Issn 1110 -6352

THE EGYPTIAN JOURNAL OF FERTILITY AND STERILITY

Volume 29 Number 3 May-June, 2025

EFSS

The Egyptian Journal Of Fertility And Sterility

The Official Journal of the Egyption Fertility and Sterility Society (**EFSS)**

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The title page should contain:

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Acknowledgments

Acknowledgments should only be made to funding institutions and organizations and, if to persons, only to those who have made substantial contributions to the study.

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1- Standard journal article

List all authors when six or less. When seven or more, list only first six and addetal. Toppozada MK, Gaafar AA, Shaala SA. In - vivo inhibition of the human non pregnant uterus by prostaglan din E2. Prostaglandins, 1974; 8: 401 - 406.

2- Books:

(a) Personal author: Speroff L, Glass RH, Kase NO. clinical gynecologic endocrinology and infertility. 4th edition, Baltimore, Williams & Wilkins; 1988: 105

(b) Chapter in book; Wilhelmsson L, Norstrom A, Tjugum 1, Hamberger L. Interaction between prostaglan dins and catecholamines on cervical collagen. In: Toppozada M., Bygdeman '. M., Hafez ESE, Eds. Prostaglandins and fertility regulation. Advances in reproductive health care. Lancaster, England, MTP Press Ltd., 1985 : 75 - 80.

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National Center for Health Statistics. Acute conditions: incidences and associated disability, United States July 1908 - June 1909. Rockville. MD.: National Center for Health Statistics, 1972.

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Contents :

Letter from the Editor:

Dear colleagues,

very interesting subjects are included in this edition. More than 50% of women who have a twin pregnancy (TP) will give birth before 37 GW, making multiple pregnancies a significant risk factor for PTB. Most females with bacterial vaginosis are asymptomatic & are at a greater probability of adverse infectious complications because of cesarean delivery. The identification of bacterial vaginosis (BV) is rapid, uncomplicated, & cost-effective. BV is a significant issue of public health that is frequent among pregnant females & is related to surgery site infections. Performing bacterial vaginosis screening on women before undergoing a cesarean section can help to further decrease the occurrence of infections following surgery in a manner that is both safe and economical.

A fetal main pulmonary artery Doppler measurement, an amniotic fluid lamellar body count (LBC) and pulmonary artery index can be used to anticipate neonatal respiratory distress syndrome (RDS). In conjunction with lower LBC and higher Pulmonary Artery Pulsatility Index (PI) and Resistance Index (RI), these non-invasive methods of fetal lung maturity evaluation are very helpful in assessing the risk of RDS.

In patients with thin endometrium undergoing frozen-thawed embryo transfer cycles, the treatment of vaginal sildenafil citrate significantly enhances the biochemical pregnancy rate, the clinical pregnancy rate, and endometrial thickness. It also modifies the endometrial pattern. Furthermore, it was demonstrated that a combined evaluation of endometrial thickness and pattern was more beneficial for patient counseling than individual analysis and was a greater predictor of the outcome of IVF/ICSI-ET.

By screening women for the presence of psychiatric disorders during pregnancy, psychiatric disorders are common among pregnant women (45.8%), and screening during antenatal care (ANC) is so important to discover and give appropriate treatment early.

Intrauterine adhesions can result from surgical or hysteroscopic intervention, it can be corrected effectively and safely with the use of hysteroscopy to restore menstrual pattern and fertility.

The retroverted position of the uterus has been associated with an increased risk of developing more significant and larger CSN in terms of depth and width. This could be due to the altered angle and mechanical stress on the cesarean scar site, potentially affecting healing. These results may help in the counseling of patients regarding their subsequent pregnancies when a decision involving a Cesarean section is being considered.

Best regards.

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Twin Pregnancy, High Cervicovaginal Fluid Inflammatory Cytokines and Preterm Birth: A Vicious Cycle Endangers Pregnancy Outcome

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Abstract

Objectives: To compare outcome of management of women had twin pregnancy (TP) and presented at beginning of 2nd trimester with threatened preterm birth (PTB) and preterm short cervical length (CL) .

Patients & Methods: 52 women had CL>25 mm were grouped as Control group and received expectant management (EM) and 91 women had CL<25 mm and were randomly divided into Group C received cervical cerclage (CC) alone, Group P received vaginal progesterone (VP) therapy only and group C/P received both CC and VP therapy. Two cervicovaginal fluid (CVF) samples, before starting management (S1) and at time of labor (S2) were obtained for ELISA estimation of tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and vitamin D binding protein (VDBP) levels. Study outcomes included frequency of PTB, pregnancy duration (PD) and differences in cytokines' levels between women of study groups and between S1 and S2 samples.

Results: PTB rates at <35 and 35-<37 weeks are 20.3% and 44.7%, respectively with non-significant difference between studied groups. Mean PD was significantly longer and mean CVF cytokine's levels in S2 sample were significantly decreased with interventions than EM. Percentage of decreased IL-6 and TNF-α levels showed positive significant correlation with PD and was significantly higher in C/P than C and P groups and in C than P group. Estimated CVP levels of VDBP in S2 samples were non-significantly increased in women of control, but significantly increased in study women, but with non-significant correlation with outcomes.

Conclusion: PTB is a frequent co-accident with TP with an incidence of 20-45%. CC allows prolonged PD and reduce PTB rate especially if combined with VP therapy. Disturbed local CVF inflammatory milieu is closely related to shorter PD and having PTB. CC allowed reduction of CVF cytokines' levels by 50-60% of its levels at time of placement of cerclage.

Keywords: Twin pregnancy, cervical cerclage, Progesterone therapy, Preterm birth, cervicovaginal cytokines.

Introduction

The frequency of twin births has grown in 1980, one in every 53 births was a twin[1]; by 2014, that number had dropped to one in every 29 births [2]. Preterm birth (PTB) at less than 37 gestational weeks (GW) is still associated with a substantial risk of early mortality and a host of health and developmental issues [3]. Numerous genetic, biochemical, environmental, and sociodemographic variables are linked to complex etiology of PTB [4].

More than 50% of women who have a twin pregnancy (TP) will give birth before 37 GW, making multiple pregnancies a significant risk factor for PTB [5]. One of the biggest risk factors for recurrent PTB is a history of PTB [6]. It's interesting to note that the likelihood of recurrent PTB after spontaneous PTB in TP was higher than that of triplet delivery [6], and twin pregnancies are much more likely to experience PTB than singleton pregnancies [7].

A well controlled balance of immune cell interactions at the mother-fetal interface is necessary for a successful pregnancy [8]. The ability of the mother's immune system to modify itself in order to tolerate the fetus through immunoregulation is necessary for such balance to occur [9]. Furthermore, this balance is crucial to the inflammatory process of labor [8], and the pathophysiology of PTB may be underlying by the mother's shift from an anti- to a pro-inflammatory state at the decidual tissues [10].

The risk and incidence of PTB can be decreased by screening women who are at risk of developing PTB spontaneously. But only a small percentage of cases are found using traditional techniques such maternal cervical length (CL) screening and obstetrical history [11]. During pregnancy, the cervicovaginal region is metabolically active [12], and proteins found in its fluid play a role in immunological control [13]. As a result, cervicovaginal fluid (CCV) is a practical, easily accessible biological fluid that may be used to learn important details about the female reproductive system in both pregnant and non-pregnant individuals [14]. Additionally, early in pregnancy, changes in CVF elements may be noticed before any clinical symptoms appear [11]. These changes, by themselves or in conjunction with clinical risk factors, may aid in identifying women who are most at risk of PTB [15].

Objectives

To compare the outcome of management of women had TP and presented by preterm short cervical length (CL).

Design

Prospective clinical interventional study.

Setting

Departments of Obstetrics & Gynecology & Clinical Pathology, Faculty of Medicine, Tanta University.

Patients & Methods

The present investigation commenced in January 2017 according to the Local Ethical Committee's acceptance of the study methodology. All expectant mothers who had more than one pregnancy and showed signs of impending preterm birth (TPTB) were qualified for assessment. The definition of TPTB was described as the onset of painful, regular, and persistent uterine contractions that lasted longer than 30 minutes at a rate of one contraction per ten minutes (16), with or without cervical changes (17). Other symptoms that were observed at gestational age (GA) of 28–35 GW included pelvic pressure, backache, increased quantity and/or consistency of vaginal discharge, menstrual-like cramps, and bleeding/show (18).

Pregnancy with more than two fetuses, a singleton fetus, a dilatation of the cervical canal of at least three centimeters, bleeding from the vagina, discharge that could be the result of a ruptured membrane, uterine overdistention due to polyhydramnios, fever exceeding 38 degrees Celsius, fetal distress, intrauterine growth restriction, blood pressure of at least 140/90 and 100/60 mmHg, any obstetric contraindications to tocolytic agents, and a history of thromboembolic diseases were among the exclusion criteria. The absence of exclusion criteria, twin pregnancy, and presentation of TPTB symptoms at the start of the second trimester were among the inclusion criteria. The study only included women who gave written agreement to be involved and to receive the designated line of intervention.

Grouping & Randomization

All women eligible for inclusion were examined clinically and underwent transvaginal ultrasonography (TVU) to estimate the cervical length (CL) at the beginning of 2nd trimester and were categorized accordingly into intervention group that will include women who had CL <25 mm and Control group that will include women who had CL >25 mm and received expectant management (EM).

According to the line of management plan, the randomization of women in the intervention group was based on the preparation of 90 dark-sealed envelopes with 30 cards for each group that had a group label. The assistant responsible for the envelops had no knowledge about the meaning of the symbols. Following their selection of an envelope, participating ladies were divided into three groups based on the label symbol: Group C will include study women who will receive cervical cerclage (CC) alone, Group P will include women who will receive vaginal progesterone (VP) therapy only and group C/P will include women who will receive both CC and VP therapy.

Management lines

1. After the ladies in groups A and C were enrolled, cervical cerclage (CC) was done using the Shirodkar technique and a non-absorbable suture within four days. Following the CC process, women were instructed not to engage in any sexual activity, use of tampons or douching, stand for longer than four hours, perform strenuous physical labor, lift heavy weights, strain, or engage in any activity that aggravates their pelvic pressure or discomfort. At 37 weeks of gestation, the cerclage suture was removed, provided there was no membrane rupture, bleeding, or labor.
2. Women in groups B and C received progesterone therapy in the form of VP pills (Crinone 200 mg, Akorn Inc., Millbrook, USA), which were to be injected once a day at night.

Laboratory investigation

CVF sampling

Women who met the requirements for inclusion were instructed to stay off the vaginal toilet in order to prevent the spread of infection, and to come to the outpatient clinic four days following the initial exam in order to provide a CVF sample before beginning treatment (S1 sample). After that, women were instructed to follow up biweekly at the outpatient clinic. Additionally, another CVF sample (S2 sample) was taken at the moment of spontaneous labor or the removal of the CC suture.

CVF sample obtaining and processing

The CVF material was acquired and handled in the manner previously detailed by Jung et al. (19): In order to obtain a high vaginal smear of CVF, a sterilized vaginal speculum was applied. Two Dacron swabs were then inserted in the posterior vaginal fornix and left there for 10 seconds to achieve satura-

tion. Afterward, the swabs were transferred into 750 ml of standard phosphate-buffered saline solution and combined with a freshly made protease inhibitor solution. After that, the swab was taken out, put in a clean tube, vortexed for ten seconds, then centrifuged at 2500 g for ten minutes at 4 oC. The fluid that was left over was then collected, properly mixed, and centrifuged for an additional ten minutes to get rid of any remaining cell debris. Before being analyzed, cell-free supernatants were gathered, separated into aliquots, and kept at -80°C.

Investigations

Using ELISA kits and a 96-well microplate ELISA reader (Dynatech, MR 7000), the CVF concentrations of tumor necrosis factor-α (TNF-α), IL-6, and vitamin D binding protein (VDBP) were determined. The instructions provided by the manufacturer were followed for the measurement process.

1. Using the quantitative sandwich enzyme immunoassay technique (20), human TNF-α was quantified using the enzyme linked immunoassay (ELISA) kit (catalogue no. ab179886, abcam Inc., Cambridge, USA).
2. IL-6 using the quantitative sandwich enzyme immunoassay approach (21) and the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46042, abcam Inc., Cambridge, USA).
3. VDBP using the quantitative sandwich enzyme immunoassay approach (22) and the enzyme linked immunoassay (ELISA) kit (catalogue no. ab108853, abcam Inc., Cambridge, USA).

Study Outcomes

1. Primary outcome: the frequency of PTB among enrolled women
2. Secondary outcomes included

   a.The difference in cytokines' levels between women of intervention groups and control women and between S1 and S2 samples.

   b.The impact of applied lines of managements on cytokines' levels as judged by difference in cytokines' levels between the two obtained samples in relation to line of management

   c.The relation between studied cytokines and the percentage of change in levels estimated in S2 sample in relation to S1 sample cytokines' levels calculated as ([S2 level]-[S1 level])/[S1 level] and multiplied by 100 and pregnancy duration.

Statistical analysis

The data collected were displayed as percentages, numbers, and mean±SD. The paired t-test, one-way ANOVA test, and chi-square test (X2 test) were used to examine the results. We used Pearson linear regression analysis to look into any links. A stepwise regression analysis approach was employed to stratify the parameters under investigation as particular predictors. **The IBM SPSS (Version 23, 2015; IBM, South Wacker Drive, Chicago, USA)** for Windows statistical program was used to do the statistical analysis. A P-value of less than 0.05 was deemed statistically significant.

Results

The study included 174 women who were presented by twin pregnancy and were eligible for evaluation; 31 women were excluded and 143 women fulfilled the inclusion criteria. TVU detected 52 women had CL>25 mm and were categorized as control group, while 91 women had CL<25 mm and were randomly divided into three study groups (Fig. 1). There was non-significant ($p>0.05$) differences between the study groups and control group concerning the inclusion criteria apart from the CL, despite the non-significant difference between the three study subgroups regarding CL (Table 1)..

Table (1): Enrolment data of studied patients

Group Data		Control group	Study group				P1 value	P2 value
			Total	Group C	Group P	Group C/P		
Age (years)		27.5±5.9	27.7±3.3	27.7±3.4	27.3±3.7	28.1±2.9	0.783	0.661
Weight (kg)		81.4±6.7	83.1±7.1	82.5±8.6	84.9±4.4	82.1±7.4	0.151	0.267
Height (cm)		169.9±2	170±2.2	170±2.6	170.3±1.7	169.8±2.2	0.728	0.741
BMI (kg/m^2)		28.2±2.5	28.6±3.3	29.3±1.5	28.5±2.6	28.2±2.5	0.398	0.513
SBP (mmHg)		117.6±3.6	117.3±4.3	117±3.4	118.8±4.7	116.2±4.5	0.677	0.064
DBP (mmHg)		81±4.6	81.3±4.9	80.5±5	81.2±5.3	82.2±4.3	0.742	0.395
FBG (mg/dl)		90±9.3	92.7±9.2	92.3±9.4	91.9±7.9	93.8±10.2	0.103	0.693
CL (mm)	<20	0	29 (31.9%)	8 (25%)	12 (41.4%)	9 (30%)		
	20-<25	0	62 (68.1%)	24 (75%)	17 (58.6%)	21 (70%)		
	>25	30 (100%)	0	0	0	0		
	P2			0.377				
	Mean	27.5±1	21.4±1.7	20.8±1.9	20.7±1.5	21±1.7	<0.0001	0.199

Data are presented as mean±SD; P1 value indicates significance of difference between control and study groups; P2 value indicates variance between the three study subgroups; P>0.05 indicates non-significant difference; P<0.05 indicates significant difference

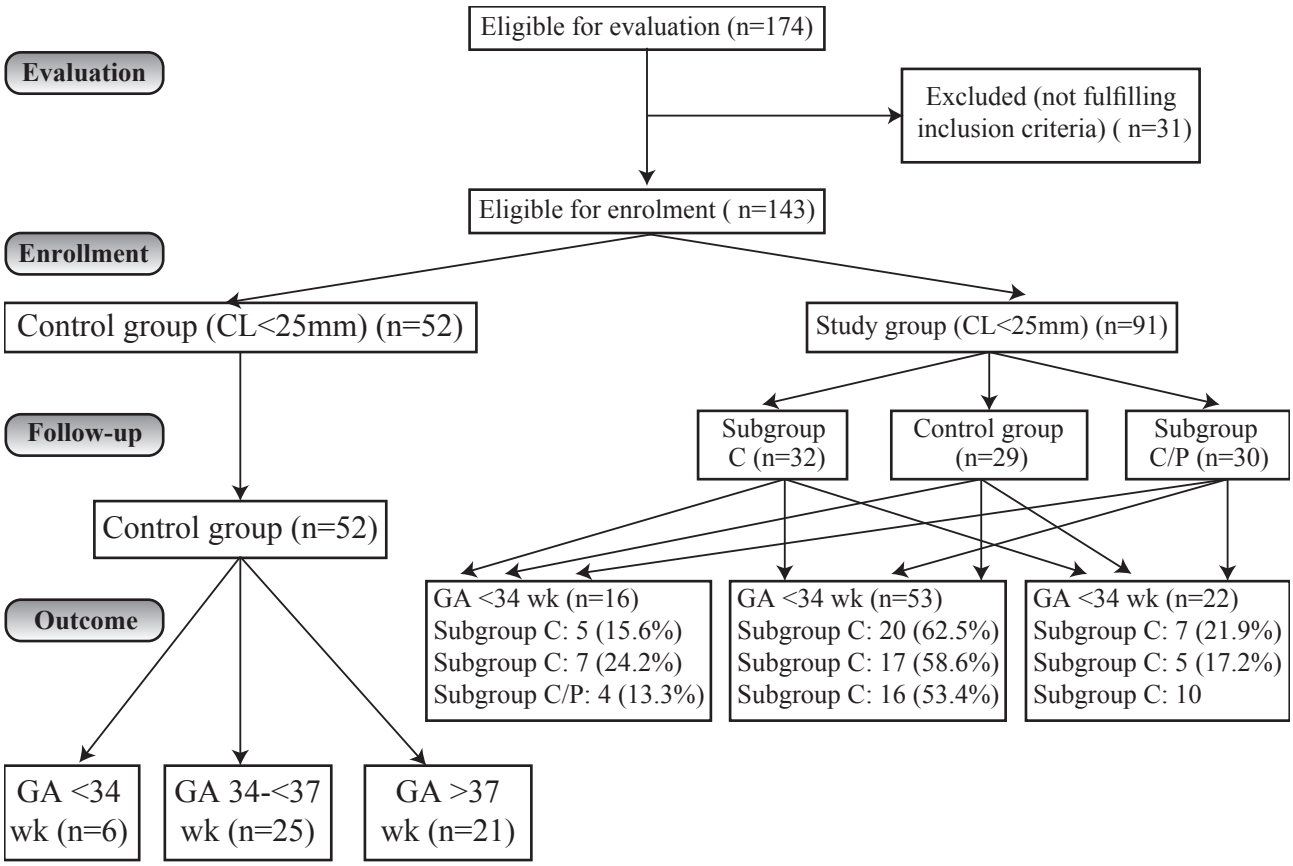

Figure 1: Consort Flow sheet

Thirty-seven women (25.9%) had birth before 35 GW, 64 women (44.8%) had birth between 35 and <37 GW and 42 women (29.3%) had birth after 37 GW with non-significant difference between control and study women as total and in subgroups. Mean duration of pregnancy was non-significantly shorter in control women in comparison to total study women and women of groups C and C/P, while was non-significantly longer than that of women of group P. Also, mean duration of pregnancy of women of group C/P was non-significantly longer in comparison to that of women in groups C and P with non-significantly longer pregnancy duration of women of group C in comparison to women of group P (Table 2).

Table (2): Pregnancy duration data of patients of studied groups

Groups Data			Control group	Control group			
				Total	Group C	Group P	Group C/P
Patients' distribution	<35 GW	No. (%)	11 (21.2%)	26 (28.6%)	6 (18.8%)	12 (41.4%)	8 (26.7%)
		P1		0.267	0.901	0.095	0.569
		P2				0.071	0.542
		P3					0.145
	35-<37 GW	No. (%)	22 (42.3%)	42 (46.2%)	18 (56.2%)	12 (41.4%)	12 (40%)
		P1		0.751	0.214	0.701	0.838
		P2				0.153	0.201
		P3					0.851
	≥37 GW	No. (%)	19 (36.5%)	23 (25.2%)	8 (25%)	5 (17.2%)	10 (33.3%)
		P1		0.155	0.271	0.068	0.77
		P2				0.46	0.474
		P3					0.156
Mean duration (GW)	Mean		35.5±2.6	36±2.7	36.2±2.6	35.3±2.6	36.5±2.7
	P value	P1		0.311	0.262	0.685	0.121
		P2				0.181	0.661
		P3					0.156

Data are presented as mean±SD; P1 value indicates significance of difference of PD of control and study women; P2 value indicates significance of difference of PD of women of group C and groups P and C/P; P3 indicates significance of difference of PD of women of groups P and C/P; P>0.05 indicates non-significant difference; P<0.05 indicates significant difference

Serum IL-6 levels estimated in S1 samples were significantly higher in study patients, as total and subgroups, in comparison to levels estimated in control group with non-significant differences between patients of study subgroups. On the other hand, serum levels of TNF-α and VDBP in S1 samples of study patients, as total and subgroups, showed non-significant differences in comparison to control group and in between study subgroups. Interestingly, serum levels of IL-6 and TNF-α estimated in S2 samples of control women were significantly higher than their corresponding S1 samples' levels, while S2 samples' levels of VDBP were non-significantly higher compared to their corresponding levels in S1 sample.

On contrary, in S2 samples of study women, estimated serum levels of IL-6 and TNF-α were significantly lower than their corresponding S1 sample's levels, while serum levels of VDBP

in total study women were significantly higher, but were non-significantly higher in women of control group and showed non-significant between study subgroups in comparison to their respective S1 samples' VDBP levels. Moreover, serum levels of IL-6 and TNF-α in S2 sample of patients of C/P subgroup were significantly lower with non-significantly higher serum VDBP levels than the corresponding levels in women of P subgroup. However, the difference between group C and both of groups P and C/P were non-significant regarding the estimated cytokines' levels (Table 3).

