوى لتلـك المجموعـة وذلـك عنـد حـدوث الـولادة أو عنـد إزالـة ربـاط عنـق الـرحم عـن طريـق إعـادة قيـاس طـول عنـق الـرحم وكـذلك أخـذ عينـة أخـرى مـن سـوائل المهبـل وعنـق الـرحم. وقـد تـم قيـاس معـدل مـواد السيتوكين والأنثرليوكين بإستعمال طريقة اليزا.

- وقـد تضـمنت مخرجـات البحـث الفـرق فـى مسـتوى السـيتوكينات فـى سـوائل المهبـل وعنـق الـرحم، كمـا تضـمنت تـأثير ربـط عنـق الـرحم علـى طـول عنـق الـرحم ومسـتوى السيتوكينات.

- وكـذلك تقيـيم أهميـة قيـاس مسـتوى السـيتوكينات فـى سـوائل المهبـل وعنـق الـرحم كمتنبئ لطول فترة الحمل.

النتائج:

- أظهـرت هـذه الدراسـة أن مسـتوى السـيتوكين كـان أعلـى بشـكل كبيـر فـى مجموعـة البحـث عـن المجموعـة الضـابطة وذلـك فـى فتـرة تقيـيم الدراسـة الأولـى عنـد الأسـبوع 24 مـن الحمـل. كمـا أظهـرت هـذه الدراسـة أن مسـتوى السـيتوكين كـان أعلـى بشـكل كبيـر لـدى السـيدات فـى مجموعـة البحـث أكثـر مـن السـيدات فـى المجموعـة الضـابطة (فـى فتـرة تقيـيم الدراسـة الأولـى) أى عنـد الأسـبوع 24 مـن الحمـل، وكـذلك كـان مسـتوى السـيتوكين أعلـى فـى تلـك الفتـرة مـن مسـتواه (فـى فتـرة تقيـيم الدراسـة الثـانوى) أى عنـد الـولادة أو إزالـة رباط عنق الرحم.
- وقـد أظهـرت أيضـاً أن عنـق الـرحم كـان أقصـر بشـكل كبيـر فـى سـيدات مجموعـة البحـث عـن المجموعـة الضـابطة وذلـك عنـد الأسـبوع 24 مـن الحمـل وعنـد حـدوث الـولادة بفـرق إحصائى مهم فى طول عنق الرحم (فى الأسبوع 24 من الحمل وعند حدوث الولادة)
- كمـا وجـد كـذلك أن هنـاك علاقـة عكسـية بـين طـول عنـق الـرحم ومسـتوى السـيتوكين فـى عينـة سـوائل المهبـل فـى فتـرة حـدوث الـولادة وكـذلك بـين نسـبة قصـر عنـق الـرحم ومـدة الحمـل بينمـا كانـت هنـاك علاقـة طرديـة بـين قلـة نسـبة السـيتوكين فـى إفـرازات المهبـل وفترة الحمل.
- أن تستخدم كمتنبئات لحدوث الولادة المبكرة.

الإستنتاج:

- إرتفـاع نسـبة السـيتوكينات فـى إفـرازات المهبـل وعنـق الـرحم وتجعـل السـيدات أكثـر عرضة لحدوث ا لولادة المبكرة.
- ربـط عنـق الـرحم يـؤدى إلـى تـأثير اكبـر مـن التـأثير الميكـانيكى حيـث يـؤدى إلـى خفـض نسبة السيتوكينات.
- إرتفـاع معـدل السـيتوكينات فـى إفـرازات المهبـل عنـد الأسـبوع 24 مـن الحمـل يمكـن أن يساعد فى التنبؤ بحدوث الولادة المبكرة.