Table (3): Laboratory findings of both CVS of studied patients

Group Data			Control group	Study group			
				Study group	Group C	Group P	Group C/P
IL-6 (ng/ml)	S1 level		9.93±2.1	14.45±3.79	14±4.15	14.87±3.75	14.5±3.56
	P value	P1		<0.0001	<0.0001	<0.0001	<0.0001
		P2				0.396	0.614
		P3					0.699
	S2 level		12.47±2.42	11.53±2.56	11.48±2.9	12.21±2.63	10.92±1.96
	P value	P1		0.032	0.094	0.656	0.0037
		P2				0.306	0.381
		P3					0.036
		P4	<0.0001	<0.0001	0.0065	0.0028	0.000011
TNF-α (ng/ml)	S1 level		4.5±1.77	4.89±1.76	4.69±2	4.92±1.64	5.072±1.59
	P value	P1		0.206	0.653	0.296	0.147
		P2				0.632	0.417
		P3					0.719
	S2 level		8.685±2.52	3.59±1.23	3.55±1.45	3.92±1.21	3.3±0.93
	P value	P1		<0.0001	<0.0001	<0.0001	<0.0001
		P2				0.289	0.418
		P3					0.031
		P4	<0.0001	<0.0001	0.013	0.011	<0.0001
VDBP (μg/ml)	S1 level		245.4±95.4	237.4±62.8	236±71.4	227.1±61.6	248.9±53.5
	P value	P1		0.547	0.633	0.355	0.472
		P2				0.605	0.167
		P3					0.064
	S2 level		282.1±91.8	261.2±64.8	258.9±74.7	248.2±65.4	276.4±50.2
	P value	P1		0.116	0.232	0.084	0.775
		P2				0.557	0.287
		P3					0.069
		P4	0.055	0.013	0.216	0.208	0.054

Data are presented as mean±SD; IL-6: interleukin-6, TNF-α: Tumor necrosis factor-α; VDBP: Vitamin D binding protein; S1: sample obtained before start of management; S2: sample obtained at time of labor or removal of CC; P1 value indicates significance of difference between control and total study women; P2 value indicates significance of difference between women of group C in comparison to those of groups P and C/P; P3 indicates significance of

difference between women of groups P and C/P; P>0.05 indicates non-significant difference; P<0.05 indicates significant difference

The percentages of decrease in serum IL-6 and TNF-α were significantly higher in women of subgroup C/P in comparison to that estimated in subgroups C and P. However, in S2 sample of women of subgroup C, the percentages of decrease of estimated levels of serum IL-6 and TNF-α were non-significantly and significantly, respectively, higher in comparison to subgroup P. On contrary, the percentage of increase of VDBP showed non-significant differences between the three subgroups (Table 4).

Table (4): Percentage of change of serum cytokines' levels estimated in S2 sample in relation to levels estimated in S1 sample of patients of study groups

Variables		Group C	Group P	Group C/P
IL-6 (ng/ml)	%	-16.78±7.8	-16.76±7.2	-23.34±9.3
	P2		0.951	0.001
	P3			0.004
TNF-α (ng/ml)	%	-22.6±10	-18.75±9.8	-34.72±12.1
	P2		0.038	0.001
	P3			<0.001
VDBP (µg/ml)	%	10.1±2.7	9.6±3.9	12.1±7.4
	P2		0.566	0.165
	P3			0.117

Data are presented as mean±SD; IL-6: interleukin-6, TNF-α: Tumor necrosis factor-α; VDBP: Vitamin D binding protein; P2 value indicates significance of difference between women of group C in comparison to those of groups P and C/P; P3 indicates significance of difference between women of groups P and C/P; P>0.05 indicates non-significant difference; P<0.05 indicates significant difference

Pregnancy duration showed positive significant correlation with CL and high percentage of decrease of serum IL-6 and TNF-α, while showed positive non-significant correlation with the use of CC or combined intervention and with the percentage of change of serum VDBP in S2 sample in relation to S1 sample levels. Application of combined therapy was positively correlated with high percentage of decrease of serum IL-6 and TNF-α, while application of CC only showed positive significant correlation with high percentage of decrease of serum TNF-α in S2 sample in relation to S1 sample levels. Moreover, percentages of decrease of serum IL-6 and TNF-α were positively correlated. On contrary, the percentage of change of CVF levels of VDBP showed non-significant correlation with the other variables (Table 5).

Table (5): Correlation between studied variables

Variables		Combined therapy	Cerclage	CL	% of change in serum		
					IL-6	TNF-α	VDBP
Pregnancy duration	r	0.184	0.129	0.303	0.362	0.550	0.151
	p	0.081	0.223	0.003	<0.001	<0.001	0.097
Combined therapy	r		0.480	0.123	0.355	0.525	0.133
	p		<0.001	0.244	0.001	<0.001	0.211
Cerclage	r			0.185	0.185	0.363	0.204
	p			0.079	0.079	<0.001	0.053

% of change in serum	IL-6	r					0.525	0.038
		p					<0.001	0.723
	TNF-α	r						0.083
		p						0.433

r: Pearson's correlation coefficient, CL: Cervical length; IL-6: interleukin-6, TNF-α: Tumor necrosis factor-α; VDBP: Vitamin D binding protein; P value indicates significance of the coefficient; P>0.05 indicates non-significant difference; P<0.05 indicates significant difference

Regression analysis defined increased percentage of change in serum TNF-α (β=0.526, p<0.001) and CL (β=0.228, p=0.01) as the positive significant predictors for prolonged duration of pregnancy, while the increased percentage of change in serum IL-6 (β=0.116, p=0.275) and VDBP (β=0.022, p=0.813) and application of cervical cerclage (β=0.136, p=0.163) and combined therapy (β=0.131, p=0.218) as positive non-significant predictors for prolonged duration of pregnancy. Cox regression analysis defined decreased risk of PTB with decreased serum TNF-α and IL-6 levels by >50% and >60%, respectively (Fig. 2&3).

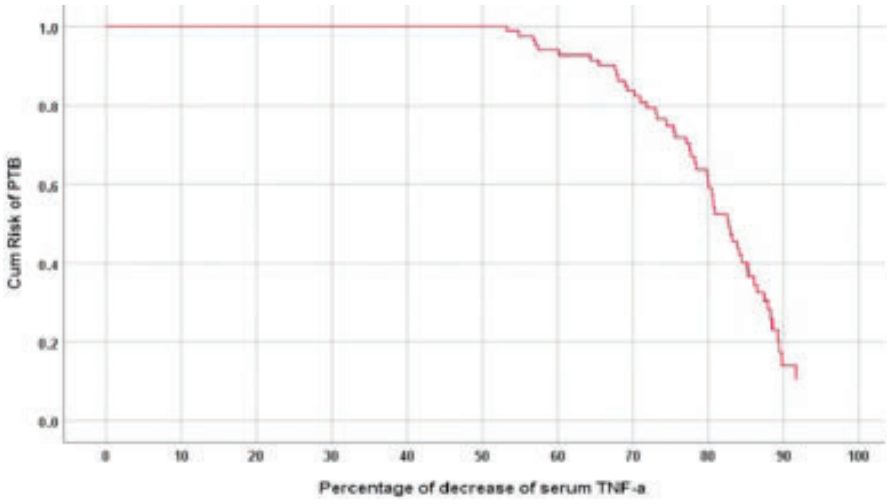

Fig. (2): Cumulative risk of PTB in relation to the percentage of change in serum TNF-α level

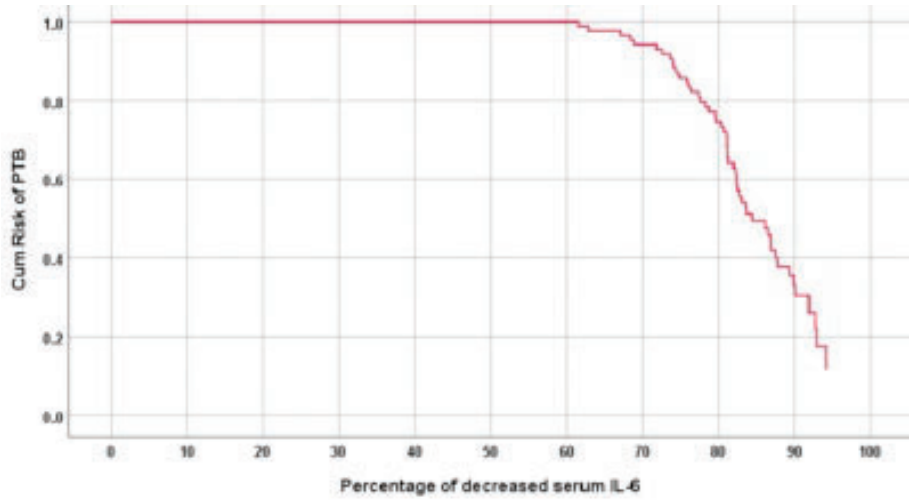

Fig. (3): Cumulative risk of PTB in relation to the percentage of change in serum IL-6 level

Discussion

The current study reported PTB rates at <35 and 35-<37 GW of 20.3% and 44.7% of enrolled patients; these figures go in hand with Berveiller et al. [23] who recently reported PTB in 43.4% of women had twin pregnancy (TP). However, there was non-significant difference between women received either interventional or expectant management (EM) regarding the frequency of PTB. Similarly, Qureshey et al. [24] reported non-significant difference between the effectiveness of EM, cervical cerclage (CC) and vaginal progesterone (VP) in decreasing the spontaneous PTB rate in twin gestations with mid-trimester cervical shortening.

Mean pregnancy duration was significantly longer in women of intervention groups than in women received EM. This finding was in accordance with previous studies documented that rescue CC for asymptomatic women with TP and cervical dilatation before 25 GW, can prolong pregnancy duration and reduce PTB rate [25, 26, 27, 28]. Moreover, mean pregnancy duration was significantly longer in women received CC than in those received VP; a finding indicated a more favorable outcome with mechanical than with drug-induced prophylaxis. In support of this assumption, women of C/P group had longer pregnancy duration than those received VP or CC separately. In line with the efficiency of mechanical prophylaxis provided by CC, multiple previous studies reported significantly longer pregnancy duration, lower rates of PTB before 28 and 32 GW with prophylactic [29], physical examination-indicated [30] or ultrasound-indicated [31] CC in asymptomatic twin pregnancies with a short [31] or very short or dilated [32] cervix during the 2nd trimester. In support of the efficacy of mechanical prophylaxis, Zimerman et al. [33] found combined treatment with Arabin cerclage pessary and VP had a lower PTB rate before gestational age (GA) of 28 weeks in comparison to EM and recently, Le et al. [34] reported that cervical pessary significantly improves the rate of morbidity-free neonatal survival in women with TP and a short cervix, in comparison to VP.

Interestingly, CVF inflammatory cytokine's levels were increased in S2 sample of control women, while were decreased in S2 sample of women of intervention groups; a finding that indicated a possible relation between the initiation of the process of labor and increased inflammatory local milieu. The period of pregnancy and the percentage of change in CVF levels in the S2 sample of intervention women showed a positive significant connection, supporting this hypothesis. These results are consistent with earlier research that found high concentrations of CVF inflammatory cytokines in term pregnancies [36] and variations in cytokine profiles across trimesters in women delivering term (35) and with Stokkeland et al. [37] who proposed that high levels of CVF inflammatory cytokines indicated that a normal pregnancy is connected to an active inflammatory state.

Numerous experimental studies attempted to investigate the relationship between elevated levels of inflammatory mediators and PTB. Nadeau-Vallée et al. [38] discovered that prenatal exposure to IL-1β induced TNF-α and IL-6 expression in placenta and fetal membranes with raised levels of IL-1β, IL-6, IL-8, and PGF2α, leading to PTB and significant mortality in neonates. Cappelletti et al. [39] noted significantly greater levels of expression of inflammatory cytokines, specifically IL-6 and induction of PTB on induction of Toll-like receptor2-driven inflammation. Deng et al. [40] discovered a role of IL-10's anti-inflammatory cytokine for PTB prevention.

Furthermore, the C/P group's women had a greater proportion of decreased CVF levels of IL-6 and TNF-α than the C and P study groups, with a significant difference favoring the C group. This suggests that CC may play a role other than its mechanical one in extending pregnancy duration through a down-regulating effect on the local synthesis of inflammatory cytokines, which has been

shown to be conversely related to pregnancy period and directly related to the PTB rate. Kiefer et al. [41] and Monsanto et al. [42] found that CC significantly decreased the amount of pro-inflammatory cytokines in CVF, which is consistent with this data and suggests that cerclage may be useful in reducing local inflammation in cervical incompetence. Subsequently, Liu et al. [43] discovered that the parameters influencing the pregnancy outcome in women with cervical incompetency prior to CC that significantly lowered these levels in contrast with EM were peripheral blood leucocyte counts, amniotic fluid TNF-α, and IL-8 level.

The calculated CVP levels of VDBP in S2 samples revealed differences between study participants; women in the control group had non-significantly higher levels, whereas women in the study groups had significantly higher levels. Yoo et al. [44] discovered that women with PTB at <32 GW had considerably greater CVF levels of VDBP than women without PTB, which is consistent with these contradictory results. Additionally, Cook et al. [45] discovered a strong correlation between increased VDBP levels in CVF samples of women with developed PTB and intact membranes and impending PTB. Conversely, D'Silva et al. [46] discovered that a drop in VDBP might lead to an inconsistency in the ideal intrauterine environment for the growing baby and a successful, straightforward pregnancy; as a result, it could be a potential indicator of spontaneous PTB.

Conclusion

Preterm birth (PTB) is a frequent co-accident with TP with an incidence rate ranging between 20% and 45%, irrespective of CL at start of the 2nd trimester. CC allows prolongation of pregnancy duration and reduce PTB rate especially if combined with VP therapy. Disturbed local CVF inflammatory milieu is closely related to shorter pregnancy duration and having PTB. CC allowed reduction of CVF inflammatory cytokines' levels by 50-60% of its levels at time of placement of cerclage and this may underlie the reported effect of CC on pregnancy duration.

Limitation

One of limitations of the current study was it is a single-center study and absence of determination of CVF levels of anti-inflammatory cytokines is another limitation.

Recommendation

Wide scale multicenter studies are mandatory to establish these results and to determine cutoff points for inflammatory and anti-inflammatory cytokines to be used for early prediction of PTB.

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A Cohort Study to Evaluate Correlation of Pre-Operative CA-125 and Intra-Operative Stage of Endometriosis

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Abstract

Background: Endometriosis is an estrogen-dependent gynecologic illness that affects many women's ability to conceive, their physical well-being, and their general quality of life.

Objectives: To ascertain if the stage of endometriosis after surgery and preoperative CA125 were correlated.

Methods: Our study was conducted at Obstetrics and Gynaecology Department, Ain Shams university hospitals and conducted on 60 patients with pelvic pain, endometriotic cysts, known endometriosis.

Results: The study involved patients aged 30.58 ± 6.19 years with a mean preoperative CA125 of 57.74 ± 127.09, with 93.3% ovarian, 1.7% ovarian, uterosacral ligament, 1.7% tubal, and 1.7% tubal & Douglas P. Adhesion types included no adhesion, filmy, dense, superficial, deep, and bowel wall infiltration. Stages were 25.0% stage 1, 8.3% stage II, 56.7% stage III, and 10.0% stage IV. Median preoperative CA125 was 22 in stage 1 and 48.5 in stage 4.

Conclusion: The study found that ovarian lesions were the most common site of lesion in 93.3% of patients, with Stage III being the most common stage in 56.7%. The mean preoperative CA125 was 57.74±127.09, and no significant difference was found between endometriosis stages.

Keywords: Endometriosis, Intra-Operative Stage, Pre-Operative, CA -125.

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INTRODUCTION

A gynecologic condition that is dependent on estrogen, endometriosis affects many women's ability to conceive, their physical well-being, and their general quality of life. Approximately 10-15% of adult women between the ages of 25 and 35 have endometriosis. The uterine lining cells, or endometrial tissue, can proliferate outside of it, leading to this disease. Infertility and a range of agonizing symptoms are the results of endometriosis infection (1).

For women who have finished having children, surgery is

the usual course of therapy for endometriosis (including hysterectomy & bilateral salpingo-oophorectomy) (2).

A gynecologic condition that is dependent on estrogen, endometriosis affects many women's ability to conceive, their physical well-being, and their general quality of life. Approximately 10-15% of adult women between the ages of 25 and 35 have endometriosis. The uterine lining cells, or endometrial tissue, can proliferate outside of it, leading to this illness. Infertility and a range of agonizing symptoms are the results of endometriosis infection (3).

Women with mild to severe endometriosis have been found to have significantly higher levels of CA-125. It has been proposed that preoperative blood CA-125 levels are a useful predictor for patients with endometrial diseases. The most prevalent benign gynecological disorders linked to elevated serum CA-125 levels are deeply infiltrating endometriosis and ovarian endometrioma (4). Consequently, this study was conducted to Find out if there is a relation between preoperative CA125 and postoperative endometriosis stage was the purpose of this study.

PATIENTS AND METHODS

This is a cohort study conducted at Obstetrics and Gynaecology Department, Ain Shams University hospitals on 60 patients with pelvic pain, endometriotic cysts, or known endometriosis from November 2023 to March 2024.

20-40 years old patients, despite their parity with chronic pelvic pain, severe dysmenorrhea and endometriotic cysts were included in the study. While, patients admitted with infertility due to other causes, fibroid, pelvic inflammatory disease, any malignancy and previous surgeries for endometriosis were excluded from the study.

All patients underwent a complete history taking, general examination (Vital signs, signs of Pallor, Cyanosis, Jaundice, and Lymph node enlargement), local examination (general inspection, abdominal examination, pelvic examination, lower abdominal tenderness and pelvic tenderness).

CA-125 blood sample 3 ml was drawn pre-operative from patients who fulfilled the enrolment criteria, then were underwent Diagnostic Laparoscopy or mini-laparotomy for cystectomy which done by staff of Ain Shams University Hospitals according to ASRM endometriosis classification.

A correlation of intraoperative stage with preoperative CA125 was done. Then after identifying stage of endometriosis, correlation of preoperative CA125 with the stage of endometriosis was done.

A wide-band 3.5 to 9 MHz transducer was used for transvaginal sonography. A 50 Hz wall filter, a high-priority color setup, and a pulse repeating frequency of 1000 to 1500 Hz are used in color Doppler examinations. Every exam must be understood right away. The transducer was inserted into the posterior cul-de-sac of the vagina and then slowly withdrawn through the vagina to view the posterior sub peritoneal space, which is anatomically identified by the presence of a small transverse thickening connecting the USL, the posterior wall of the uterus, and the posterior fornix of the vagina. By repeatedly sliding the probe up and down from the anal canal to the posterior fornix of the vagina, the rectovaginal septum and bowel wall were examined. For the purpose of identifying posterior endometriotic lesions, the probe must rotate. The transducer was then inserted to evaluate the bladder and vescico-uterine septum in the anterior cul-de-sac of the vagina.

Approval of the ethical committee to retrieve data of the patients in the database was sought provided that the patient was not consented preoperatively to include his data in clinical study assuring patient privacy and dignity not stating their personal identity. Prior to starting the trial, all subjects provided written in-

formed consent. People were given the information they needed to freely choose whether or not to take part in the study. Consent was given in a way that was morally acceptable; specifics regarding the nature and goal of the study would be provided.

Statistical analysis: Using the SPSS software, the gathered data was coded, tabulated, and statistically examined. For qualitative variables, the data were expressed as a number and a percentage, and for quantitative variables, as mean + standard deviation (SD). Subsequently, relevant statistical analyses were conducted. The significance level was established at P = 0.05, meaning that findings > 0.05 were considered non-significant, and results < 0.05 were considered significant.

RESULTS

Table (1): Distribution of patient characteristics in the studied group

`	Mean ± SD	Median (IQR)	Range
Age	30.58 ± 6.19	30.5 (25 - 35.5)	20 - 43
Preoperative CA125	57.74 ± 127.09	32 (15.7 - 51.5)	8 - 226

Table (1) shows that, the mean age of the patients was 30.58 ± 6.19, ranged from 20 to 43 years while mean of Preoperative CA125 was 57.74 ± 127.09 and ranged from 8 to 226.

Table (2): Distribution of endometriosis characteristics in the studied group.

		N	%
Site of lesion	ovarian	56	93.3%
	Ovarian & Douglas p	1	1.7%
	ovarian, uterosacral ligament	1	1.7%
	tubal	1	1.7%
	tubal & Douglas P	1	1.7%
Type of adhesions	No	42	70.0%
	Filmy	16	26.7%
	dense	2	3.3%
superficial or Deep	superficial	57	95.0%
	deep	3	5.0%
Infiltration of the bowel wall	No	59	98.3%
	Yes	1	1.7%
Stage	I	15	25.0%
	II	5	8.3%
	III	34	56.7%
	IV	6	10.0%

Table (2) shows that, there were 56 (93.3%) of patients who had ovarian endometriosis, which is the most common site in this study. 1 (1.7%) of patients had Ovarian & Douglas p endometriosis, 1 (1.7%) of patients had ovarian, uterosacral ligament, 1 (1.7%) of patients had tubal endometriosis while 1 (1.7%) of patients had tubal & Douglas Pouch endometriosis. Regarding Type of adhesions, there were 16 (26.7%) who had filmy adhesions which are loosely connected to pelvic organs, transparent and may be vascular or avascular. While 2 (3.3%) who had dense adhesions which are thick fibrous bands and opaque. 57 (95%) their endometriosis

was superficial while 3 (5%) their endometriosis was deep, 1 (1.7%) of them had Infiltration of the bowel wall. Regarding the stages of endometriosis, 15 (25%) of patients with stage I , 5 (8.3%) with stage II , 34 (56.7%) with stage III while 6 (10%) with stage IV.

Table (3): Relation between stages of endometriosis and Preoperative CA125 in the studied group.

	Stage				Kruskal Wallis test		
	1 N=15	2 N=5	3 N= 34	4 N= 6			
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	value	p value	sig.
$_{\text{Preoprative}}$ CA125	22 (11 – 51)	28 (16 – 63)	26.5 (18.1 – 49)	48.5 (43 – 114)	4.12	0.244	NS

Table (3) shows that, there was no statistically significant association between stages of endometriosis and Preoperative CA125. These outliers indicate data points that belong to a different CA125 than the rest of the sample set. Every outlier is a data point that differs significantly from other observations.

DISCUSSION

Our study was conducted at Obstetrics and Gynaecology Department, Ain Shams university hospitals and conducted on 60 patients with pelvic pain, endometriotic cysts, or known endometriosis.

Our research aimed to ascertain if the preoperative CA125 level and the postoperative endometriosis stage were correlated.

Our results showed that the mean age of the studied patients was 30.58 ± 6.19 years, with range 20 – 43 years. The mean preoperative CA125 was 57.74 ± 127.09, with range 8 – 226.

This was consistent with the findings of Mishra et al. (2015), who aimed to determine the frequency and clinical features of endometriosis in infertile women. They found that the patients' average age was 29± 4.3 years (range: 19–40 years).

Similar to this, Satyanarayana et al. (5) stated that 29 patients with an endometriosis diagnosis were included in their investigation. Their objectives were to analyze endometriosis patients with a single underlying condition presenting in a variety of clinical presentations and to emphasize the use of Fine Needle Aspiration Cytology (FNAC) in the preoperative diagnosis of scar endometriosis. The age range of the patient was 20 to 45 years, with a median age of 28.8 years and an average age of 29.4±7.7 years.

Furthermore, the participants' mean age varied from 21 to 54 years old, with a mean of 32.22±6.91, according to Karimi-Zarchi et al. (6). In addition to evaluating the association between preoperative serum CA-125 levels and clinic pathological features in women with endometriosis, the study sought to establish the ideal cut-off levels for serum CA-125 in pre- and postmenopausal women. In women with endometriosis, the mean blood CA-125 level was 49.93±4.30 U/mL (range: 2-191 U/mL).

In correlation to our study, the mean age of the patients was 30.58 ± 6.19, ranged from 20 to 43 years while mean of Preoperative CA125 was 57.74 ± 127.09 and ranged from 8 to 226.

Furthermore, the mean age of the endometriosis group was 29.2 ± 5.6 years, according to Amaral et al. (7), who sought to correlate the levels of CA-125 and 17β-estradiol (E2) in blood and peritoneal fluid from women with and without pelvic endometriosis. The

women with pelvic endometriosis had serum CA-125 levels ranging from 8.3 to 115.3 U/ml, with a mean of 39.1 ± 45.8 U/ml.

In the present study, regarding site of lesion, we found that there were 93.3% ovarian, there were 1.7% Ovarian & Douglas pouch, there were 1.7% ovarian, uterosacral ligament, there were 1.7% tubal and there were 1.7% tubal & Douglas P. Regarding type of adhesion, there were 70% with no adhesion, there were 26.7% Filmy and there were 3.3% dense. There were 95.0% superficial and 5.0% were deep. There were 98.3% with no Infiltration of the bowel wall and there were 1.7% with Infiltration of the bowel wall.

According to Abd El-Kader et al. (8), who attempted to determine the effect of endometriosis-related adhesions on infertile women's quality of life, 68 (62.38%) of the 109 women in their study had endometriosis without adhesions. These findings are consistent with our findings. Apart from that, adnexal adhesions (51.2%) and anterior abdominal wall adhesions (24.4%) were the most frequent sites for endometriotic adhesions.

Of the 29 cases, 16 cases (or 50%) had scar endometriosis involving the anterior abdominal wall at the Lower Segment Caesarean Section (LSCS) scar site (56.25% by cytology and 43.75% by histopathology); 10 cases (37.5%) had ovarian endometriosis (bilateral in one case), 2 cases (8.3%) had bladder endometriosis, and 1 case (4.2%) had bowel endometriosis involving the sigmoid colon of the colon.

Additionally, Busard et al. (9) reported that 28 patients were enrolled in their study, of which 20 had ovarian endometrial cysts (71%), 28 had retro-cervical DIE (100%), and 3 had bladder wall infiltration (11%). The aim of the research was to assess the morphologic and signal intensity abnormalities associated with deep infiltrating endometriosis (DIE) of the intestinal wall and ascertain its predictive usefulness in terms of the depth and extent of infiltration into the bowel wall. In a single case, histopathology did not show any intestinal wall invasion.

Regarding stages, our findings revealed that there were 25.0% stage 1, there were 8.3% stage II, there were 56.7% stage III and there were 10.0% stage IV.

Our findings are in opposition to those of Laila et al. (10), who examined the relationship between the severity of pelvic endometriosis and the level of serum cancer antigen (CA-125). Of the 70 patients in their study, 7 had stage 1, 14 had stage II, 17 had stage III, and 32 had stage IV.

Additionally, Szubert et al. (11), who evaluated CA-125 in serum and peritoneal fluid (PF) as an indicator of endometriosis, reported that in a group of 44 women, endometriosis was confirmed laparoscopically in 18.52 percent of cases, 31.48% of cases in stages II, 20.37 percent in stages III, and 1.85 percent in stages IV.

Our current study showed that the Median (IQR) Preoperative CA125 in stage 1 was 22 (11 - 51), in stage 2 was 28 (16 - 63), in stage 3 was 26.5 (18.1 - 49) and in stage 4 was 48.5 (43 - 114). There was no statistically significant difference between stages regarding the Median (IQR) preoperative CA125, p= 0.244.

Our results disagree with Karimi-Zarchi et al. (12) who reported that the mean CA 125 in stage 1 was 29.38 ± 5.81, in stage 2 was 53.20 ± 6.64, in stage 3 was 56.12 ± 6.88 and in stage 4 was 49.91 ± 7.51, with statistically significant difference, $p \leq 0.001$

Furthermore, according to Laila et al. (10), the mean CA125 was 21.8 ± 15.1, 26.0 ± 17.3, 83.2 ± 48.9, and 117.0 ± 41.6 in stages 1 through 4. There is a significant correlation (r= 0.729; p= 0.001) between the various phases of endometriosis and blood CA-125 levels.

Furthermore, Szubert et al. (11) found a significant correlation ($R = 0.5993$, $p < 0.001$) between the endometriosis stage and plasma levels of CA-125.

Moreover, de Luna Ramos et al. (13) discovered that the mean concentrations of CA-125 in both collections were greater in advanced endometriosis (stage III or IV) compared to beginning endometriosis (stage I or II) and in the absence of endometriosis. Their goals were to assess the amounts of soluble CD-23 and CA-125 in the blood and relate them to clinical symptoms, the location and stage of pelvic endometriosis, and the histological classification of the disease.

According to Amaral et al. (7), women with more advanced stages of pelvic endometriosis had higher serum and peritoneal fluid CA-125 levels ($p = 0.0001$) when the levels of the disease were compared with the serum levels of women with pelvic endometriosis. The mean CA-125 in stage 1 was 8.3 ± 4.1, in stage 2 was 14.8 ± 8.0, in stage 3 was 37.8 ± 37.9, and in stage 4 was 115.3 ± 35.4.

The strength points of this study included that its prospective study design and its setting at a single tertiary care center. However, the findings of this study should be interpreted in light of its limitations, including relatively smaller sample size relative to the previous studies, not being a multicentric study and this represents a significant risk of publication bias. Another limitation is lack of control group and lack of association of high CA-125 levels clinical symptoms such as chronic pelvic pain, dysmenorrhea, deep dyspareunia, bowel symptoms, which may contribute to the diagnosis of ovarian endometriosis. A greater number of samples could contribute to a better accuracy of the parameters evaluated.

CONCLUSION

According to the findings, preoperative serum CA-125 is an important marker for endometriosis patients and should be considered when surgical therapy is advised, particularly if evaluations of adhesion score, lesion size, and disease stage are conducted. When diagnosing endometriosis, CA125 can be useful in differentiating the disease's severity, tracking the impact of treatment, and identifying malignant change.

Additional Information

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosures

Conflicts of Interest: The authors have no conflicts of interest to declare.

Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of data from patients.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Financing Support: All authors have declared that no financial support was received from any organization for the submitted work.

Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.

Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work

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Bacterial Vaginosis and Its Relation to Caesarean Wound Infection

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Abstract

Background: Postoperative infections frequently occur as consequences of obstetric & gynecologic surgeries. The pathogenesis of following-cesarean endometritis and following-surgery cuff cellulitis includes the upward dissemination of potentially harmful bacteria present in the vagina. BV is related to elevated vaginal levels of specific facultative and anaerobic bacteria. BV is a potential risk factor for infections following surgery in gynecology and obstetrics.

Objectives: to assess the correlation between bacterial vaginosis & cesarean infection of the wound.

Methods: This prospective cohort investigation has been carried out at the Obstetrics & Gynecology department of Ain Shams University Maternity Hospital, from October 2023 to June 2024. The investigation included a total of 230 pregnant females who were scheduled for elective Caesarean Section & met the specified criteria for exclusion & inclusion. The incidence of infection in the caesarean wound was significantly greater in the group with bacterial vaginosis.

Results: Statistically insignificant variances were observed among the study groups in terms of body mass index, age, previous cesarean section, parity, & fetal gestational age. The scoring of wounds in the Southampton group was significantly elevated in the bacterial vaginosis group. The occurrence of Grade zero was significantly lower in the bacterial vaginosis group, however Grades IV and V were significantly greater in the bacterial vaginosis group. The incidence of infection of deep cesarean wound was slightly higher in the BV group, but this difference was statistically insignificant.

Conclusion: Most females with bacterial vaginosis are asymptomatic & are at a greater probability of adverse infectious complications because of cesarean delivery. The identification of bacterial vaginosis (BV) is rapid, uncomplicated, & cost-effective. BV is a significant issue of public health that is frequent among pregnant females & is related to surgery site infections. Performing bacterial vaginosis screening on women before undergoing a cesarean section can help to further decrease the occurrence of

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infections following surgery in a manner that is both safe and economical.

Keywords: Caesarean Wound Infection, Bacterial Vaginosis.

Introduction

Caesarean section is a frequently done major surgical surgery on females that is becoming more prevalent. A global rise in the rate of caesarean sections, reaching up to 31.2 percent, was observed in the past decade. Caesarean section carries a greater possibility of maternal mortality & morbidity compared to vaginal birth, with infections following surgery being a frequently observed component of morbidity. Given the rising prevalence of caesarean section, it is crucial to reduce the potential dangers to the mother to the greatest extent feasible (1).

Females who have caesarean section are susceptible to infection from both internal (endogenous) and exterior (exogenous) sources throughout birth. These females are susceptible to hospital-acquired infections because of their exposure to a hospital environment. The incidence of post-caesarean infection is expected to be twice higher compared to that following vaginal birth (2).

Infections following surgery frequently occur as consequences of obstetric & gynaecologic operations. The pathogenesis of both post-caesarean endometritis and following surgery cuff cellulitis is characterized by the upward spread of potentially harmful germs present in the vagina. Bacterial vaginosis is related to elevated levels of specific anaerobic & facultative bacteria in the vagina. Bacterial vaginosis is a known risk factor for infections following surgery in gynaecology & obstetrics (3).

Bacterial vaginosis is a condition that affects the vaginal microecosystem & appears to be associated with puerperal endometritis & infection at the surgical site (3, 4). Bacterial vaginosis constitutes fifty percent of all cases of vaginitis (5).

Therefore, the objective of the investigation was to assess the correlation among BV & infection of caesarean wound.

Patients and Methods

This prospective cohort investigation has been carried out at the Obstetrics & Gynecology department of Ain Shams University Maternity Hospital, following permission from an ethical committee as well as obtaining written consents from the patients. The investigation occurred from October 2023 to June 2024 and included a total of 230 pregnant females who were scheduled for elective Cesarean Section.

Study population: Pregnant females who meet the specified criteria for inclusion and exclusion criteria:

Inclusion criteria: Pregnant females between the ages of twenty and forty who had a cesarean delivery at or after thirty-seven weeks of pregnancy (elective), with a single living fetus, agreed to undertake a standardized vaginal speculum examination. These women had BMI ranging from twenty to thirty kilograms per square meter & tested positive for bacterial vaginosis.

Exclusion criteria: Diabetes mellitus is a medical condition characterized by high levels of sugar in the blood. A patient has a high body temperature, The case is utilizing steroids to manage a long-term medical condition. Preterm premature rupture of membranes, the variation between preoperative & postoperative hemoglobin levels exceeds ten percent. Cases with coagulopathies, obstructed or labor prolonged, and obesity type two or higher, Criteria for vaginal birth eligibility include the presence of chorioamnionitis, other infections that require postpartum antibiotic treatment, or fetal mortality. Additionally, cases with mental conditions that prevent them from understanding the nature, extent, and potential implications of the investigation are also excluded.

Study Procedures: All participants underwent the following:

All patients in the current investigation provided informed consent & had a detailed clinical evaluation, which involved a thorough medical history, & general, abdominal, and pelvic investigations.

History: involving age, personal, medical, obstetric, surgical, & family history.

Examination: involving (general, pelvic and abdominal examination).

Investigations: involving (labs; coagulation profile, blood group, kidney and liver functions, CBC, & imaging; two-dimensional obstetric ultrasound).

Before operation vaginal cultures were collected from pregnant cases undergoing CS. To maintain confidentiality, each case was designated only by a code. Cases have been categorized into two categories:

- The first group consisted of 115 pregnant females who tested positive for bacterial vaginosis.

- The second group consisted of 115 pregnant females who tested negative for bacterial vaginosis.

Vaginal swabs were immediately applied onto glass slides to generate vaginal smears, which have been subjected to Gram staining & evaluated using the Nugent method.

The Nugent approach involved using microscopic examination to assess the bacterial morphotypes and determine the general characteristics of the vaginal flora.

The Nugent scoring system assigns a numerical value between zero and ten, which is determined by the number of colonies of three different types of bacteria: Lactobacillus, Bacteroides or G vaginalis, & Mobiluncus.

The abundance of extended rod-shaped bacteria that are Gram-positive was assessed on a scale of zero to four, where a score of zero represents a high number of Lactobacillus. Small rod-shaped bacteria that are Gram-negative or Gram-variable, such as Bacteroides as well as G. vaginalis, were also scored on a scale of zero to four, with a score of four indicating the highest noticed numbers of these bacteria. Curved rods, such as Mobiluncus spp, were scored on a scale of zero to two, with a score of two representing the greatest detected numbers.

To maintain uniformity in the quantity of vaginal fluid on each slide, as well as in the Gram-staining & Nugent scoring processes, all swabs were rolled by a single technician, & all slides were stained & scored by the same technician.

A surgical incision was made during a Cesarean section procedure to investigate and diagnose the presence of infection in the wound. The septic wound has been subjected to culture & sensitivity testing to detect the specific pathogenic organism & determine the appropriate treatment.

Female cases have been monitored until they were released from the hospital for any surgical site infections. Detailed evaluation of the wound has been conducted using Southampton wound grading (6). The depth of the wound was classified based on the guidance provided by the CDC (7).

The CDC has established a definition for surgical site infection that differentiates between superficial incisional surgical site infection, deep incisional surgical site infection, and organ/space surgical site infection (7).

Superficial incisional SSI is an infection that arises within thirty days following surgery. It specifically affects the subcutaneous tissue or skin of the incision & includes a minimum one of the following criteria.:

1. Presence of pus discharged, with or without lab verification, from the superficial incision.
2. Organisms detected from a specimen acquired using aseptic techniques from the superficial incision.
3. The presence of a minimum of one of the

following indications of infection: pain or sensitivity, redness, localized swelling, or heat AND the surgeon intentionally opens the surface incision, unless the incision shows no signs of infection when tested.

4. The surgeon or attending physician diagnoses superficial incisional surgical site infection.

Do not report the following conditions as surgical site infection:

- Stitch abscess (minimal inflammation and discharge that are restricted to the sites of suture invasion).
- Infected burn wound.
- A localized stab wound, or pin site infection would be classified as a skin or soft tissue infection, depending upon its depth.
- Infection incisional surgical site that penetrates the fascial and muscular layers (refer to deep incisional surgical site infection).
- Infected circumcision.

Deep incisional surgical site infection typically occurs between thirty and ninety days after the surgery. It includes infection in the deep soft tissues, like the fascial & muscle layers, of the incision. Additionally, it must meet a minimum of one of the following criteria:

There is pus coming out of the deep incision, but not from the organ/space part of the surgery site.

- A deep incision may naturally dehisce or may be opened by a surgeon on purpose when the case demonstrates at least one of the following symptoms or signs: temperature (greater than thirty-eight degrees Celsius), localized discomfort, or tenderness, unless the region is culture negative.
- The discovery of an abscess or any other indication of infection in the deep incision can be made through direct examination, reoperation, radiologic or histopathologic evaluation, or any combination of these methods.
- The detection of an infection at the surgical site of a deep incision by a surgeon or attending physician or physician.

Note:

- Deep incisional surgical site infections refer to infections that affect both deep and superficial incision areas.
- A deep incisional surgical site infections is classified as an organ/space infection that drains via the incision.

Surgical site infection in the organs or space happens during a period of between thirty and ninety days. This type of infection affects any portion of the body that is deeper than the layers of fascia or muscle and has been opened or manipulated throughout a surgical procedure. Additionally, a minimum one of the following criteria must be met: (7):

1. The discharge containing pus from a drain inserted into the organ or space.
2. Organisms are recognized by a microbiologic testing approach, either culture-based or nonculture-based, which is carried out on aseptically acquired fluid or tissue from the organ/space. This testing is done for clinical diagnostic or therapeutic purposes.
3. The presence of an abscess or any other indication of infection in the organ/space, which can be identified through direct inspection or through radiologic or histopathologic investigation.
4. The identification of a deep incision surgical site infection by an attending physician or surgeon.

Sample Size: By utilizing the PASS 15 software for sample size calculation, an analysis of the findings from a prior investigation conducted by Alkady et al. (8) revealed that

56.8% of cases in the bacterial vaginosis group had Cesarean wound infection prior to discharge. After adjusting for a ten percent dropout rate, these findings indicate that a sample size of at least 230 cases is needed to obtain a two-sided confidence interval of ninety five percent with a width of 0.140, assuming a sample proportion of 0.568.

Outcome measures: Correlation among bacterial vaginosis and infection at the surgical site following caesarean section.

Ethical Considerations: The case data was anonymized. The information has been presented based on the diagnosis rather than the patient's name, ensuring the protection of case confidentiality. A complete informed permission was obtained from all participants, written in Arabic language & properly acknowledged with the date and time. To maintain confidentiality, patients' initials were assigned a numerical value, which was only known to the investigator.

Conflict of interest: The candidate asserted that there isn't any conflict of interest & personally covered the expenses for the investigation.

Statistical analysis: The analysis will be conducted by SPSS for Windows version 20.0. The data will be presented using the mean, range, & standard deviation for numeric parametric variables. For numeric non-parametric variables, the median, range, & inter-quartile range will be used. For categorical variables, the percentage & number will be reported. The analysis of the variation among both independent groups will be conducted utilizing the independent student's t-test for numeric parametric variables, which will also include the calculation of the mean difference and its ninety-five percent confidence interval. For categorical variables, the chi-squared test will be used, along with the calculation of the risk ratio and its ninety-five percent confidence interval. The purpose of the analysis is to use binary logistic regression to estimate the correlation among good/poor response & the measured variables. The construction of ROC curves is necessary to estimate the validity of measured variables as predictors of great or poor response. The validity of the data will be assessed using specificity, sensitivity, negative and positive predictive values, with a significance level of 0.05 and corresponding ninety-five percent confidence interval .

Results

Throughout this investigation, A total of 293 cases have been evaluated for their eligibility , out of that 230 cases have been chosen to participate in the investigation, with an equal distribution of 115 cases in each group. Out of all the cases who were eligible, twenty-three cases have been excluded from the control investigation and twenty-two cases were excluded from the investigation group because they didn't meet the inclusion criteria. Additionally, eighteen cases refused to participate in the trial.

Ultimately, the analysis has been conducted using data from 230 pregnant females who have been scheduled for a cesarean section and separated into two groups. (115 BV group and 115 control group) resulting in a 50% prevalence rate bacterial vaginosis in our investiagation.

Table (1): Demographic characteristics between the studied groups

Variables		Bacterial vaginosis group (Total=115)	Control group (Total=115)	p-value
Age (years)	**Mean± standard deviation**	29.6±3.8	30.3±3.7	^0.206
	Range	21.0–39.0	20.0–38.0	
BMI (kg/m^2)	**Mean±SD**	26.7±1.5	26.5±1.5	^0.374
	Range	23.6–29.5	23.3–29.5	

Parity (n, %)	**Primi**	23 (20.0%)	28 (24.3%)	#0.427
	Multi	92 (80.0%)	87 (75.7%)	
Previous cesarean section, (n, %)		43 (37.4%)	48 (41.7%)	#0.500
GA (weeks)	**Mean± standard deviation**	39.7±0.8	39.6±0.9	^0.357
	Range	37.0–41.0	37.0–41.0	

Table (1) illustrates statistically insignificant distinction among the examined groups according to age, body mass index, parity, previous cesarean section & fetal gestational age.

Table (2): Operation characteristics between the examined groups

Variables		**Bacterial vaginosis group (Total=115)**	**Control group (Total=115)**	**p-value**
Duration (minutes)	**Mean±SD**	46.9±5.4	47.3±5.9	^0.635
	Range	33.0–60.0	33.0–63.0	
Blood loss (mL)	**Mean±SD**	558.0±64.0	550.4±65.3	^0.373
	Range	454.0–764.0	390.0–688.0	

Table (2) demonstrations statistically insignificant variance among the examined groups regarding operation duration and blood loss.

Table (3): Southampton wound scoring between the studied groups.

Grade	**Bacterial vaginosis group (Total=115)**	**Control group (Total=115)**	**p-value**
0	71 (61.7%) a	100 (87.0%) b	**# 0.001**
I	10 (8.7%) a	6 (5.2%) a	
II	9 (7.8%) a	5 (4.3%) a	
III	8 (7.0%) a	2 (1.7%) a	
IV	8 (7.0%) a	1 (0.9%) b	
V	9 (7.8%) a	1 (0.9%) b	

#Chi square test. *Significant. Homogenous groups had the same symbol "a,b" dependent on post hoc Bonferroni test

Table (3) shows that Southampton wound scoring significantly higher has been observed in bacterial vaginosis group. Grade 0 was significantly less common in bacterial vaginosis group, while grades IV and V were significantly more common in bacterial vaginosis group.

Table (4): Infection of cesarean wound between the examined groups.

Findings	**Bacterial vaginosis group (Total=115)**	**Control group (Total=115)**	**#p- value**	**Relative risk 95% CI**
Positive	44 (38.3%)	15 (13.0%)	**<0.001***	2.93 (1.73–4.96)
Negative	71 (61.7%)	100 (87.0%)		Reference

Table (4) illustrates that infection of cesarean wound was significantly more common in bacterial vaginosis group.

Table (5): CDC site of surgical infection between the studied groups.

Findings	Bacterial vaginosis group (Total=44)	Control group (Total=15)	p-value	Relative risk 95% CI
Superficial	35 (79.5%)	14 (93.3%)	§0.426	Reference
Deep	9 (20.5%)	1 (6.7%)		3.07 (0.42–22.25)
Organ space	0 (0.0%)	0 (0.0%)		Not applicable

Table (5) Demonstrate that there was statistically insignificant variation in the occurrence of deep cesarean wound infection among the bacterial vaginosis group and other groups.

DISCUSSION

Bacterial vaginosis is a prevalent illness in females, with around 33% of females aged fourteen to forty-nine testing positive for bacterial vaginosis (9). Among women seeking medical attention, bacterial vaginosis is the most frequent reason for having vaginal discharge or unpleasant odor. Nevertheless, it is worth noting that most females with bacterial vaginosis do not exhibit any symptoms (10).

In addition to causing discomfort, bacterial vaginosis has been related to various obstetric and gynecologic complications, involving preterm premature rupture of membranes, preterm labor and delivery, chorioamnionitis, spontaneous abortion, post-Caesarean delivery wound infections, postpartum endometritis, postsurgical infections, as well as subclinical pelvic inflammatory illness (11).

Infections of the surgical site in obstetric & gynecological operations occur when bacteria from the vagina & cervix move upwards to the upper genital tract. The surgical sites involve the endo-myometrium, which is incised during a cesarean section; the vaginal cuff & paravaginal tissues, which are incised during a hysterectomy; or the endometrium, which is removed through uterine curettage for the treatment of early pregnancy abnormalities such as incomplete, missed, or elective abortion. Bacterial vaginosis bacteria are frequently found in the surgical sites of females' who have developed a surgical site infection (12).

Regarding pregnancy, it has been demonstrated that Bacterial vaginosis raises the possibility of progressing intrapartum chorioamnionitis, a well-established risk factor for postpartum endometritis. Moreover, Bacterial vaginosis was related to following-cesarean endometritis, where Bacterial vaginosis microorganisms are found in the endometrial cavity of these cases (13). In females who had a cesarean delivery, giving them metronidazole gel in the vagina before the surgery decreased the chances of developing endometritis after the cesarean by fifty-eight percent (14).

Given that surgical site infections following cesarean sections are a significant issue and frequently related to bacterial infections, the focus has been on screening and treating Bacterial Vaginosis as a means of reducing postoperative surgical site infections. This has been identified as a key area of concern (15).

Therefore, this investigation was done with the objective of assessing the correlation among BV & infection of cesarean wound.

This prospective cohort investigation was carried out at the Obstetrics & Gynecology department of Ain Shams University Maternity Hospital, from October 2023 to June 2024. The investigation included a total of 230 pregnant females who were scheduled for elective Cesarean Section.

A total of 293 cases were evaluated for eligibility in this investigation, out of whom 230 individuals (115 in each group) were finally involved. Out of all the eligible patients,

twenty-three cases have been excluded from the control investigation and twenty-two cases were disqualified from the investigation group due to failing to satisfy the inclusion criteria. Additionally, eighteen cases declined to participate in the trial.

In our investigation, the analysis was conducted using information from 230 pregnant females who were scheduled for CS. These women were separated into two groups: 115 in the Bacterial vaginosis group and 115 in the control group. The prevalence rate of bacterial vaginosis in our investigation has been discovered to be fifty percent. The rate of prevalence mentioned is greater than the range observed amongst females of reproductive age globally (twenty-three to twenty-nine percent) However, it is important to note that there are greater variances in rates of prevalence among females from low-income households & various ethnic and racial groups (16).

Sadek, & Soliman (17) performed a randomized double-blinded, single center interventional investigation. The investigation enrolled 150 females to determine if screening and treating bacterial vaginosis at cesarean section decreases infection rate. The goal was to determine if routine screening & management should be recommended or discouraged for all cases planning a cesarean section late in pregnancy or throughout CS. The investigation found a bacterial vaginosis prevalence of 37.4 percent.

The present investigation found statistically insignificant differences among the groups being evaluated according to age, body mass index, prior cesarean section, parity, & gestational age (p values equal 0.206, 0.374, 0.427, 0.500, 0.357) correspondingly.

Our findings indicate that there statistically insignificant variations have been observed in the length of the operation and the amount of blood loss among the groups that were evaluated (p values = 0.635, 0.373) respectively.

These findings corroborate prior investigations. Alkady et al. (8) carried out a prospective clinical investigation involving two hundred pregnant females who underwent elective cesarean section. The goal of the investigation was to investigate the correlation among BV as well as infection of caesarean wound. The results showed that there were insignificant variations among the groups being analyzed according to surgery indication, length, & blood loss (p values equal 0.321, 0.217, and 0.541, correspondingly).

Multiple classifications for surgical site infections exist, involving those provided by the Centers for Disease Control & Prevention & the ASEPSIS wound score system. Nevertheless, the Southampton wound scoring system is likely the simplest scoring method. The grading system assigns wound complications on a scale ranging from 0 to V, where V indicates a deep or severe wound infection, and zero reflects the typical healing process. In addition, the Southampton Scale includes wound management after being released from the hospital, which is divided into four categories: The healing process can be categorized into four stages: (A) normal healing, (B) minor complications, (C) wound infection (specifically Southampton scoring system IV or V, or a cesarean wound that required antibiotics after being discharged), and (D) major hemorrhage that needed drainage or aspiration (6).

Our findings indicate that the Southampton wound scoring was significantly elevated in the group with bacterial vaginosis. The occurrence of Grade zero was significantly lower in the bacterial vaginosis group, however Grades IV and V were significantly higher in the bacterial vaginosis group (p value equal 0.001).

Our findings indicate that there was a significantly higher occurrence of Cesarean wound infection in the bacterial vaginosis group (38.3%) compared to the control group (13%) (p value less than 0.001).

As per the CDC recommendations, superficial incisional surgical site infection refers to an infection that arises within thirty days and affects just the skin & subcutaneous tissue of the incision. Deep incisional surgical site infection occurs either within thirty days or within ninety days after the surgery, and it affects the deeper layers of the incision, such as the fascial and muscular tissues. Organ/space surgical site infection occurs within either thirty or ninety days after the operation and affects any portion of the body except for the fascia, skin incision, or muscle layers that were opened or manipulated throughout the operation (7).

The investigation found that 79.5 percent of females with surgical site infection in the bacterial vaginosis group (thirty-five cases) had only superficial surgical site infections, as contrast to 93.3 percent in the control group. 20.5 percent of the female participants had deep surgical site infection, characterized by serious wound infection with tissue breakdown or hematoma requiring aspiration. In comparison, only 6.7 percent of the cases in the control group had this condition.

Nonetheless, there was a significant difference in the frequency of superficial and deep cesarean wound infection between the BV group and the control group (p value equal 0.426).

Several research have been conducted to examine and establish the correlation among bacterial vaginosis & surgical site infections. While some investigations support our findings, others present conflicting outcomes.

Consistent with our research, Alkady et al. (8) demonstrated that the occurrence of infection of cesarean wound was significantly greater in the BV group than the control group (p value less than 0.001).

In agreement with our findings, Soper DE, (10) performed an investigation which involved 134 cases who had abdominal hysterectomy. The incidence of vaginal-cuff cellulitis or abscess was significantly greater in cases with before surgery bacterial vaginosis compared to cases without BV (thirty four percent vs eleven percent).

The connection between bacterial vaginosis and infections following surgery is reinforced by the detection of bacterial vaginosis -associated organisms, like G. vaginalis, in cases who have developed post-hysterectomy cuff infections (10).

In a study conducted by Soper DE (10), it was shown that preoperative administration of metronidazole led to a significant reduction in the occurrence of vaginal-cuff infections and the length of hospital stay in women with bacterial vaginosis who underwent hysterectomy. The rate of infections was 0% in the treated group than thirty-six percent in the untreated group ($P < .05$).

Kulkarni et al. (11) carried out an observational investigation to find out the prevalence of bacterial vaginosis in pregnant females attending the antenatal clinic. The investigation included 246 pregnant females in their second and third trimesters, with the aim of preventing perinatal complications. The investigation found that bacterial vaginosis is correlated with a higher risk of preterm labor and premature rupture of membranes. Consequently, pregnant women with bacterial vaginosis are at a higher risk of developing postoperative surgical site infections.

According to a study conducted by Hay P, (18) , it was shown that females who have bacterial vaginosis and choose to have an elective termination of pregnancy had a higher chance of developing endometritis & pelvic inflammatory disease. This condition may be present in approximately thirty percent of these females.

According to Jacobsson et al. (19), it is advisable to screen for bacterial vaginosis during the 35- to 37-week group B streptococcus culture visit & provide treatment for those who test positive. This recommendation is based on the findings that bacterial vaginosis is associated with intrapartum chorioamnio-

nitis and post-cesarean endometritis.

Nevertheless, this investigation has shown that conducting preoperative screening and treatment of bacterial vaginosis prior to elective cesarean section appears to be a potential approach for reducing the occurrence of following surgery surgical site infections.

When assessing the preventive treatment prior to to cesarean section, Sadek and Soliman (17) discovered that treating bacterial vaginosis was strongly correlated with a decrease in post-cesarean endometritis. The statistical analysis illustrated a significant association, with a P value of 0.0121 and cOR of 0.2834. The ninety-five percent confidence interval ranged from 0.2228 to 0.8627.

The strength points of this investigation

The key strengths of this investigation include its prospective study design, the inclusion of all cases, and the absence of any cases lost to follow-up after the surgery. This investigation is the first to establish a correlation between Bacterial vaginosis and particular types of SSI, specifically infection of cesarean wound. The study utilized CDC standards and Southampton wound scoring to make this correlation.

The limitations of the investigation:

The investigation has some limitations that are worth mentioning. Firstly, the sample size is rather small compared to previous investigations. Additionally, it is not a multicentric study like the one conducted by Kulkarni et al. (11), which included a total of 246 cases. Furthermore, there is no correlation between bacterial vaginosis & negative pregnancy outcomes, specifically preterm birth and neonatal sepsis. Thirdly, the investigation is conducting single-center research at a tertiary maternity care unit that primarily serves low-socio-economic females or those with complicated pregnancies. Consequently, the population represented might not be comprehensively representative.

CONCLUSION

Most females with bacterial vaginosis are asymptomatic and are at a greater probability of adverse infectious complications because of cesarean delivery. The identification of bacterial vaginosis is simple, rapid, and cost-effective. BV is a significant public health problem that is widespread among pregnant females & is related to surgical site infections. In a safe and cost-effective manner, screening females for bacterial vaginosis before a cesarean section can further reduce postoperative infections.

To prevent postoperative surgical site infections, it is recommended that all pregnant women undergo routine vaginal & cervical swab sample cultures throughout prenatal visits, especially throughout the 2nd and 3rd trimesters.

Additional Information

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosures

Conflicts of Interest: The authors have no conflicts of interest to declare.

Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of data from patients.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Financing Support: All authors have declared that no financial support was received from any organization for the submitted work.

Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.

Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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Fetal Main Pulmonary Artery Doppler and Lamellar Body Count in Amniotic Fluid as Predictors for Respiratory Distress Syndrome Development

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Abstract

Context: Insufficient surfactant in the underdeveloped fetal lungs can cause severe respiratory distress syndrome (RDS), potentially necessitating specialized management during delivery.

Aims: Investigate the lamellar body count in amniotic fluid and fetal main pulmonary artery doppler measures as possible markers for the onset of respiratory distress syndrome.

Settings and Design: This cross-sectional study included 118 pregnant women scheduled for elective Cesarean section. Participants were recruited from Menoufia University Hospital and El-Tahrir General Hospital in Imbaba.

Methods and Material: All the studied cases were subjected to: Detailed personal and obstetric histories included information such as:(Age, BMI, Sex, Gravidity, Parity, Gestational age, Previous CS, Previous PTL, Corticosteroids and Corticosteroids dose time), Ultrasound Examinations, Cesarean section and delivery assessment, Post-Delivery Neonatal Evaluation.

Statistical analysis used: Data analysis was conducted using SPSS version 24, which stands for the Statistical Program for the Social Sciences.

Results: Patients with respiratory distress syndrome (RDS) can be reliably identified using the pulmonary artery pulsatility index (PI) when the cut off value is more than 2.55. It shows 81.8% sensitivity, 100% specificity, 100% positive predictive value (PPV), and 98.2% negative predictive value (NPV) with an area under the curve (AUC) of 0.96 and a p-value less than 0.001. Also, with a cutoff value higher than 0.89, the pulmonary artery resistance index (RI) can distinguish RDS in these patients. It has a 0.9 area under the curve (AUC) and a p-value of less than 0.001, in addition to 81.8% sensitivity, 85% specificity, 36% PPV, and 97.8% NPV.

Conclusions: A fetal main pulmonary artery Doppler measurement, an amniotic fluid lamellar body count (LBC) and pulmonary artery index can be used to anticipate neonatal respiratory distress syndrome (RDS).

In conjunction with lower LBC and higher Pulmonary Artery Pulsatility Index (PI) and Resistance Index (RI), these non-invasive methods of fetal lung maturity evaluation are very helpful in assessing the risk of RDS.

Key-words: Fetal Main Pulmonary Artery Doppler, Lamellar Body Count, Respiratory Distress Syndrome.

Introduction

Insufficient surfactant in the fetus's immature lungs can lead to serious respiratory distress syndrome (RDS), potentially requiring targeted interventions during delivery. In order to reduce the possibility of respiratory distress syndrome (RDS), it is essential to detect fetal lung immaturity before delivery and prescribe surfactant medication. Recently, assessing fetal lung maturity (FLM) has become a key focus in the weeks leading up to delivery [1].

Extensive efforts have been made to predict fetal lung maturity to evaluate neonatal complications risk caused by pulmonary immaturity, such as respiratory distress syndrome or death. Several approaches to assess fetal lung maturity are available, with amniocentesis being the standard procedure used today. However, Amniocentesis carries complications and risks in about 0.7% of cases. These complications can include preterm labor and delivery, preterm premature rupture of membranes, placental abruption, and fetomaternal hemorrhage [2].

Pregnant women would prefer a non-invasive way to determine when their fetuses' lungs are fully developed. At present, fetal lung maturity is typically evaluated indirectly through ultrasound imaging of the fetus's overall structure and Doppler measurements of blood flow patterns. At the present time, there is no reliable non-invasive method for predicting fetal lung maturity prior to delivery. Foetal pulmonary artery flow velocity waveforms changed with increasing gestational age [3].

Other methods to assess fetal lung maturity include biological studies. The Lecithin to Sphingomyelin (L/S) ratio, as developed by The Gluck assay is a commonly used test for evaluating fetal lung maturity. Although it is reliable, it is also costly, time-consuming, and not available everywhere. Furthermore, fluids contaminated with blood or meconium cannot be used to reliably evaluate the L/S ratio [4].

In 1989, Dubin initially introduced the lamellar body count (LBC), an abbreviation for lamellar body number density. Type II pneumocytes secrete their surfactant-containing structures as lamellar bodies. Growing lungs in utero, there is an increase in lamellar body production, which is reflected in an increase in the L/S ratio and elevated phospholipid levels in the amniotic fluid [5].

Lamellar bodies are about the same size as platelets and can be counted using the platelet channel on most electronic cell counters. This makes the test quick, simple, and cost-effective. This measurement is expressed as the quantity of amniotic fluid lamellar bodies per microliter (μL). Indicators of fetal lung maturity (FLM) that have been shown to be reliable in multiple investigations are lamellar body counts [6].

The purpose of this study was to identify potential risk factors for respiratory distress syndrome in pregnant women by analyzing doppler signals from the foetus's primary pulmonary artery and the number of lamellae in the amniotic fluid.

Subjects and Methods

This cross-sectional study included 118 pregnant women scheduled for elective Cesarean section. Participants were recruited from Menoufia University Hospital and El-Tahrir General Hospital in Imbaba during the period from 1st June 2023 to 30th April2024.

The Research Ethics Committee (REC) at Menoufia University's Faculty of Medicine

gave their approval under number: 6/2023 obgs 35. Furthermore, before any patient could take part in the study, they were asked to sign a document indicating that they were fully informed.

Inclusion criteria:

Women who are over 18 years, will be having a baby boy or girl, are 26–39 weeks along in their pregnancies, and are scheduled to have a spinal anesthesia-induced elective caesarean section.

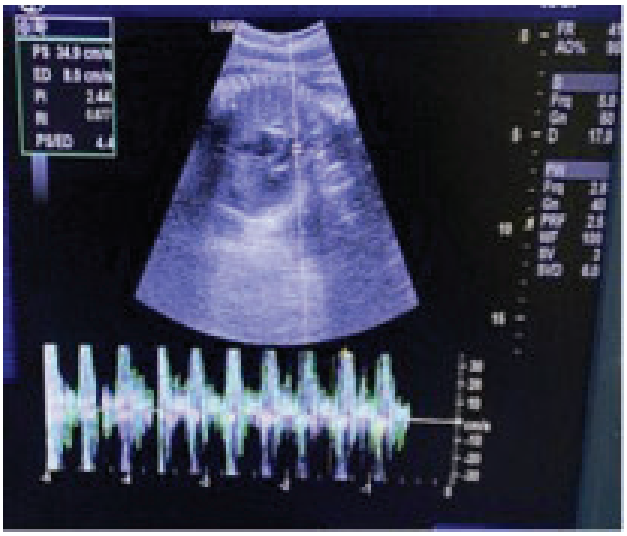
Exclusion criteria:

Chorioamnionitis, fever, antepartum hemorrhage, congenital anomalies, less than 24 weeks, and patients under general anesthesia for LSCS.

All the studied cases were subjected to: Detailed personal and obstetric histories included information such as:(Age, BMI, Sex, Gravidity, Parity, Gestational age, Previous CS, Previous PTL, Corticosteroids and Corticosteroids dose time), Ultrasound Examinations, Cesarean section and delivery assessment, Post-Delivery Neonatal Evaluation.

Ultrasound Examinations:

In a systematic manner, the examiner assessed the foetal heart using the four-chamber view, the three-vessel view, and the outflow pathways. From the midpoint between the pulmonary valve and the point where the main pulmonary artery (MPA) divides into the right and left branches, the examiner followed the MPA under an axial view of the thorax. The fetus was at rest and not moving because of fetal respiration. With the insonation angle maintained below 15 degrees and the pulsed Doppler sample gate set to 3 mm, The study was conducted. The peak systolic velocity (PS) and early diastolic notch are components of the velocity waveform, was clearly shown after adjusting the Doppler gain and scale. There was a slight, inverted flow notch at the end of systole and a sharp systolic peak in the MPA Doppler waveform, which is known as the "spike and dome" pattern. The ductus arteriosus waveform is more commonly rounded, fuller, and triangular, and it has a higher diastolic flow rate; its unique shape helps to distinguish it from it. Once the optimum MPA waveform was generated, the Doppler velocity variables were manually traced three times and an average was determined. One of these variables was the resistance index (RI), and another was the pulsatility index (PI) [7].



Doppler US scan of the fetal main pulmonary artery showing the characteristic PA wave with normal values of pulsitility index (PI) and resistive index(RI)

Cesarean section and delivery assessment:

Following the completion of ultrasound studies, all women enrolled in the study were scheduled for a lower segment Cesarean section (LSCS) based on obstetric indications which were documented in the patient's medical records. Regional anesthesia, typically spinal or epidural, was administered to ensure patient comfort and safety during the operation.

Amniotic Fluid Extraction and Analysis:

During the Cesarean section, once the uterus was opened and before the delivery of the fetus, approximately 5 cm^3 of amniotic fluid was carefully aspirated from the amniotic sac using a sterile technique. The collected amniotic fluid was immediately transferred into

a pre-labeled, sterile, capped plastic syringe. This syringe was then placed in a secure, temperature-controlled container to prevent any alteration in sample integrity.

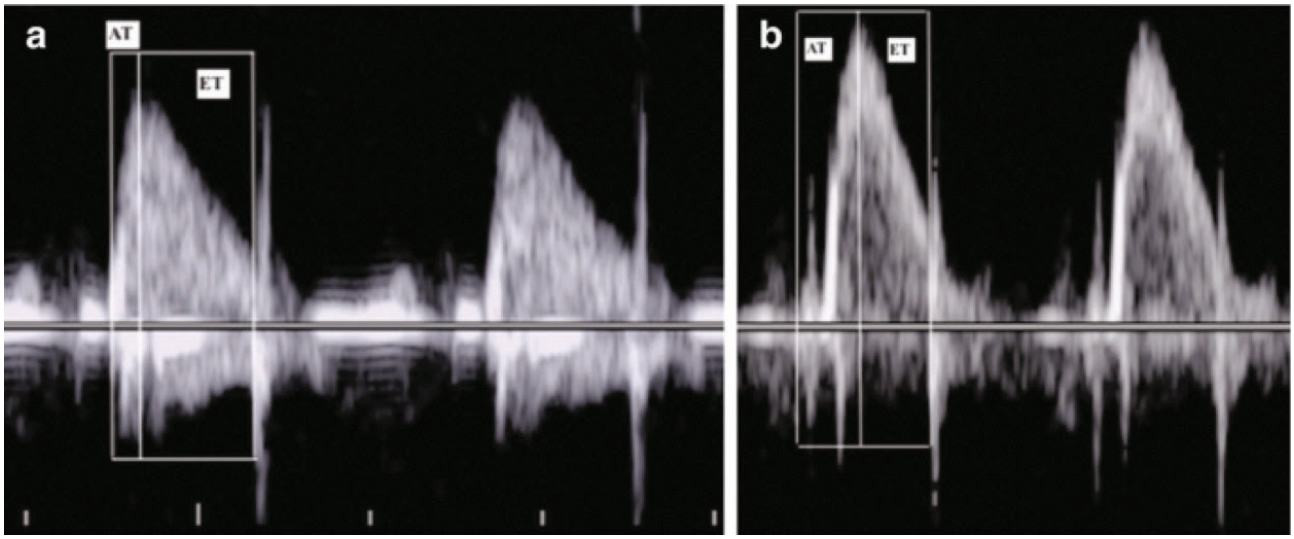
Laboratory Analysis:

The sample was transported promptly to the clinical laboratory to minimize the time between collection and analysis. Upon arrival at the laboratory, the amniotic fluid was analyzed for lamellar body count (LBC) using a standardized electronic counting system [8].

Statistical Analysis

Data were analysed using Statistical Program for Social Science (SPSS) version 24. Qualitative data were reported as frequencies and percentages. Quantitative data were presented as means ± standard deviations (SD) for data that followed a normal distribution, or as medians with interquartile ranges (IQR) for data that did not follow a normal distribution. Test of Mann Whitney U (MW) when comparing among 2 groups (for abnormally distributed data). Test of Chi-square was used when comparing between non-parametric data. In order to determine the strength of the connection between the variables, we utilized Pearson's correlation coefficient (r). One such curve is the ROC, or receiver operating characteristic was employed to determine the cutoff values, as well as to evaluate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).P-value < 0.05 was considered significant.

Results



Measurement of the acceleration (AT) and ejection time (ET) in the main pulmonary artery Doppler waveform. (a) Representation of an immature-type wave; (b) representation of a mature-type wave

Table 1 shows description of US data, LBC and neonatal status in all patients of study.

Table 1: Description of US data, LBC and neonatal status in all patients of study.

(n = 118)	Studied patients (N = 118)
BPD (cm)	9.32±0.23
FL (cm)	7.40±0.21
AC (cm)	33.02±0.98
AFI	11.45±3.12
Pulmonary A. PI	2.46±0.09
Pulmonary A. RI	0.88±0.01
EFW (gm)	3003.6±227.5
LBC	67822.03 ± 23784.8
Neonatal status	
APGAR (1 min)	5.8 ± 1.18
APGAR (5 min)	7.93 ± 1.54
RDS	11 (9.3%)
NICU admission	11 (9.3%)

Statistically significant (p-value = 0.039) increased BMI in patients with RDS when compared with patients of no RDS. • Statistically significant correlation (p-value = 0.002) between RDS and Corticosteroid doses of the studied patients. There were 71 patients (66.4%) with Corticosteroid doses in patients without RDS versus 2 patient (19.2%) in patients with RDS. Statistically significant decreased BPD, AC and AFI in patients with RDS when compared with patients of no RDS. High statistically significant (p-value < 0.001) increased pulmonary artery PI, and pulmonary artery RI in patients with RDS when compared with patients of no RDS. Table 2.

Table 2: Correlation study between RDS and demographic data of the studied patients and between RDS and US data of the studied patients.

		RDS				P-value
		No (N = 107)		Yes (N = 11)		
Age (years)		27(24 – 32)		26(20 – 33)		0.717
BMI (kg/m²)		27(25 – 29)		30(26 – 32)		0.039*
G. Age (weeks)		38(37 – 38)		38(38 – 39)		0.076
Fetal sex	**Male**	54	50.5%	4	36.4%	0.373
	Female	53	49.5%	7	63.6%	
Gravidity	**Gravida 1**	18	16.8%	5	45.5%	0.081
	Gravida 2	35	32.7%	0	0%	
	Gravida 3	31	29%	4	36.4%	
	Gravida 4	16	15%	1	9.1%	
	Gravida 5	7	6.5%	1	9.1%	

Parity	**Nulliparous**	20	18.7%	5	45.5%	0.06
	Para 1	35	32.7%	0	0%	
	Para 2	35	32.7%	5	45.5%	
	Para 3	12	11.2%	0	0%	
	Para 4	5	4.7%	1	9.1%	
Previous CS		83	77.6%	6	54.5%	0.091
Previous NICU		6	5.6%	2	18.2%	0.114
Previous PTL		4	3.7%	1	9.1%	0.401
Corticosteroids		71	66.4%	2	19.2%	0.002*
BPD (cm)		9.37(9.23 - 9.43)		8.91(8.61 - 9.37)		0.033*
FL (cm)		7.45(7.32 - 7.51)		7.42(6.99 - 7.79)		0.270
AC (cm)		33.2(32.7 - 33.6)		31.9(30 - 33.2)		0.037 *
AFI		12(10 - 14)		7(3 - 12)		0.001 *
Pulmonary A. PI		2.43(2.42 – 2.45)		2.68(2.57 – 2.83)		< 0.001*
Pulmonary A. RI		0.879(0.872 – 0.886)		0.901(0.896 – 0.912)		< 0.001*
EFW		3043(2905 - 3100)		3107(3017 – 3220)		0.065

*: p-value < 0.05 considered substantial.

High statistically substantial (p-value < 0.001) decreased pulmonary artery PI, and pulmonary artery RI in patients with corticosteroids when compared with patients of no corticosteroids. Table 3

Table 3: Correlation study among corticosteroid administration and other studied data in the studied patients.

		Corticosteroid		**P-value**
		No (N = 45)	**Yes (N = 73)**	
Pulmonary A. PI	Median	2.51	2.43	< 0.001*
	IQR	2.43 – 2.53	2.41 – 2.44	
Pulmonary A. RI	Median	0.898	0.878	< 0.001*
	IQR	0.875 – 0.902	0.872 – 0.884	
LBC	Median	74000	74000	0.881
	IQR	63500 - 84000	58000 - 84000	

*: p-value < 0.05 considered substantial

Discussion

Respiratory Distress Syndrome (RDS) presents a major health challenge for newborns, particularly when associated with the immaturity of the fetal lungs. Such immaturity often necessitates urgent therapeutic interventions at birth, including surfactant replacement therapy, to mitigate the risk of severe respiratory complications. Early and accurate prediction of fetal lung maturity (FLM) is thus critical for optimizing neonatal outcomes and preparing appropriate delivery room interventions [9].

The mean number of lamellar bodies (LBC)

among all participants in this study was 67,822.03 ± 23,784.8, ranging from 2,000 to 99,000.

The lamellar body counts in amniotic fluid can be measured using cell-counting equipment commonly used in clinical laboratories. This method is simple, fast, and inexpensive. Additionally, it is the most feasible method for determining the probability of neonatal respiratory distress syndrome (RDS) and evaluating the fetal lung maturity during pregnancy.

When the LBC count falls within 0 to 15,000, it is categorized as "Immature." Ranges between 15,000 and 50,000 are labeled "Indeterminate," while counts exceeding 50,000 are termed "Mature." [10].

As regard 1 min Apgar score, the mean score in all patients of study was 5.8 ± 1.18 with 2 minimum score and 8 maximum score. As regard 5 min Apgar score, the mean score in all patients of study was 7.93 ± 1.54 with 2 minimum score and 10 maximum score. As regard RDS and NICU admission, there were 11 patients (9.3%) developed RDS and needed NICU admission.

In close proximity, [11]. demonstrated a study that evaluated the validity and reliability of non-invasive approaches for predicting the possibility of neonatal respiratory distress syndrome (RDS) using fetal lung volume measurements using VOCAL and the pulmonary artery resistance index (PA-RI) in preterm pregnancies involving 80 eligible women and found that Apgar 1-min and 5-min shoed means of (4.56± 0.63) and (5.79 ± 0.43), respectively [11].

Also [12]. conducted research to identify the most sensitive and specific cutoff points for fetal main pulmonary artery Doppler indices (MPA indices) in predicting fetal lung maturity (FLM). Near the 38-week mark of gestation, 200 pregnant women participated in the study; 46 (or 23% of the total) fetuses were found to have respiratory distress syndrome (RDS), whereas 154 (or 77% of the total) did not. The RDS group (Group I) had an APGAR score ranging from 6 to 9, with an average of 8.05 ± 0.86, according to the results, while the non-RDS group (Group II) had an APGAR score ranging from 5 to 8, with a mean of 6.91 ± 0.87. At 5 minutes, the APGAR scores for Group I's scores were between 8 and 10, with a mean of 8.92 ± 0.76, while Group II's scores were between 5 and 8, with a mean of 6.98 ± 0.84 [12].

No statistically significant correlation between RDS and age, gestational age, sex, gravidity, parity, previous CS, previous NICU and previous PTL of the studied patients (p-value = 0.717, 0.076, 0.373, 0.081, 0.06, 0.06, 0.114 and 0.401 respectively). While statistically significant (p-value = 0.039) increased BMI in patients with RDS (median = 30, IQR = 26 - 32 kg/m2) when compared with patients of no RDS (median = 27, IQR = 25 - 29 kg/m2). Also, statistically significant correlation (p-value = 0.002) between RDS and Corticosteroid doses of the studied patients. There were 71 patients (66.4%) with Corticosteroid doses in patients without RDS versus 2 patient (19.2%) in patients with RDS.

Similar to our study, [11]. found a statistically substantial difference among groups of RDS and non-RDS in neonatal birth weight (mean of 2460 ± 125.7 vs 2886 ± 342.2, P value= 0.04) [11].

In the present study, no statistically substantial difference was noticed (p-value = 0.270) of FL as regard RDS. Statistically substantial (p-value = 0.037) decreased AC in patients with RDS (median = 31.9, IQR = 30 – 33.2) was found when compared with patients of no RDS (median = 33.2, IQR = 32.7 – 33.6). While a statistically substantial (p-value = 0.033) decreased BPD was noticed in patients with RDS (median = 8.91, IQR = 8.61 – 9.37) when compared with patients of no RDS (median = 9.37, IQR = 9.23 – 9.43). Statistically substantial (p-value = 0.001) decreased AFI was observed in patients with RDS (median = 7, IQR = 3 – 12) when com-

pared with patients of no RDS (median = 12, IQR = 10 – 14). High statistically significant (p-value < 0.001) increased pulmonary artery PI in patients with RDS (median = 2.68, IQR = 2.57 – 2.83) when compared with patients of no RDS (median = 2.43, IQR = 2.42 – 2.45). High statistically significant (p-value < 0.001) increased pulmonary artery RI in patients with RDS (median = 0.901, IQR = 0.896 – 0.912) when in correlation with patients of no RDS (median = 0.879, IQR = 0.872 – 0.886) [14].

The decreased BPD observed in patients with RDS suggests that these infants might have experienced some degree of growth restriction or developmental delays, particularly in head growth, which is often a sensitive indicator of overall fetal health. Smaller BPD measurements in the context of RDS could reflect a compromised intrauterine environment affecting the fetus, potentially influencing lung development and function at birth [13]. Also, a smaller AC in fetuses that develop RDS might indicate less glycogen and fat storage, which are crucial for energy supply and growth. Like BPD, a smaller AC may suggest a suboptimal intrauterine environment, which could be linked to impaired development of fetal organs, including the lungs [15].

A high statistical substantial (p-value < 0.001) reduced LBC in patients with RDS (median = 4000, IQR = 3000 – 6000) when compared with patients of no RDS (median = 74000, IQR = 64000 – 84000).

The number of lamellar bodies in the amniotic fluid is an excellent indicator of the fetal lung maturity and surfactant production. The reduced number of lamellar bodies indicates that the fetal lungs may not be sufficiently matured, leading to an increased risk and prevalence of RDS. This immaturity results in the lungs' inability to properly inflate and function post-delivery, necessitating medical intervention to manage the respiratory distress [1].

High statistically significant (p-value < 0.001) decreased 1 min Apgar score in patients with RDS (median = 3, IQR = 3 – 3) when compared with patients of no RDS (median = 6, IQR = 6 – 7). High statistically significant (p-value < 0.001) decreased 5 min Apgar score in patients with RDS (median = 4, IQR = 3 – 4) when compared with patients of no RDS (median = 8, IQR = 8 – 9).

In parallel to our results, [16]. conducted research to determine the efficacy of main pulmonary artery (MPA) Doppler indices in predicting clinical respiratory distress syndrome (RDS) in late preterm and early term fetuses. In this study, 342 pregnant women who were between 34 and 39 weeks along in their pregnancies participated by going in for their regular third-trimester ultrasounds. This study found a substantially lower score of Apgar was observd in foetuses with RDS at 1-min and 5-min showing means of (4.02 ± 0.87) and (5.87 ± 0.61), respectively than non-RDS foetuses showing means of (7.27 ± 0.55) and (9.45 ± 0.62), respectively (P value < 0.05) [16].

By utilizing a cutoff level higher than 12,000, the ROC curve analysis proved that lamellar body count (LBC) successfully differentiates respiratory distress syndrome (RDS) in the population under study. Thanks to this cutoff, we were able to attain perfect sensitivity, specificity, PPV, and NPV, as well as an AUC of 1.0 and a p-value below 0.001.

Fetal lung maturity can be predicted, and the risk of neonatal respiratory distress syndrome (RDS) can be reduced with an LBC cutoff value of ≥20,000/μL. One easy, quick, and inexpensive way to measure LBC in amniotic fluid is using cell-counting equipment, which is available in most clinical laboratories. On top of that, it is the best and most practicable method for assessing foetal lung maturity and predicting the likelihood of RDS in newborns when done during pregnancy [10].

Pulmonary artery PI Using a cutoff value

larger than 2.55, which provides 81.8% sensitivity, 100% specificity, 100% PPV, and 98.2% NPV, identify respiratory distress syndrome (RDS) in the patients under study. (AUC = 0.96 & p-value < 0.001).

Pulmonary artery RI can distinguish between patients with respiratory distress syndrome (RDS) when the cutoff level is higher than 0.89. This approach provides 81.8% sensitivity, 85% specificity, 36% positive predictive value (PPV), and 97.8% negative predictive value (NPV), with an area under the curve (AUC) of 0.9 and a p-value of less than 0.001.

Similarly, [11]. demonstrated that a PA-RI cutoff value of 0.77 results in an area under the curve (AUC) of 0.83, a sensitivity of 83.3%, and a specificity of 85.26% when it comes to predicting neonatal respiratory distress syndrome (RDS). Furthermore, with a PA-RI cutoff of ≥0.77, one may anticipate the upcoming occurrence of RDS with an accuracy of 85.3% and a sensitivity of 83.3% [11].

Also, [17]. found that with a PI cutoff value of 2.33 for MPA, the diagnostic accuracy is 94.4%, the sensitivity is 79.0%, the specificity is 100.0%, the PPV is 100.0%, the NPV is 92.9%, and the NPV is 100. Not only that, with an MPA resistance index (RI) cutoff value of 0.89, the diagnostic accuracy is 88.1%, the sensitivity is 84.2%, the specificity is 89.5%, the PPV is 74.4%, and the NPV is 94.0% [17].

Conclusions In fetuses with elevated Doppler indices of the major pulmonary artery, such as Pulsatility Index (PI), Resistance Index (RI) and pulmonary artery index, there's a higher risk of neonatal respiratory distress syndrome (RDS). Analyzing amniotic fluid for these indices and lamellar body count (LBC) can help predict the likelihood of RDS. A decrease in LBC also suggests an increased risk of RDS in these cases, significantly correlate with the development of RDS, underscoring their utility as non-invasive tools for assessing fetal lung maturity. Pulmonary artery index is a good predictor of neonatal RDS.

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Acknowledgement: Nil

Effect of Sildenafil Citrate on endometrial preparation before thawed embryo transfer cycles in treatment of infertility

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Abstract

Background: The utilization of assisted reproductive technology has become more common as a helpful therapy for infertile women. An endometrium in a receptive condition, an embryo with implantation competency, and coordinated development of the embryo and endometrium are the three prerequisites for a successful implantation.

Objectives: to assess the efficacy of sildenafil citrate on the ultrasound endometrial thickness and pattern during endometrial preparation before transfer of thawed embryos in treatment of infertility.

Methods: The Assisted Reproductive Technology (ART) Unit at Ain Shams University Maternity Hospital conducted prospective randomized clinical research from November 2023 to May 2024 with 80 women who underwent endometrial preparation before frozen embryo transfer. There were inclusion and exclusion criteria for the study.

Results: There was no statistically significant difference seen in age or BMI between the study groups. There was no statistically significant difference between the analyzed groups' parity, cause of infertility, type of infertility, and period of infertility. There was no statistically significant difference between the groups under study when it came to the lab investigations. Between the research groups, there was a statistically significant difference in the total number of sacs, clinical pregnancy, and pregnancy test results. There was a highly significant difference in the groups under research when it came to the last ultrasound (endometrial thickness) and last ultrasound (endometrial type), but there was no statistically significant difference in the Day 7 Ultrasound.

Conclusion: In patients with thin endometrium undergoing frozen-thawed embryo transfer cycles, the treatment of vaginal sildenafil citrate significantly enhances the biochemical pregnancy rate, the clinical pregnancy rate, and endometrial thickness. It also modifies the endometrial pattern. Furthermore, it was demonstrated that a com-

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bined evaluation of endometrial thickness and pattern was more beneficial for patient counseling than individual analysis and was a greater predictor of the outcome of IVF/ICSI-ET.

Keywords: Thawed embryo transfer cycles, Endometrial preparation, Sildenafil Citrate.

INTRODUCTION

The utilization of assisted reproductive technology has become more common as a helpful therapy for infertile women. An endometrium in a receptive condition, an embryo with implantation competency, and coordinated development of the embryo and endometrium are the three prerequisites for a successful implantation (1).

The endometrium is the special epithelial lining of the uterus that is above the level of internal os. It is made up of two layers: the functional layer on top and the deeper base layer underneath. Endometrial growth requires both uterine blood flow and angiogenesis. Uterine blood flow and the vascular development of the endometrium are closely related. The uterine artery branches into coiled arteries, which serve the surface layer of the endometrium, while small, straight, short arteries nourish the base layer. Usually measuring 8 to 10 mm, the endometrium is in the secretory phase. Angiogenesis has a major impact on the growth of the endometrium, the formation of a corpus luteum, and the development of a dominant follicle. Angiogenesis is required to sustain endometrial growth after menstruation and to provide a vascularized, responsive endometrium that is prepared for implantation (2).

An important part of assisted reproduction is endometrial evaluation. The success of assisted reproduction can be predicted by endometrial thickness. Since adequate endometrial receptivity is required for a healthy pregnancy, it is the main reason why embryo implantation attempts fail (3).

The endometrium is often not an embryo's receptive environment, with the exception of the window. The window of opportunity for blastocyst implantation into the endometrium occurs between cycle days 20 and 24, and is known as the "implantation window." Refractory endometrial conditions are indicative of it (1).

It is believed that endometrial receptivity is required for a fruitful pregnancy. When progesterone is utilized in frozen-thawed embryo transfer cycles, or on the day of ovulation or human chorionic gonadotrophin (HCG) injection in fresh IVF cycles, an endometrium is considered thin if its measurement is less than 7 mm. Regardless of ovarian stimulation, a thin endometrium can be a distinct unfavorable predictor of pregnancy outcomes. The endometrium's thickness and pattern are two different aspects that influence the outcome of a pregnancy (4).

Since 1998, sildenafil citrate, sometimes referred to as Viagra VR, has been utilized as a vasoactive treatment for male erectile dysfunction. This specific inhibitor of phosphodiesterase type 5 activates the cyclic guanosine monophosphate (cGMP) pathway in the penile erectile tissue, which can augment the effects of nitric oxide (NO) on smooth muscle relaxation and vasodilation (1).

In patients with a thin endometrium, sildenafil citrate helps to improve endometrial development and achieve successful pregnancy outcomes. However, studies have also demonstrated that sildenafil citrate has little effect on the endometrium (1). In order to determine the impact of sildenafil citrate on endometrial thickness and pattern on ultrasonography during endometrial preparation prior to the transfer of frozen embryos for the purpose of treating infertility, we are carrying out the current study.

PATIENTS AND METHODS

With permission from the ethical committee and patient consent in writing, this prospec-

tive randomized clinical study involved 80 women who underwent endometrial preparation before thawed embryo transfer at the Assisted Reproductive Technology (ART) Unit at Ain Shams University Maternity Hospital between November 2023 and May 2024.

Study population: Women undergoing endometrial preparation before thawed embryo transfer with the following inclusion criteria:

Inclusion criteria: Frozen-thawed embryo transfer cycles, High quality embryos and Women aged 21–40 years. Oocytes: all were MII (metaphase II) before ICSI and Embryos grading: grade I and grade II were included.

Exclusion criteria: Women with pelvic pathology including endometrial polyps, adenomyosis, ovarian cysts, fibroids or congenital uterine anomaly (high risk for implantation failure), Women with an abnormal hormonal profile (high risk for failed IVF Cycles), Significant cardiovascular, liver, or renal disease (high risk for maternal mortality), Smoking (high risk for failed IVF Cycles) and Hydrosalpinx (high risk for implantation failure).

Indication of IVF/ICSI:

1. Unexplained infertility

Unaccounted-for infertility, also known as subfertility, is the diagnosis given to a couple that does not meet the criteria for oligo/anovulatory infertility, a diagnosis of male factor infertility or anatomical concerns such as endometriosis, blocked fallopian tubes, uterine cavity defects, or cervical/vaginal obstruction (5).

2. Male factor

The World Health Organization (WHO) defines male infertility as the inability of a male to conceive a fertile female for at least a year after engaging in regular, unprotected sexual activity (6).

3. Anovulation

Anovulation, or the lack of ovulation, is typically brought on by a change in hormone levels that impairs follicular growth. More precisely, there is a decrease in progesterone and estrogen levels; still, the synthesis of estrogen is adequate to promote some expansion of the uterine lining, which frequently results in bleeding (7).

4. Tubal factor

When sperm and egg cannot meet due to a blockage in the fallopian tubes, it results in tubal factor infertility. About 25–30% of cases of infertility are due to tubal factor infertility. This syndrome encompasses situations where all fallopian tubes are obstructed, situations when just one tube is blocked, and situations where scarring narrows the tubes (8).

Study Procedures: All participants were submitted to the following:

History: including

Full detailed history focusing on present & past history, age, gravidy, Parity, BMI, Drug history, history of previous operations.

Examination: including

General examination to exclude systemic diseases.

Pelvic examination to exclude any pelvic pathology.

Investigational Studies:

CBC, Blood group, PT and INR, FSH, E2, Serum progesterone, TSH and Prolactin.

Radiological investigation:

Transvaginal sonography (TVS) was performed to exclude any uterine position, pathology and to assess the endometrial thickness.

The endometrial thickness assessment by TVUS was done by single operator, Lecturer Dr Mohamed Mokhtar at the Assisted Reproductive Technology (ART) Unit.

Transvaginal ultrasonography was used to measure the endometrial thickness after 7 days, and then every other day after that. The assessments were completed by a skilled investigator.

Gray-scale ultrasonography was used to measure endometrial thickness before and after sildenafil citrate therapy, and the results were associated with the control group's conception rate.

The patients were instructed to empty their bladders before to TVS. The patient was positioned supine, with a cushion beneath their buttocks, for the examination. The condom with the coupling gel inside was sealed around the probe. The covered probe was coated with more gel. The uterus was scanned both transversely and longitudinally after the transducer was inserted into the posterior vaginal fornix. The thickest area in the longitudinal plane was used to calculate the endometrial thickness. The highly reflecting contact where the myometrium and endometrium meet, were used to quantify it.

Two endometrial layers were indicated by this measurement. The two-half thickness endometrial measures were combined when there was fluid present in the endometrial canal. 400 mg of vaginal progesterone were given when the endometrial thickness exceeded 8 mm, and this treatment continued for three or five days until the embryo was transferred. Progesterone and estradiol valerate were then given until two weeks following the transfer of the embryos. Progesterone and estradiol valerate were administered until the thirteenth week of pregnancy in the event of a positive BHCG test. Two weeks after the embryo transfer, vaginal ultrasonography was used to determine the number of gestational sacs (9). If suspected, hysteroscopy or hysterosalpingography to rule out uterine or tubal pathology

Procedure

- **Endometrial preparation:**
- 0.1mg/day of gonadotropin-releasing hormone agonist Triptorelin (Decapeptyl®0.1mg/ml 7 prefilled syringe, FERRING PHARMACEUTICALS, CAIRO, EGYPT) was administrated subcutaneously on daily basis starting in the midluteal phase of the previous menstrual cycle till the day of 2 of the cycle.
- Oral E2 was started on the second or third day of the cycle to prime the endometrium. Estradiol was administered in an incremental fashion 2 mg/day during days 1-7, 4 mg/day during days 8-12, 6 mg/day during days 13 to embryo transfer.
- Follow up of endometrial thickness after 7 days then every 2 days.

Intervention procedure:

Beginning on the second day of the menstrual cycle, patients in the study group self-administered 20 mg of sildenafil citrate tablets (Pfizer Inc., New York, New York, USA) twice daily for seven days (10). Women were evaluated during treatment.

Sample Size: Based on the results Firouzabadi et al (9), with mean endometrial thickness in control group 8±3 while the mean endometrial thickness in intervention group 9.3±3.3, with alpha error 5% and power of study 80%, the required sample size is 80 patients , 40 in each group.

Sampling method: Randomized Sampling.

Randomisation:

A computer-generated database of random numbers was used for the randomization process, and Sealed Envelope Ltd.'s Simple Randomization Service with allocation concealment was used in 2017. Allocation was not altered after it was completed.

Allocation Concealment:

Sequentially-numbered, opaque, sealed envelopes enclosed the letter corresponding to the group which the patient followed. Envelopes were opened the day before starting the endometrial preparation to allocate the patient to its specific group; (case vs control group).

Detection bias was avoided by blinding the outcome assessors.

Outcome Measurements and Follow-up

- **Primary outcome:**

The clinical pregnancy rate defines as healthy gestational sac with positive fetal life

- **Secondary outcome:**

1. Endometrial thickness.
2. Endometrial pattern.
3. The morphology of the endometrium determines the classification of the endometrial pattern by B ultrasound radiography.

Ethical Considerations: The patient information was private. Patient confidentiality was maintained and data was presented according to diagnosis rather than the patient's identity. Every participant signed an informed consent form, which was verified with a date and time in Arabic. The patient's initials were given a number, and only the investigator knew this, protecting patient confidentiality.

Statistical analysis: The analysis was done with Windows v20.0 of SPSS. The data should be presented as range, mean, and standard deviation for numerical parametric variables, range, median, and inter-quartile range for numerical non-parametric variables, or as number and percentage for categorical variables. After that, appropriate statistical analyses were used.

Results

This study is a prospective randomized clinical study conducted in Assisted Reproductive Technology (ART) Unit at Ain Shams University Maternity Hospital.

The study included 80 patients which randomized and divided into 2 groups:

Group I: **Sildenafil group** included 40 women.

Group II: **Control group** included 40 women.

Table (1): Demographic data of the studied groups

	Sildenafil group (n=40)	Control group (n=40)	Test	P value
Age (years)				
Mean ±SD	31.5±6.19	31.8±4.67	t= 0.244	0.807
BMI (kg/m^2)				
Mean ±SD	29.4±5.75	30.1±5.83	t= 0.54	0.590

Based on age and BMI, Table 1 indicates that there was no statistically significant difference between the study groups.

Table (2): Patient's characteristics of the studied groups.

	Sildenafil group (n=40)	Control group (n=40)	Test	P value
Period of infertility				
Mean ±SD	2.88±1.18	2.85±0.83	t=0.131	0.717
Cause of infertility				
Unexplained	27 (67.5%)	33 (82.5%)	**X^2=4.695**	0.196
Male factor	3 (7.5%)	4 (10%)		
Tubal factor	5 (12.5%)	1 (2.5%)		
Anovulation	5 (12.5%)	2 (5%)		
Type of infertility				
1ry	25 (62.5%)	26 (65%)	**X^2=0.131**	0.895
2ry	15 (37.5%)	14 (35%)		

Parity				
NG	25 (62.5%)	26 (60%)	**X^2=0.676**	0.878
p0+	1 (2.5%)	2 (5%)		
P1	13 (32.5%)	11 (27.5%)		
P2	1 (2.5%)	1 (2.5%)		

x^2: Chi square test, t: Unpaired t test

Regarding the Period of Infertility, Cause of Infertility, Type of Infertility, and Parity, Table (2) demonstrates that there was no statistically significant difference between the analyzed groups.

Table (3): Lab Investigations of the studied groups.

	Sildenafil group (n=40)	**Control group (n=40)**	**Test**	**P value**
Prolactin (ng/mL)				
Mean ±SD	17.7±5.52	17.5±6.96	t= 0.142	0.887
TSH (uIU/mL)				
Mean ±SD	1.68±1.02	1.76±0.98	t= 0.357	0.721

There was no statistically significant difference in lab investigations between the examined groups (Table 3).

Table (4): Number, Quality of Embryos transferred and Transfer Day between studied groups

	Sildenafil group (n=40)	**Control group (n=40)**	**Test**	**P value**
NO. of Embryos transferred				
3	15 (37.5%)	17 (42.5%)	**X^2= 0.208**	0.648
2	**25 (62.5%)**	**23 (57.5%)**		
Quality of Embryos transferred				
Grade I	36 (90%)	35 (92.5%)	**X^2= 0**	1
Grade II	4 (5%)	5 (7.5%)		
Transfer Day				
Mean ±SD	3.9±0.84	3.92±0.82	t= 0.107	0.914

x^2: Chi square test, t: Unpaired t test

According to table 4, there was no statistically significant difference in the quantity, quality, or day of transfer of embryos between the groups under study.

Table (5): Primary out-come between studied groups

	Sildenafil group (n=40)	Control group (n=40)	Test	P value
Pregnancy test				
Positive	14 (35%)	10 (25%)	**X^2= 0.952**	0.329
Clinical pregnancy				
Positive	25 (62.5%)	17 (42.5%)	**X2= 5.07**	0.024
No. of sacs				
Nil	26 (65%)	30 (75%)	**X^2= 0.952**	0.329
1	14 (35%)	10 (25%)		

In terms of the pregnancy test, clinical pregnancy, and number of sacs, table (5) demonstrates that there was a statistically significant difference between the groups under study.

Table (6): Secondary out-come between studied groups

	Sildenafil group (n=40)	Control group (n=40)	Test	P value
Day 7 Ultra sound				
Mean ±SD	4.59±0.8	4.29±0.75	**t=1.73**	0.087
Last Ultra sound (Endometrial Thickness)				
Mean ±SD	9.74±1.16	8.19±0.76	**t=7.06**	<0.001
Last Ultra sound (Endometrial Pattern)				
Triple line	30 (75%)	11(27.5%)	**X^2= 22.83**	<0.001
Intermediate	4 (10%)	2 (5%)		
Echogen	6 (15%)	27 (67.5%)		

According to Table (6), there was a highly statistically significant difference in the last ultra sound (endometrial type) and last ultra sound (endometrial thickness) between the studied groups, but there was no statistically significant difference in the Day 7 ultra sound between the groups.

DISCUSSION

The primary goal of this study was to assess the impact of sildenafil citrate on the ultrasonography endometrial thickness and pattern during endometrial preparation prior to the transfer of frozen embryos in the treatment of infertility.

This prospective randomized clinical study was conducted at tertiary care hospital at Assisted Reproductive Technology (ART) Unit at Ain Shams University Maternity Hospital from November 2023 till May 2024 and performed on total 80 women who underwent endometrial preparation before thawed embryo transfer.

Eighty participants (40 in each group) were enrolled in the trial after 112 of them had their eligibility evaluated. Out of all eligible patients, 4 patients declined to take part in the trial, and 28 patients were excluded from the study due to inclusion requirements.

Ultimately, the analysis was based on the data of total 80 women who underwent endometrial preparation before thawed embryo transfer and divided into sildenafil group and control group.

The current study revealed that there was no statistically significance difference between the studied groups regarding age and BMI (p value= 0.807, 0.590) respectively with no

statistically significance difference regarding medical and surgical history.

The current research study revealed that the most common cause of infertility was unexplained infertility (67.5% vs 82.5%) in sildenafil group and control group, respectively. Primary infertility was the most common type of infertility in sildenafil group (62.5%) and control group (65%). However, there was no statistically significance difference between the studied groups regarding Period of Infertility, Cause of Infertility type of Infertility and Parity.

Regarding pregnancy outcome, our study results revealed that the pregnancy rate was 62.5% in sildenafil group, compared to 45% in the control group (p value= 0.044). Furthermore, the clinical pregnancy rate was 62.5% in sildenafil group, compared to 42.5% in the control group (p value= 0.024). However, there was no statistically significance difference between the studied groups regarding no. of sacs.

These results are consistent with earlier studies. In order to assess the impact of vaginal sildenafil citrate on enhancing the endometrial central line (trilaminar) pattern, implantation rate, and chemical pregnancy rate in frozen embryo transfer cycles, Kansouh et al. (11) carried out prospective randomized clinical research comprising ninety sub-fertile women. The study did not find any statistically significant differences in age, length of infertility, number of prior attempts, or number of transplanted embryos between the sildenafil and control groups. If the sildenafil group had a greater biochemical pregnancy rate than the control group, it was not statistically significant (33.3% vs. 20%, p=0.15). The biochemical pregnancy rate increased by 13.3% when sildenafil citrate was added. The number required to treat (NNT) was 8, meaning that out of every 8 women receiving sildenafil citrate in addition to normal therapy, 1 would benefit by getting pregnant and the other 7 would not benefit in any way (11).

Our results are consistent with Firouzabadi et al. (9), who conducted a randomized clinical controlled trial involving 80 patients with a history of poor endometrial response. They estimated the effect of sildenafil citrate on ultrasonographic endometrial thickness and pattern and investigated the implantation and chemical pregnancy rates in frozen embryo transfer cycles. The endometrial thickness and endometrial triple line patterns were found to be considerably larger ($p<0.0001$) and higher, respectively, in the sildenafil citrate group in the research. However, the intermediate patterns of the endometrium did not differ significantly between the two groups. The control group had significantly more endometrial echogenic patterns ($p<0.0001$). Lastly, despite the fact that the group using sildenafil citrate had greater rates of chemical pregnancy and implantation, these differences were not statistically significant (9).

Our findings align with those of Firouzabadi et al. (2013), who carried out a clinical trial that was randomized and controlled, encompassing eighty patients who had previously experienced poor endometrial response. They examined the implantation and chemical pregnancy rates in frozen embryo transfer cycles, as well as the impact of sildenafil citrate on ultrasonographic endometrial thickness and pattern. These findings were obtained from a meta-analysis carried out in 2020 by Li et al., (1) to compare the effectiveness of sildenafil citrate for patients with a thin endometrium with controls.

Our study's findings regarding endometrial thickness showed that there was a highly statistically significant difference (p value < 0.001) between the studied groups regarding last ultrasound endometrial pattern and thickness, but no statistically significant difference between the studied groups regarding Day 7 Ultrasound. The control group's echogen pattern was significantly greater (67.5%) than that of the sildenafil group (15%), and the sildenafil group's triple line endometrial

pattern (trilaminar) was significantly higher (75%), compared to (27.5%) in the control group (p value < 0.001).

Therefore, it was discovered that a combined examination of endometrial thickness and pattern was a stronger predictor of the success of IVF/ICSI-ET and more helpful for patient counseling than a solo analysis.

Kansouh et al. (11) found that on cycle day 8, endometrial thickness was significantly greater ($p<0.001$) in the sildenafil citrate group, which is consistent with our results. The endometrium's triple line pattern was significantly greater in the sildenafil citrate group ($p<0.001$), but the control group's echogen and intermediate endometrial patterns were significantly higher ($p<0.001$).

According to a number of studies, including Aisaka et al. (12), Morad et al. (14), and Eid (15), vaginal sildenafil was given to 22 patients for a total of 7 days between the time of ovulation and the transfer of embryos, with an endometrial thickness (EMT) of 7 mm and an elevated pulsatility index (PI>0.3). Sixty-eight percent (15/22) of the patients saw a decrease in their PI and an improvement in endometrial thickness. Patients who responded to therapy had greater rates of pregnancy and implantation (26% vs. 7% and 40% vs. 14%, respectively).

These results are in line with those of Al-Assadi et al., (15), who found that women who had previously failed assisted reproductive cycles due to poor endometrial response improved in terms of uterine artery blood flow, endometrial thickness, and pattern when using vaginal sildenafil citrate (15).

In line with our findings, Li et al. (1) discovered that patients taking sildenafil citrate had significantly thicker endometrium compared to the control group (placebo or no medication) (weighted mean difference: 1.22; 95% confidence interval [CI]: 1.07–1.38). Estrogen-induced endometrial development depends on blood flow to the basal endometrium (1).

Most studies show that the occurrence of a thin endometrium during IVF ranges from 1% to 2.5% (16). The advantages of sildenafil citrate usage for infertile women with thin endometria have been documented in a number of studies. Citrate sildenafil has been shown by Firouzabadi et al. (9) to enhance the triple line pattern and successfully increase endometrial thickness.

Numerous studies have been published in the literature to support this study. Malinova et al. (17) demonstrated that giving infertile women sildenafil citrate by vaginal injection increased uterine blood flow and endometrial thickness and may be a useful treatment strategy for ovulation induction.

Another research by Sher & Fisch (10) shown that infertile women became pregnant when sildenafil was administered vaginally in addition to a 70% increase in embryo transfers. When combined, the aforementioned research points to the significance of sildenafil citrate for both maternal–fetal immunotolerance and endometrial receptivity.

Additionally, Yavangi et al. (18) conducted a clinical randomized controlled trial (RCT) and discovered that there were no statistically significant variations between the control and sildenafil citrate groups regarding the quantity of transferred embryos, prior pregnancies, abortions, cycles, retrieved eggs, and fertilized eggs.

According to Takasaki et al. (19), sildenafil citrate medication enhanced patients' RI (< 0.81) and endometrial thickness (> 8 mm). According to Ashoush et al. (20), women who have thin endometria—possibly as a result of uterine radial artery impedance—benefit from vaginal sildenafil citrate treatment, which increases the rate of conception and endometrial development.

These results corroborate and enhance the trustworthiness of our study findings, which are identical to these findings.

During IVF therapy, vaginal ultrasound mea-

surements of the endometrial and sub-endometrial blood flows are a reliable indicator of pregnancy. The current findings support and expand on earlier findings that poor in vitro fertilization and embryo transfer (IVF-ET) outcomes are linked to an advanced hyperechogenic alteration of the endometrium (22).

The strength points of this study:

The prospective randomized clinical trial design and the fact that no patients were lost to follow-up during the study period are the study's strong characteristics. Moreover, the study was performed at a single institution with the same IVF team, IVF lab and the same induction protocols, which likely increased the validity of our results.

The limitations of the study:

This study's limitations should be taken into consideration when interpreting the results, as it was not a multicentric study and had a comparatively smaller sample size than prior research, which has a high risk of publication bias. The inability to assess sub-endometrial and uterine artery blood flow with vaginal color doppler ultrasonography is another drawback. Furthermore, no information on the percentage of live births or abortions was taken from the women who were included, which might have compromised the validity of this research.

CONCLUSION

The current study unequivocally demonstrates that vaginal sildenafil citrate usage enhances clinical pregnancy rate, endometrial thickness, and biochemical pregnancy rate in patients undergoing frozen-thawed embryo transfer cycles who have thin endometria. It also modifies the endometrial pattern in these individuals. Additionally, it was shown that a combined assessment of endometrial thickness and pattern was a stronger predictor of the success of IVF/ICSI-ET and more helpful for patient counseling than a solo analysis.

Patients undergoing frozen embryo transfer are highly advised to routinely utilize vaginal sildenafil citrate if they had previously failed cycles of assisted reproduction technologies because of inadequate endometrial thickness.

Additional Information

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosures

Conflicts of Interest: The authors have no conflicts of interest to declare.

Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of data from patients.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Financing Support: All authors have declared that no financial support was received from any organization for the submitted work.

Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.

Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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Psychiatric Disorders in Pregnant Women: A hospital–based Study

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Short running title:

Psychiatric Disorders in Pregnant Women

Conflict of interest Statement:

Declarations of interest none

Abstract

Objective: To estimate the level of different psychiatric disorders in pregnant females and their associated factors.

Methods: The current study was carried out at obstetrics and gynecology outpatient clinics, Mansoura University Hospitals, Mansoura, Egypt during March 2023 to March 2024. A total number of 384 pregnant females who fulfilled the inclusion criteria were enrolled and investigated by using MINI international interview for presence of any psychiatric disorder.

Results: our study showed that 45.8% of the studied women had one or more psychiatric disorders, 22.9% had dysthymia, 9.9% had generalized anxiety disorder,4.9% had major depressive episode, 3.4% had social phobia, 1.3% had major depressive episode with melancholic features, 0.3% had suicidality, manic disorder, hypomanic disorder, post-traumatic stress disorder (PTSD), drug dependence non-alcohol and there was a correaltion between presence of one or more psychiatric disorders and job of woman's husband as 176 women had one or more psychiatric disorders, 37 of them whose husband were employee and 139 were manual workers (p=0.03) and number of gravidity as 13 women were primi-gravida,112 were second and third gravida and 51 were ≥4th gravida (p=0.005).There were independent predictor of presence of psychiatric disorders and gravidity (2-3) = 2.19 and gravidiy ≥4 = 3.16.There were no significant association between women having major depressive episode and the studied risk factors (p >0.05).There were significant association between presence of generalized anxiety disorder and gestational age of the pregnant woman as 5 women were in first trimester, 11 women in the second trimester , 22 women in the third trimester (P=0.037) and there were significant association between presence of generalized anxiety disorder and associated risk of pregnancy as 5 women had high risk pregnancy and 22 women had

low risk pregnancy (P=0.002). There were significant association between presence of dysthymia and age of the pregnant women as there were no dysthymia below 20 years old, 81 women were between 20-35 years, 7 women were >35 years (p=0.002) and There were significant association between presence of dysthymia and women's work as 5 women were working and 83 women were housewives (p=0.039).

Conclusion: By screening women for presence of psychiatric disorders during pregnancy, we found that psychiatric disorders are common among pregnant women (45.8%) and screening during antenatal care (ANC) is so important to discover and give appropriate treatment early.

Keywords: Screening, Psychiatric disorders, pregnancy, ANC.

Introduction

Psychiatric illness during pregnancy is a serious public health issue as it influence the health of the mother and of her child too [1]. In affluent nations, the prevalence of psychiatric problems in pregnancy is 7 -15% [2]. There is a higher burden of psychiatric illness in low and medium income countries, where 15.6% of pregnant females and 19.8% of females in the postpartum period are expected to have mental problems [3].

Common mental disorders (CMDs) are non-psychotic mental illnesses including depression, anxiety, adjustment, and somatoform disorders that impair daily functioning. These disorders have a greater prevalence among females as compared to males [4].

Pregnant females who have CMDs uncommonly seek prenatal care and might experience reduced weight gain throughout gestation [1]. This increases the probability of complicated labor [5] and neonatal problems including low birth weight, prematurity, and even death of the neonate [6].

CMDs might also have a deleterious influence on neonatal neurobiological development [7]. If neglected during the pre-natal period, they might persist in the post-natal period [8]. As a result, there is less emotional attachment and hostility toward the infant. Mental problems during pregnancy might potentially be linked to child's malnutrition [9] and influence child's behavioral [10] emotional, and cognitive development [11].

With the advancement of medical care, the public's perception of pregnant females has moved from solely physical health to more psychological health [12]. There are different perspectives on what might create prenatal psychological hardship, such as whether hormonal variations in pregnancy lead to alterations in inflammatory markers that raise the likelihood of psychiatric disorders [13]. Pregnant women's health and personal behavior, such as worry and illnesses, can also lead to depression [14], smoking [15] and excessive alcohol consumption [16]; and the educational state and employment (housewife/unemployment) [17]. Social support also has a vital role, particularly when the pregnant women receive Insufficient support from their husbands (domestic violence) [18] or Friends and live in difficult familial situations [19], the decision of induction of labour [20]; etc).

Population and Methods

This was a cross-sectional study which was conducted through the period from March 2023 to March 2024 at Mansoura University Hospital (Obstetrics and Gynecology Outpatient clinics) on 384 pregnant women who Pregnant women who attended the above mentioned clinic who fulfilled the following ; 1-Age over 18 years. 2-Women with confirmed pregnancy either by laboratory investigations or ultrasound 3- Women willing to join the study.

An arabic questionnaire was constructed to collect the following socio-demographic characteristics: age, marital state , education-

al level, residency, employment and income, type of family, environmental exposure, husband (age, education, job) , polygamy. History of the participants including: Gravidity, parity, gestational age and pregnancy-related complications, the participating women were asked about any medical disorder that complicated their pregnancies (hypertension, diabetes, hypothyroidism, FMF , pre-eclampsia.), planned mode of delivery , pregnancy outcome was taken from the participants.

The Mini International Neuropsychiatric Interview (MINI): This section was an instrument with a standard model of a short (15-30 min) structured interview to evaluate the presence of Axis I psychiatric disorders in accordance with Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition [21]. The MINI provides a diagnostic structured interview compatible with DSM-III-R and ICD-10 criteria. It focuses mainly on current diagnoses and only determines lifetime diagnoses where it is clinically relevant to the present. For most diagnostic sections, one or two questions are utilized to exclude the diagnosis if answered negatively. Using decision tree logic, positive answers to questions are explored by further investigations of other diagnostic criteria. In a sample of psychiatric patients and controls, kappa coefficient, sensitivity and specificity were good or very good for all diagnoses except for the generalized anxiety disorder

(kappa = 0.36), agoraphobia (sensitivity = 0.59) and bulimia (kappa = 0.53). Inter-rater and test-retest reliability were good. The MINI could rule out patients who dis not have a disorder. The MINI provided reliable DSM-III-R diagnoses within a short time. As the MINI is modularized, the module for any one disorder can be administered within few minutes [22].

Statistical analysis

Data were analyzed sing SPSS software (SPSS Inc., PASW statistics for windows version 25. Chicago: SPSS Inc.). Qualitative data were represented as numbers

and percenst. Quantitative data were represented as means± SDs for normally distributed data after testing normality by Kolmogrov-Smirnov test. The significance of a result was set at the (≤0.05) level.

Chi-Square , Fisher exact test , Monte Carlo tests were utilized for comparison of qualitative data between groups as appropriate.

Student t test was utilized for comparison between 2 independent groups for normally distributed data.

Results

The mean age of participants was 27 years, 95% of women were between 20 years – 35 years, the 62% of them were resident in rural areas, 62% of them had only secondary education, 97% of them were housewifes, the mean age of their husbands was 31.5 years, 73% of their husbands were above 30 years, 60% of their husbands had secondary education, 73% of the husbands were manual workers, the income was enough in 94% , 50% were living in nuclear families (Table 1) . The mean number of gravidity in the studied females was 3, 12.8% were primigravida, 63.3% were second and third gravida, 24% were more than or equal to fourth gravid. 14.3% of the women had one or more medical disorders and 12.2% of them had undergone one or more surgical procedures and 30% of the studied women were in the first trimester, 19.5% were in the second trimester, 50% were in the third trimester, 3.9% were high risk pregnancy and 66.9% would be delivered by cesarean section (Table 2). 45.8% of the studied women had one or more psychiatric disorders, 22.9% had dysthymia, 9.9% had generalized anxiety disorder, 4.9% had major depressive episode, 3.4% had social phobia, 1.3% had major depressive episode with melancholic features,0.3% had suicidality,manic disorder, hypomanic disorder, PTSD, drug dependence non-alcohol. (Table 3)

There were significant association between women whose husbands were manual workers and presence of one or more psychiatric disorders and there is also significant association between number of gravidity more than or equal to 4 and presence of one or more psychiatric disorders and There were no significant associationbetween presence of one or more psychiatric disorders and age of the women, their residency,educational level, job, age of their husband, husband education, income, type of family, gestational age, special care, medical history, surgical history and planned mode of delivery (Table 4). The independent predictor of presence of psychiatric disorders and gravidity (2-3) = 2.19 and gravidiy ≥4 = 3.16 (Table 5)

There were no significant associations between existence of major depressive episodes and age of the women, their residency, educational level, job, age of their husband, husband education, job of the husband, income, type of family, gestational age,number of gravidity, special care, medical history, surgical history and planned mode of delivery (Table 6). There were significant association between presence of generalized anxiety disorder and gestational age of the pregnant woman and those who had high risk pregnancy. There were no significant associations between existence of generalized anxiety disorder and age of the women, their residency, educational level, job, age of their husband, husband education, job of the husband, income, type of family, number of gravidity, medical history, surgical history and planned mode of delivery (Table 7). There were significant association between presence of dysthymia and age and job of the studied pregnant women. There were no significant association between presence of dysthymia and women's residency,educational level, age of their husband, husband education, job of the husband, income, type of family, gestational age, number of gravidity, special care, medical history, surgical history and planned mode of delivery. (Table 8).

Discussion

Psychiatric disorders are common in women through the childbearing period. Psychiatric problems are twice more common among females as compared to males. Mental illness in pregnant females can deleteriously affect both the mother and the child [23]. In our study and by using the MINI international interview, the prevalence of psychiatric problems in pregnant females was 45.8%. Such high rate highlights the importance of psychiatric problems during gestation as a public health concern in the community of El Mansoura city, Dakahlia Governorate.

The most prevalent psychiatric disorders among the study participants were dysthymia (22.9%), generalized anxiety disorder (9.9%), major depressive episode (4.9%), Social phobia (3.4%), OCD (3.4%), major depressive episode with melancholic features (1.3%), [Suicidality, Hypomanic episode , manic episode, drug dependence non-alcohol, post-traumatic stress disorder each was (0.3%)] . A study done in rural eastern Ethiopia found that the prevalence of psychiatric disorders during pregnancy was 37.5% [24]. which is nearer to our results may be due to similar socioeconomic circumstances.

There were also association with the job of the husband, as psychiatric disorders were common among those whose husbands were manual workers and this relies to that manual workers have low education mostly and don't give their women good psychological support during pregnancy and ask women to help them in work and this makes women under stress during pregnancy and more vulnerable to psychiatric disorders. Low pre-natal support from the family showed no correlation with an enhanced risk of depression, whereas low pre-natal support from husband and parents showed a correlation with an enhanced risk of depression [25].

There were no association between major depressive episodes and any of the studied risk factors which is different from a study done in Taiwan by Pan-Yen Lin which found that females with low educational levels, fam-

ily history of psychiatric problems, lack of post-partum recuperation, and family-bond stress were more likely to have MDEs and this difference may be due to different cultural and social factors between Egypt and Taiwan and that our study was done on pregnant women only not including postpartum women like that done in Taiwan [26].

Generalized Anxiety Disorder was found to be increased among women in the third trimester and this means that when pregnant women get nearer to their delivery date, they become more anxious due to fear from any complications during delivery [27]. Also, women who were having high risk pregnancy had high prevalence of GAD and this was due to fear of them to lose their pregnancy. The Prevalence of GAD during pregnancy was between 8.5% and 10.5% [28] which is in agreement with this study which demonstrated that the prevalence of GAD was 9.9%

The prevalence of dysthymia in our study was 22.9% which is much higher compared to that in a study done in Mumbai, India which was 9.18% and it was significantly linked to many risk factors such as gravidity (P = 0.0092), unintended pregnancy (P = 0.001), history of abortion (P = 0.0001) [29] which is different from this study results and this may be due different cultures and different socio-demographic features between Egypt and India.

Conclusion

The study demonstrated the prevelance psychiatric disorders in pregnant women attending MUH outpatient clinics and revealed risk factors for psychiatric disorders including increased gestational age, high risk pregnancy, unembloyment.Pregnant females should have a screening for psychiatric disorders during pregnancy

Conflict of interest: No conflict of interest.

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Table (1): Demographic characteristics of the studied sample

	n	%
Age / years **mean±SD** **(Min-Max)**	27.09±3.65 (18-38)	
Age group **<20 years** **20-35years** **>35 years**	 6 367 11	 1.6 95.6 2.9
Residence **Rural** **Urban**	 238 146	 62.0 38.0
Educational level **Less than secondary** **Secondary** **Higher Education**	 49 238 97	 12.8 62.0 25.3
Job **Working** **Housewife**	 10 374	 2.6 97.4
Age of husband (years) **mean±SD** **(Min-Max)**	31.53±3.88 (22-45)	
<30 years **≥30 years**	103 281	26.8 73.2
Husband education **Less than secondary** **Secondary** **Higher Education**	 64 231 89	 16.7 60.2 23.2
Job of husband **employee** **manual worker**	 101 283	 26.3 73.7
Income **Enough** **Not Enough**	 363 21	 94.5 5.5
Type of family **Extended** **Nuclear**	 189 195	 49.2 50.8

Table (2): Obstetric history of the studied sample and medical and surgical history of the studied cases and history distribution among studied sample.

Obstetric history	N=384	%
Gravidity	3(1-11)	
Primi **2-3** **≥4**	49 243 92	12.8 63.3 24.0
	N	**%**
Medical history **-ve** **+ve**	 329 55	 85.7 14.3
Surgical history **-ve** **+ve**	 337 47	 87.8 12.2
	N	**%**
Gestational age (weeks)		
First trimester **Second trimester** **Third trimester**	117 75 192	30.5 19.5 50.0
Special care **High risk** **Low risk**	 15 369	 3.9 96.1
Planned Mode of delivery **Normal vaginal delivery** **CS**	 127 257	 33.1 66.9

Table(3) :The Mini International Neuropsychiatric Interview (MINI)

	n	%
Major depressive episode **Current** **Lifetime**	 6 13	 1.6 3.4
Major depressive episode with melancholic features **Current**	 5	 1.3
Dysthymia **Current (past 2 years)**	 88	 22.9
Suicidality	1	0.3
Hypomanic episode	1	0.3
Manic episode	1	0.3
Panic disorders	0	0.0
Agoraphobia	0	0.0
Social phobia	13	3.4

Obsessive compulsive disorders	13	3.4
Post traumatic disorders	1	0.3
Alcohol abuse	0	0.0
Drug dependence	0	0.0
Drug dependence non alcohol	1	0.3
Psychotic disorders	0	0.0
Anorexia nervosa	0	0.0
Bulimia nervosa	0	0.0
GAD	38	9.9
Antisocial personality disorders	0	0.0
Total#	176	45.8

having one or more psychiatric disorders

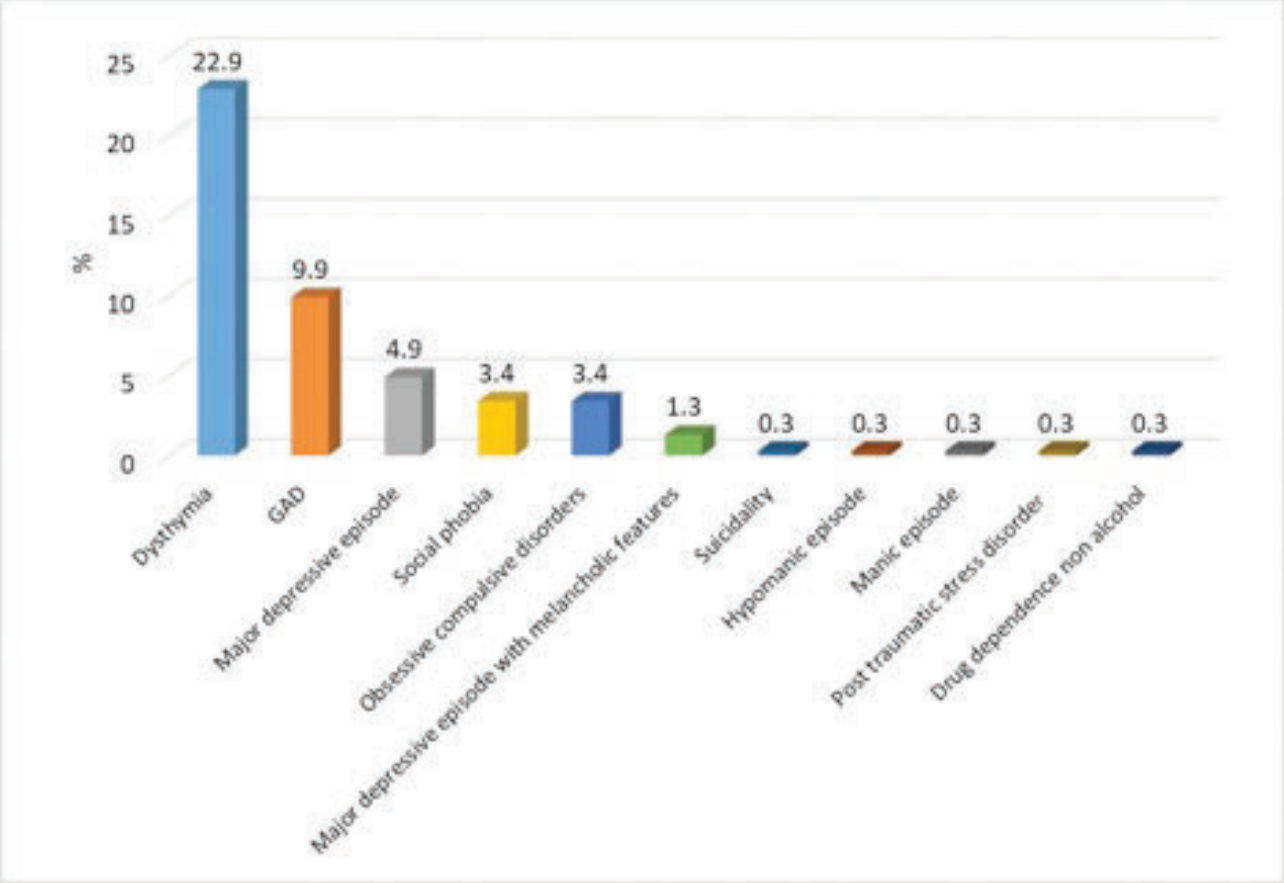

Figure (1): shows the prevalence of psychiatric disorders among the studied women.

Table(4): Association between studied risk factors and presence of any psychiatric disorders

risk factors	Total	Total psychiatric disorders		Test of significance
		No N=208	Yes N=176	
Age group				
<20 years	6	4(66.7)	2(33.3)	
20-35years	367	201(54.8)	166(45.2)	x^2=3.64
>35 years	11	3(27.3)	8(72.7)	p=0.162
Residence				
Rural	238	123(51.7)	115(48.3)	x^2=1.56
Urban	146	85(58.2)	61(41.8)	p=0.212
Educational level				
Less than secondary	49	25(51.0)	24(49.0)	
Secondary	238	122(51.3)	116(48.7)	x^2=3.98
Higher Education	97	61(62.9)	36(37.1)	p=0.137
Job				
Working	10	3(30.0)	7(70.0)	x^2=2.42
Housewife	374	205(54.8)	169(45.2)	P=0.120
Age of husband (years)				
<30 years	103	61(59.2)	42(40.8)	x^2=1.45
≥30 years	281	147(52.3)	134(47.7)	p=0.229
Husband education				
Less than secondary	64	36(56.2)	28(43.8)	
Secondary	231	118(51.1)	113(48.9)	x^2=2.52
Higher Education	89	54(60.7)	35(39.3)	P=0.284
Job of husband				
employee	101	64(63.4)	37(36.6)	x^2=4.67
manual worker	283	144(50.9)	139(49.1)	P=0.03*
Income				
Enough	363	194(53.4)	169(46.6)	x^2=1.395
Not Enough	21	14(66.7)	7(33.3)	P=0.237
Type of family				
Extended	189	104(55.0)	85(45.0)	x^2=0.111
Nuclear	195	104(53.3)	91(46.7)	P=0.739
Gestational age				
First trimester	117	64(54.7)	53(45.3)	
Second trimester	75	42(56.0)	33(44.0)	x^2=0.199
Third trimester	192	102(53.1)	90(46.9)	p=0.905
Special care				
High risk	15	6(40.0)	9(60.0)	$x\square^2$=1.26
Low risk	369	202(54.7)	167(45.3)	p=0.261
Medical history				
-ve	329	181(55.0)	148(45.0)	x^2=0.666
+ve	55	27(49.1)	28(50.9)	p=0.414
Surgical history				
-ve	337	184(54.6)	153(45.4)	x^2=0.208
+ve	47	24(51.1)	23(48.9)	p=0.649

Gravidity **Primi** **2-3** **≥4**	 49 243 92	 36(73.5) 131(53.9) 41(44.6)	 13(26.5) 112(46.1) 51(55.4)	 x^2=10.78 p=0.005*
Planned Mode of delivery **Normal vaginal delivery** **CS**	 127 257	 73(57.5) 135(52.5)	 54(42.5) 122(47.5)	 □^{2}=0.839 p=0.385

Table (5): Binary logistic regression for predictors of presence of any psychiatric disorders among studied cases

	β	**P value**	**AOR (95%CI)**
Gravidity **Primi(R)** **2-3** **≥4**	 0.785 1.15	 0.026* 0.003*	 1 2.19(1.09-4.36) 3.16(1.47-6.79)

Table (6): Association between studied risk factors and presence of major depressive episodes.

risk factors	Total	Major depressive episode		Test of significance
		No N=365	Yes N=19	
Age group **<20 years** **20-35years** **>35 years**	 6 367 11	 6(100.0) 348(94.8) 11(100.0)	 0 19(5.2) 0	x^2=0.926 p=0.629
Residence **Rural** **Urban**	 238 146	 227(95.4) 138(94.5)	 11(4.6) 8(5.5)	x^2=0.142 P=0.707
Educational level **Less than secondary** **Secondary** **Higher Education**	 49 238 97	 …. 230(96.6) 90(92.8)	 8(3.4) 7(7.2)	x^2MC6.50 P=0.165
Job **Working** **Housewife**	 10 374	 9(90.0) 356(95.2)	 1(10.0) 18(4.8)	x^2=0.557 P=0.455
Age of husband (years) **<30 years** **≥30 years**	 103 281	 99(96.1) 266(94.7)	 4(3.9) 15(5.3)	x^2=0.339 P=0.560
Husband education **Less than secondary** **Secondary** **Higher Education**	 64 231 89	 59(92.2) 222(96.1) 84(94.4)	 5(7.8) 9(3.9) 5(5.6)	 x^2=1.75 P=0.418
Job of husband **employee** **manual worker**	 101 283	 96(95.0) 269(95.1)	 5(5.0) 14(4.9)	x^2=0.0 P=0.999
Income **Enough** **Not Enough**	 363 21	 344(94.8) 21(100)	 19(5.2) 0	x^2=1.16 P=0.282

Type of family **Extended** **Nuclear**	189 195	179(94.7) 186(95.4)	10(5.3) 9(4.6)	x^2=0.093 P=0.760
Gestational age **First trimester** **Second trimester** **Third trimester**	117 75 192	110(94.0) 71(94.7) 184(95.8)	7(6.0) 4(5.3) 8(4.2)	x^2=0.539 P=0.764
Special care **High risk** **Low risk**	15 369	15(100.0) 350(94.9)	0 19(5.1)	x^2=0.813 P=0.367
Medical history **-ve** **+ve**	329 55	313(95.1) 52(94.5)	16(4.9) 3(5.5)	x^2=0.035 P=0.852
Surgical history **-ve** **+ve**	337 47	322(95.5) 43(91.5)	15(4.5) 4(8.5)	x^2=1.45 P=0.229
Gravidity **Primi** **2-3** **≥4**	49 243 92	47(95.9) 231(95.1) 87(94.6)	2(4.1) 12(4.9) 5(5.4)	x^2=0.125 P=0.940
Planned Mode of delivery **Normal vaginal delivery** **CS**	127 257	122(96.1) 243(94.6)	5(3.9) 14(5.4)	x^2=0.412 P=0.521

Table (7): Association between studied risk factors and presence of generalized anxiety disorders

risk factors	Total	GAD		Test of significance
		No N=346	Yes N=38	
Age group **<20 years** **20-35years** **>35 years**	6 367 11	6(100) 329(89.6) 11(100)	0 38(10.4) 0	x^2=1.95 p=0.377
Residence **Rural** **Urban**	238 146	212(89.1) 134(91.8)	26(10.9) 12(8.2)	x^2=0.743 P=0.389
Educational level **Less than secondary** **Secondary** **Higher Education**	49 238 97	44(89.8) 210(88.2) 92(94.8)	5(10.2) 28(11.8) 5(5.2)	x^2=3.38 P=0.184
Job **Working** **Housewife**	10 374	10(100) 336(89.8)	0 38(10.2)	x^2=1.13 P=0.288
Age of husband (years) **<30 years** **≥30 years**	103 281	95(92.2) 251(89.3)	8(7.8) 30(10.7)	x^2=0.715 P=0.398

Husband education				
Less than secondary	64	58(90.6)	6(9.4)	
Secondary	231	203(87.9)	28(12.1)	x^2=4.22
Higher Education	89	85(95.5)	4(4.5)	P=0.122
Job of husband				
Employee	101	95(94.1)	6(5.9)	x^2=2.40
Manual worker	283	251(88.7)	32(11.3)	P=0.121
Income				
Enough	363	326(89.8)	37(10.2)	x^2=0.657
Not Enough	21	20(95.2)	1(4.8)	P=0.418
Type of family	189			
Extended	195	169(89.4)	20(10.6)	x^2=0.197
Nuclear		177(90.8)	18(9.2)	P=0.658
Gestational age				
First trimester	117	112(95.7)	5(4.3)	
Second trimester	75	64(85.3)	11(14.7)	x^2=6.59
Third trimester	192	170(88.5)	22(11.5)	P=0.037*
Special care				
High risk	15	10(66.7)	5(33.3)	x^2=9.62
Low risk	369	336(91.1)	33(8.9)	P=0.002*
Medical history				
-ve	329	299(90.9)	30(9.1)	x^2 =1.56
+ve	55	47(85.5)	8(14.5)	P=0.212
Surgical history				
-ve	337	303(89.9)	34(10.1)	x^2=0.115
+ve	47	43(91.5)	4(8.5)	P=0.734
Gravidity				
Primi	49	46(93.9)	3(6.1)	
2-3	243	221(90.9)	22(9.1)	x^2 =2.83
≥4	92	79(85.9)	13(14.1)	P=0.243
Planned Mode of delivery				
Normal vaginal delivery	127	111(87.4)	16(12.6)	x^2 =1.55
CS	257	235 (91.4)	22(8.6)	P=0.212

Table (8): Association between studied risk factors and presence of dysthymia

risk factors	Total	Dysthymia		Test of significance
		No N=296	Yes N=88	
Age group				
<20 years	6	6(100)	0	
20-35years	367	286(77.9)	81(22.1)	x^2=12.26
>35 years	11	4(36.4)	7(63.6)	P=0.002*
Residence				
Rural	238	180(75.6)	58(24.4)	x^2=0.748
Urban	146	116(79.5)	30(20.5)	P=0.387

Educational level				
Less than secondary	49	37(75.5)	12(24.5)	
Secondary	238	180(75.6)	58(24.4)	x^2=1.39
Higher Education	97	79(81.4)	18(18.6)	P=0.497
Job				
Working	10	5(50.0)	5(50.0)	x^2=4.26
Housewife	374	291(77.8)	83(22.2)	P=0.039*
Age of husband (years)				
<30 years	103	82(79.6)	21(20.4)	x^2=0.509
≥30 years	281	214(76.2)	67(23.8)	P=0.475
Husband education				
Less than secondary	64	48(75.0)	16(25.0)	
Secondary	231	178(77.1)	53(22.9)	x^2=0.281
Higher Education	89	70(78.7)	19(21.3)	P=0.869
Job of husband				
employee	101	83(82.2)	18(17.8)	x^2=2.01
manual worker	283	213(75.3)	70(24.7)	P=0.156
Income				
Enough	363	281(77.4)	82(22.6)	x^2=0.402
Not Enough	21	15(71.4)	6(28.6)	P=0.526
Type of family				
Extended	189	150(79.4)	39(20.6)	x^2=1.09
Nuclear	195	146(74.9)	49(25.1)	P=0.295
Gestational age				
First trimester	117	84(71.8)	33(28.2)	
Second trimester	75	65(86.7)	10(13.3)	x^2=5.78
Third trimester	192	147(76.6)	45(23.4)	P=0.06
Special care				
High risk	15	10(66.7)	5(33.3)	x^2=0.59
Low risk	369	286(77.5)	83(22.5)	P=0.327
Medical history				
-ve	329	255(77.5)	74(22.5)	x^2=0.234
+ve	55	41(74.5)	14(25.5)	P=0.629
Surgical history				
-ve	337	259(76.9)	78(23.1)	x^2=0.082
+ve	47	37(78.7)	10(21.3)	P=0.775
Gravidity				
Primi	49	42(85.7)	7(14.3)	
2-3	243	185(76.1)	58(23.90	x^2=2.42
≥4	92	69(75.0)	23(25.0)	P=0.299
Planned Mode of delivery				
Normal vaginal delivery	127	102(80.3)	25(19.7)	x^2=1.12
CS	257	194(75.5)	63(24.5)	P=0.290

Role of Hyaluronic Acid Intrauterine Injection in Asherman's Syndrome in Women Undergoing Hysteroscopy

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Abstract

Background: Asherman's syndrome an acquired disorder resulting from pregnancy, childbirth, infection, uterine intervention or repair of abnormalities in the uterus (e.g., intrauterine septum, bicornuate uterus or myomectomy).

Aim of the Work: Investigating the efficacy of injecting Hyaluronic acid intrauterine in Asherman's patients using hyster oscopic adhesiolysis.

Patients and Methods: This randomized clinical trial was conducted at Early Cancer Detection & Endoscopy Unit maternity hospital Ain Shams University from January 2023 till March 2024.

Results: The Cases Group showed an increase from 8.3% before treatment to 41.7% after treatment, while the Control Group remained relatively stable at 33.3% both before and after treatment. Similarly, the proportion of participants experiencing mild symptoms in the Cases Group increased from 33.3% before treatment to 50.0% after treatment, whereas the Control Group remained consistent at 33.3%. However, the reduction in amenorrhea in the case group compared to the control group, but an insignificant difference between the two groups.

Conclusion: Intrauterine adhesions can result from surgical or hysteroscopic intervention, it can be corrected effectively and safely with the use of hysteroscop to restore menstural pattern and fertility.

Keywords: Hyaluronic Acid Intrauterine Injection; Asherman's Syndrome; Hysteroscopy.

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INTRODUCTION

Women diagnosed with Asherman's syndrome suffer from menstrual abnormalities specifically amenorrhea as well as infertility hence the need for a treatment, they can be treated by cutting and removing adhesions hysteroscopically or laparoscopically [1].

The main management is to try to restore a healthy endometrium and obtain physiological conditions inducive to pregnancy. Currently drugs containing hyaluronic acid

are used for the treatment of the adhesions [2].

Hyaluronic acid (hyaluronan) is a polymeric acid which is an organic compound. It is composed of N-acetyl glucosamine and D-glucuronic acid. These chains can contain up to 25,000 units or more, with a molecular weight ranging from 5000 to 20million Dalton s. This acid is synthesized by hyaluronan synthase enzyme, of which there are at least three types: HAS1, HAS2, and HAS3. HAS1 & HAS2 both produce hyaluronic acids with high molecular weight, while HAS3 makes lower molecular weight hyaluronic acids.

The hyaluronidase enzyme is responsible for breaking down hyaluronic acid. The anti-adhesive properties of hyaluronic acid can be assessed by examining its molecular weight and concentration. A gel composed entirely of hyaluronic acid (HA) is highly adhesive and serves as a physical barrier in the uterus, helping to prevent the formation of adhesions. The hyaluronic acid stays intact for seven days before being fully absorbed, with adhesions typically forming between the third- and fifth-day post-surgery.

Numerous research explored the impact of HA on the prevention of adhesion formation following interventions in the uterine cavity, both in human and in animal models. These studies have found that HA gel is of adequate effectiveness in maintaining the position of the uterine walls for up to 72 hours. [3].

Additionally, several hysteroscopic studies have demonstrated a statistically significant reduction in intrauterine adhesions for several months following surgery. [4]

PATIENTS AND METHODS

After obtaining the approval of the ethical committee and the patients informed consent. This randomized clinical trial was conducted at Early Cancer Detection & Endoscopy Unit maternity hospital Ain Shams University from January 2023 till March 2024.

For this study, 34 patients under went assessment for eligibility and 24 were included (12 women in each group). Of all the eligible women, 6 were did not fit the inclusion criteria and 4 women refused to participate in the study.

24 patients were divided into two groups: Case Group (Hyaluronic acid intrauterine injection) and Control Group.

Study population:

Women diagnosed with Asherman syndrome attending the Endoscopic unit at Ain Shams University Maternity Hospital.

Inclusion criteria included Age (18 to 40 yrs), History of an intrauterine surgeries and Patients diagnosed with Asherman syndrome.

Exclusion criteria: Body Mass Index (BMI) > 35 kg/m2, Menopause, Pregnancy, Coagulation disorders and History of cervical cancer.

Study procedure:

Study Tools: patients underwent:

1. Detailed history taking and Clinical exam.
2. Hysteroscope.
3. Hyaluronic acid gel.
4. Saline.

All women were subjected to:

All participants provided Informed consent before the enrollment in the study. Patients underwent detailed clinical assessment (detailed history, general examination, abdominal examination and pelvic examinations).

A. Detailed History

1. Personal history including age, parity, BMI, and special habits etc.
2. Past medical events such as hypertension, diabetes.
3. Obstetrics history.
4. Gynaecological history including menstrual score.
5. Past surgical history.
6. Past family history.

B. Examination:

1. General examination: measurement of vital sign.
2. Abdominal Examination.
3. Local examination: vaginal examination for any abnormality.

C. Hyaluronic Acid Injection:

All patients underwent hysteroscopic adhesiolysis with scissors before injection of hyaluronic acid gel.

Using saline (NaCl 0.9%) as distension medium the hysteroscopy was conducted. The procedures were performed by experienced surgeons.

The operating surgeon described the intrauterine pathology and classified the characteristics and type of the observed pathology and complication related to the hysteroscopy if present.

Case group were injected with hyaluronic acid gel in the cervical canal and uterine cavity, using a syringe containing hyaluronic acid gel (20mg¬ /2ml) HYALGAN® (Country of Origin Italy).

Manufacturer **Fidia Pharma USA Inc)** by 3cm syringe was applied through a pediatric nelaton catheter 6 FR.

If patient experienced discomfort or intolerance, they were offered local, spinal, or general anesthesia.

D. Follow-up:

A diagnostic hysteroscopy follow-up was scheduled 8 to 12 wks after the operation, it was performed at early-to mid-follicular stage of the menstrual cycle. A routine pregnancy testing was included prior to the procedure and if positive, the procedure was canceled.

Intrauterine adhesion score was examined through clinico-hysteroscopic classification criteria of American Fertility Society (AFS). Scoring points (1 to 3) were assigned to the characteristics and also staging of AS was done (stage I/II/III: mild/moderate/severe) according to the obtained scoring.

Table (1): American Fertility Society (AFS) classification [5]

	Characteristics		
Extent of cavity involved	<1/3 1	<1/3-2/3 2	>2/3 4
Type of adhesions	Flimsy 1	Filmy and Dense 2	Dense 4
Menstrual pattern	Normal 0	Decreased 2	Amenorrhoea 4
Prognostic classication		HSG score	Hysteroscopy score
Stage I (Mild) Stage II (Moderate) Stage III (Severe)	1-4 5-8 9-12		

Outcome Measures:

1. Menstrual score after and before the procedure.
2. IUA staging before and after the procedure
3. Pain score.
4. Complications of hysteroscopy.

Sample Size:

By using Power Analysis and Sample Size Software (PASS11.) (Version11.0.08.) for the calculation of the sample size, confidence level was set at 80%, error margin ±0.20 and after the revision of the previous study results **Pellicano et al.** [5] showed that Effectiveness of HA gel use following the laparo-

scopic myomectomy in patients of infertile in preventing adhesions at second look laparoscopy was lower in those took hyaluronic acid gel than the control group who didn't took hyaluronic acid gel (27.7% versus 73.6% respectively); based on that, a sample size of at least 34 patients with Asherman's syndrome in patients that will undergo hysteroscopy divided randomly into 2 groups (17 patients in each group) were sufficient to achieve study objective.

Statistical Analysis:

Data was analyzed with the Social Sciences Statistical Package, version 23.0 (SPSS Inc., Chicago, Illinois, USA). Quantitative data with a normal distribution was presented as mean ± standard deviation and ranges, while non-parametric data (non-normally distributed variables) were expressed as median with interquartile range (IQR). Qualitative variables were reported as numbers and percentages. Normality of the data was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests.

The statistical tests performed were:

- **Independent - samples t - test:** was used for the comparison of two means.
- **Mann – Whitne y U test:** Applied for comparisons between two groups in non-parametric data.
- **Chi-square test:** Employed for comparisons involving qualitative data. Fisher's exact test was used as an alternative to the Chi-square test was used when the expected count in any cell was < 5.

Confidence interval was set at 95%, with an accepted error margin of 5%.

The significance of the p-value was interpreted as follows:

- significant P-value< 0.05.
- highly significant P-value < 0.001
- not significant P-value > 0.05.

Results

Table (2): Comparison between cases group and control group according to demographic data.

Demographic Data	Cases Group (n=12)	Control Group (n=12)	Test value	p-value	Sig.
Age "years"					
Mean±SD	31.75±6.43	30.58±6.44	0.197	0.661	NS
Range	21-40	20-40			
BMI [(wt/(ht)^2]					
Mean±SD	22.83±1.95	21.88±1.08	0.151	0.701	NS
Range	20-27	20-23			
Parity					
Nulliparous	4 (33.3%)	0 (0.0%)	2.700	0.103	NS
Parous	8 (66.7%)	12 (100.0%)			

t-Independent Sample t-test for Mean±SD;
Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate
NS: Non significant; S: Significant; HS: Highly significant

This table shows no statistically significant difference between the case group and the control group according to demographic data about age "years," BMI [(wt/(ht)^2], and parity, with a p-value > 0.05.

Table (3): Comparison between cases group and control group according to obstetric history

Obstetric history	Cases Group (n=12)	Control Group (n=12)	Test value	p-value	Sig.
Non	7 (58.3%)	8 (66.7%)	0.173	0.677	NS
Pre. C.S	5 (41.7%)	9 (75.0%)	2.624	0.105	NS
No. of Prev.	I:2 (16.7%) II:3 (25.0%) III: 0 (0.0%)	I:4 (33.3%) II:4 (33.3%) III:1 (8.3%)	0.726	0.697	NS
PPH	5 (41.7%)	3 (25.0%)	0.721	0.396	NS
PROM	0 (0.0%)	1 (8.3%)	0.996	0.318	NS
Rupture uterus	0 (0.0%)	1 (8.3%)	0.996	0.318	NS

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate
NS: Non significant; S: Significant; HS: Highly significant

There is no statistically significant difference between the case group and the control group according to obstetric history, with a p-value >0.05.

Table (4): Comparison between cases group and control group according to past surgical history

Past surgical history	Cases Group (n=12)	Control Group (n=12)	Test value	p-value	Sig.
Non	6 (50.0%)	4 (33.3%)	0.660	0.417	NS
D&C (Post Abortive)	2 (16.7%)	6 (50.0%)	2.869	0.090	NS
Hysterotomy	0 (0.0%)	1 (8.3%)	0.996	0.318	NS
Myomectomies	1 (8.3%)	0 (0.0%)	0.996	0.318	NS

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate
NS: Non significant; S: Significant; HS: Highly significant

There is no statistically significant difference between the case group and the control group according to past surgical history, with a p-value >0.05.

Table (5): Comparison between cases group and control group according to IUA score pre and IUA score post

	Cases Group (n=12)	Control Group (n=17)	Test value	p-value	Sig.
IUA score pre					
Stage I	2 (16.7%)	5 (41.7%)	1.829	0.401	NS
Stage II	6 (50.0%)	4 (33.3%)			
Stage III	4 (33.3%)	3 (25.0%)			
IUA score post					
Stage I	3 (25.0%)	4 (33.3%)	1.393	0.498	NS
Stage II	0 (0.0%)	1 (8.3%)			
None	9 (75.0%)	7 (58.3%)			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate
NS: Non significant; S: Significant; HS: Highly significant

There was improvement in case group but it was statistically insignificant with a p-value > 0.05.

Evaluation variable	Stage
Extent of cavity involved	
<1/3	I
1/3-2/3	II
>2/3	III

Table (6): Comparison between cases group and control group according to pain score 1 hr. after

Pain score 1 hr. after	Cases Group (n=12)	Control Group (n=12)	Test value	p-value	Sig.
Mean±SD	3.58±0.79	3.00±0.74	1.477	0.076	NS
Median (IQR)	3 (2-4)	3 (3-4)			
Range	2-5	2-4			

Using: U-Mann- Whitney test
NS: Non significant; S: Significant; HS: Highly significant

The pain was less in the case group than in the control group but was statistically insignificant with a p-value > 0.05.

Table (7): Comparison between cases group and control group according to menstrual score

Menstrual score	Cases Group (n=12)	Control Group (n=12)	Test value	p-value	Sig.
Menstrual score pre					
Mean±SD	16.92±3.21	18.25±4.02	1.279	0.270	NS
Range	0-20	0-25			
Menstrual score post					
Mean±SD	20.25±3.85	25.00±5.50	0.53	0.474	NS
Range	0-30	0-28			
Amount of Change for Menstrual score					
Mean±SD	3.33±0.63	6.75±1.49	3.344	0.081	NS
Range	0-35	0-30			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate
NS: Non significant; S: Significant; HS: Highly significant

There was no statistically significant difference between the two groups in the pre-intervention menstrual score, while patients in both groups had improvement in the post intervention menstrual score, yet there was no statistically significant difference, with a p-value > 0.05.

Table (8): Comparison between cases group and control group according to complications

Complications	Cases Group (n=12)	Control Group (n=12)	Test value	p-value	Sig.
No	11 (91.7%)	12 (100.0%)	1.043	0.307	NS
Perforation	1 (8.3%)	0 (0.0%)			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate
NS: Non significant; S: Significant; HS: Highly significant

This table shows that the one patient (8.3%) was perforated in the cases group, while there were no complications in the control group, with a p-value > 0.05

Table (9): Comparison between cases group and control group according to previous adhesiolysis

Previous adhesiolysis	Cases Group (n=12)	Control Group (n=12)	Test value	p-value	Sig.
No	4 (33.3%)	7 (58.3%)	1.448	0.229	NS
Yes	8 (66.7%)	5 (41.7%)			

Using: x^2: Chi-square test for Number (%) or Fisher's exact test, when appropriate
NS: Non significant; S: Significant; HS: Highly significant

This table shows that the 8 patients (66.7%) had previous adhesiolysis in the cases group, while the 5 patients (41.7%) had previous adhesiolysis in the control group, with a p-value > 0.05.

DISCUSSION

Asherman syndrome or intrauterine adhesions (IUA); adhesions builds up inside the uterus causing less space inside it. This condition can cause pelvic pain, abnormal uterine bleeding and fertility issues.

Since various hysteroscopic surgical approaches for management of intrauterine adhesions represents major conflict and often associated with complications, a main point of interest is the evaluation of the efficacy of a new crosslinked hyaluronan gel in the decrease in formation of intrauterine adhesions (IUAs) after D&C highlighted (6).

So, the research was conducted and aimed to investigate the intrauterine injection of hyaluronic acid in women diagnosed with intra-uterine adhesions using hysteroscopic adhesiolysis.

AS is reported in 13% of routine infertility investigations in women with infertility and of 7% in women with 2ry amenorrhea so it seems important to account for primary prevention of Asherman and as well as treatment and prevention of recurrence.

Primary prevention Of Asherman Syndrome

Tafti et al. (3) A randomized double-blind clinical trial was conducted to test the efficacy of intrauterine hyaluronic acid injections in preventing Asherman's syndrome in patients having electrocautery uterine septum resection. The study found that the Asherman's syndrome incidence was significantly higher in the control group compared to the case group (p = 0.012) following the intervention. This suggests that the injection of HA intrauterine effectively reduced the Asherman's syndrome incidence in the case group without any reported complications.

In 2020, a meta-analysis was performed by Zheng et al. (7) showed that HA gel decreased incidence of IUA and its score after an intrauterine operation.

This decrease in incidence was not affected by the presence of primary diseases or even the type of intrauterine operation.

Hooker et al. (8) conducted a multicenter double-blinded prospective randomized trial that included 52 women with a previous history of intrauterine surgery (D&C) to investigate the application of auto-crosslinked hyaluronic acid (ACP) gel in the uterine cavity. There was a decrease in the incidence of intrauterine adhesions in the intervention group compared with control group (RR, 0.43; 95% CI 0.22–0.83; P=0.013), in addition to the significantly lower mean adhesion scores in the intervention group compared with the control group (P<.0001).

A prospective, randomized, controlled study was performed by Guida et al. (9) to test the

auto-crosslinked hyaluronic acid (ACP) gel efficacy in prevention of intrauterine adhesions following hysteroscopic surgery and revealed that the intervention group demonstrated a significant decrease in the formation of intrauterine adhesions compared to the control group.

Also, Acunzo et al. (10) in a randomized controlled trial found that HA significantly decreases the formation of IUA following surgical operations and is probably linked to the decrease in the occurrence of severe adhesions.

Tsapanos et al. (11) conducted a blind prospective randomized controlled trial to assess the effectiveness and safety of Seprafilm™, a new bio-resorbable membrane composed of modified HA and carboxy-methyl-cellulose. They aimed to investigate its role in preventing and reducing postoperative adhesions in the endometrial and endocervical areas following general curettage or suction evacuation for recurrent, missed, or incomplete abortion. Their findings suggest that Seprafilm™ significantly decreases the occurrence and severity of adhesions

Secondary prevention of Asherman Syndrome

Based on systematic reviews and meta-analysis, evidence is lacking in concluding that any management is effective in the preventing of IUAs after hysteroscopy (12).

The current information on ACP following operative hysteroscopy shows inconsistency with the significant heterogeneity and major risk of bias, so it is almost impossible to reach a firm conclusions ,and also the impaction on reproductive outcomes not yet evaluated (8).

Xiao et al. (13) conducted a prospective, randomized, double-blinded, and controlled clinical trial their aim to assess the effectiveness and safety of hyaluronic acid gel in preventing intrauterine adhesion recurrence following hysteroscopic adhesiolysis (using electrocautery).

Their findings demonstrated that applied of auto-crosslinked hyaluronic acid gel led to a significantly higher success rate in reducing adhesions compared to the control group. Specifically, the effective rates were 76% in the case group and 48% in the control ($P = 0.0009$).

Moreover, the American Fertility Society (AFS) score in the treatment group was notably lower than that in the control group ($P = 0.0008$).

Additionally, usage of hyaluronic acid gel post-surgical intervention significantly contributed to individual patient improvement in terms of adhesive type and menstrual pattern ($P = 0.0378$, $P = 0.0004$, respectively).

Furthermore, patients treated with hyaluronic acid gel exhibited a significant decreased proportion of severity and staging of adhesions compared to the control group ($P = 0.0006$), with no observed adverse events or complications.

Our study results about complication consistent with Hooker et al. (8) and Xiao et al. (13) who reported no adverse effects or complications related to application of HA gel in the intervention group, and the complication rate due to hysteroscopic intervention were similar in case and control group.

The difficulties of the study compared to previous studies may be attributed to the smaller sample size in our research due to patient recruitment difficulty and also study depends on surgical on performance, unlike previous studies which use injection of hyaluronic acid as a prophylactic treatment rather than curative management, which could have led to an underestimation of the incidence and diagnosis of intrauterine adhesions in the previous studies populations, compared to our study.

Included cases that underwent intrauterine surgery (such as hysteroscopic myomectomy, evacuation of remnants of pregnancy conception, and other hysteroscopic surger-

ies) regardless of whether Asherman syndrome occurred postoperatively.

Strength points of the study:

The strength points of the study are that it is randomized clinical study design, its setting at a single tertiary care center, having experienced hysteroscopic operator who performed all the examinations and having no patients lost to follow-up in three months follow-up. Detection bias was avoided by blinding the hysteroscopic outcome assessors and the participants regarding treatment assignment during follow-up. This is the first study in literature evaluating the intrauterine injection of hyaluronic acid after hysteroscopic adhesiolysis by scissors without using cervical dilatation or electrocautery in treatment of the diagnosed cases of Asherman syndrome.

The limitations of the study:

The findings of this study should be interpreted in light of its limitations, involving relatively smaller sample size than to previous researches, that study not multicentric study and this suggests a major concern for publication bias. Another limitation is that weakened generalizability owing to limited racial diversity in the study cohort, the lack of data regarding impact on fertility and long-term clinical symptoms and the relatively short-term follow-up of patients postoperatively (3 months), which may underrate the effect of injection of hyaluronic acid intra-uterine.

CONCLUSION

Hysteroscopic surgery (for example septum excision or pedunculated myoma) may result in significant intrauterine adhesions. These adhesions can be managed safely and effectively hysteroscopically for restoring menstrual pattern and fertility. However, intrauterine adhesions have a high incidence of recurrence even after treatment.

As evident from the current study, there were no statistically significant differences among the study groups regarding the IUA score, pain score and menstrual score.

We know that no absolute conclusions can be drawn about the effect of gel from results based on such a small randomized control study. However, this study is the first to show the safety of this treatment, both in terms of intraoperative complications and postoperative course in patients diagnosed with Asherman syndrome.

Additional Information

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosures

Conflicts of Interest: The authors have no conflicts of interest to declare.

Confidentiality of Data: The authors declare that they have followed the protocols of their work center on the publication of data from patients.

Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Financing Support: All authors have declared that no financial support was received from any organization for the submitted work.

Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have

an interest in the submitted work.

Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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Relation between Position of the Uterus (Anteversion or Retroversion Flexion) and Degree of Cesarean Scar Niche: Cross-sectional Study

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Abstract

Background: In recent decades, the Cesarean section rate has been significantly rising, leading to a surge in complications associated with surgical scars, notably the development of cesarean scar niche (CSN). Exploring the symptoms, correlations, and risk factors associated with its occurrence has marked now as a main point of interest.

Objectives: The aim of the study was to determine the association between the size of caesarean scar niche and uterine position in childbearing women.

Methods: This cross-sectional study was conducted at tertiary care hospital at gynecological outpatient clinic - Ain Shams University Maternity Hospital (ASUMH) from October 2023 till June 2024 and performed on total 86 women with a history of cesarean section. All of them were subjected to full history taking, and sonographic assessment of caesarean scar niche (width, depth, length, residual myometrial thickness (RMT) overlying the defect, and the adjacent myometrial thickness fundal to the defect), and the uterine position was classified as anteverted or retroverted, using transvaginal ultrasound with inclusion and exclusion criteria.

Results: The prevalence of large CSN was 40.7% and that was significantly higher in RVF uterus (80%) compared to AVF uterus (28.8%) (P= 0.001). This indicates a 2.78-fold increased risk of a large CSN in RVF uterus. Additionally, there was a statistically significant highest mean value of ratio between depth and adjacent myometrial thickness, the width, and depth of CSN in RVF group compared to AVF group. (P= 0.001, 0.038, and 0.001 respectively). On the other hand, the mean value of RMT was significantly lower in the RVF group compared to the AVF group (P= 0.013). However, no statistically significant differences were observed in the number of CSN, its length, or the adjacent myometrium between the two groups. In terms of clinical symptoms there was a statistically significant higher prevalence of severe chronic pelvic pain in large CSN group (8.6%) compared to small CSN group (3.9%) (P= 0.022). However, there was no statistically signifi-

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cant difference between large and small CSN in post-menstrual spotting, AUB, dysmenorrhea, dyspareunia and secondary infertility.

Conclusion: The retroverted position of the uterus has been associated with an increased risk of developing more significant and larger CSN in terms of depth and width. This could be due to the altered angle and mechanical stress on the cesarean scar site, potentially affecting healing. These results may help in the counseling of patients regarding their subsequent pregnancies when a decision involving a Cesarean section is being considered.

Keywords: Cesarean Scar Niche, Cesarean Scar Defect, Isthmocele, Position of the Uterus, RVF Uterus.

INTRODUCTION

In past decades the Cesarean section rate has increased markedly. At a rate of 52 percent Egypt stands out among countries with the highest CS delivery rates in the world, following Dominican Republic (56.4 percent) and Brazil (55.6 percent). Within the Arab region, rates of CS are far higher in Egypt than any other Arab country (1).

Cesarean section is associated with complications in subsequent pregnancies, such as scar pregnancy with life-threatening bleeding, placenta previa, placenta accreta, increta or percreta, dehiscence or uterine rupture (2).

Cesarean scar niche or defect (isthmocele) is a known complication after cesarean delivery. It has become more common due to a rising cesarean delivery rate (3). It is the formation of a pouch at the site of an old caesarean incision at the anterior lower uterine segment, uterine isthmus or in the upper segment of the cervical canal (4).

Cesarean scar niche (CSN) is defined as the presence of a hypoechoic area within the myometrium in the isthmus (lower uterine segment) with discontinuation of myometrium at the site of a previous caesarean section scar. A niche has been described as the indentation of myometrium of around 1-2 mm due to tethering of the endometrium. They are encountered in various shapes such as: (1) semicircular: considered commonest, (2) triangular: next commonest, (3) droplet shaped and (4) inclusion cysts (5)

A histopathological study of hysterectomy specimens with Cesarean section scars proposed three possible mechanisms underlying the pathogenesis of this condition: firstly, the presence of a congested endometrial fold and small polyps in the scar recess are potential causes of menorrhagia and abnormal uterine bleeding; secondly, lymphocytic infiltration and distortion of the lower uterine segment could contribute to chronic pelvic pain and dyspareunia; thirdly, iatrogenic adenomyosis confined to the scar could account for dysmenorrhea (6)

Residual myometrial thickness (RMT) is the vertical distance between uterine serosa and apex of defect. Large niches are defined when RMT is <50% of adjacent myometrium or ≤ 2.2 mm on transvaginal ultrasound or ≤ 2.5 mm with saline instillation sonohysterography. Marotta et al. has given cut-off of 3 mm, and ≥3mm RMT is small defect (7)

These are often detected incidentally, patients presenting with clinical symptoms such as postmenstrual spotting, dysmenorrhea, dyspareunia, chronic pelvic pain or dull sensation following menstruation (8).

It has also been noted that the CSN in patients with retroflexed uteri appear to be larger than are those in patients with anteverted uteri. Moreover, patients who have undergone multiple Cesarean sections have been observed to have larger scar defects, and often experience one or more of the symptoms mentioned above. However, previous literature lacks data addressing the association between these symptoms and the clinical findings of CSNs (9). Thus, the purpose of this study was to determine the association between the size of caesarean scar niche and uterine position (anteverted or retroverted uterus) in childbearing women.

PATIENTS AND METHODS

After ethical committee approval with number: MS 522/2023, the study was registered on Clinical Trials.Gov with the following ID: NCT06475924. and written consents from the patients, this cross-sectional study was conducted at tertiary care hospital at gynecological outpatient clinic - Ain Shams University Maternity Hospital (ASUMH) from October 2023 till June 2024 and performed on total 86 women with a history of cesarean section, in childbearing age (18 -49 years), At least six months postpartum and women with cesarean scar niche(CSN) by transvaginal ultrasound that is defined as "indentation at the site in the cesarean section scar with a depth of at least 2 mm" that was agreed upon in **2019 by a European task force (10)**, excluding women with severely obese (Body mass index; BMI ≥ 35 kg/m2), women with abnormal uterine pathology, observed during the ultrasound examination that may have been responsible for abnormal uterine bleeding, including endometrial hyperplasia, polyps, malignancy or myomas, women with any cause of pelvic pain as pelvic inflammatory diseases, endometriosis or adenomyosis, women with history of classic upper segment cesarean section or hysterotomy, women with history of previous uterine scars rather than cesarean sections as myomectomy and women with bleeding tendency disorders, taking anticoagulants or having chronic medical conditions as liver diseases, or coagulopathies.

All participants were submitted to full history taking including present & past history, age, parity, BMI, medical and surgical history. **Examination:** including general, abdominal and vaginal, clinical examinations for assessment of uterine position or any abnormal findings. **Laboratory investigations:** as CBC, coagulation profile (PT, PTT, INR) and liver profile. **Sonographic assessment of Cesarean scar Niche (CSN)**: was performed in the Fetal Medicine and ultrasound unit at Ain Shams University Maternity Hospital using Samsung H60 Ultrasound machine equipped with a 7–9 MHz transvaginal probe. Women were examined in the lithotomy position with an empty bladder. The uterus was examined in a standardized way excluding causes of abnormal uterine bleeding or pelvic pain (myoma, polyp, pelvic inflammatory disease, adenomyosis). CSN was defined as indentation at the site in the cesarean section scar with a depth of at least 2 mm. Upon detection of the SCD, the following was measured): In the midsagittal plane: the depth (the vertical distance between the base and apex of the defect), width (the length of the widest gap along the cervicoisthmic canal), residual myometrial thickness (RMT) overlying the defect, and the adjacent myometrial thickness fundal to the defect. In the transverse plane: the length of the defect. **(Figure1 and 2)**

The uterine position was classified as anteverted or retroverted: **Version:** indicated the relation between the uterus and vagina in the sagittal plane. **Anteverted:** the uterus is tilts forward at the cervix. **Retroverted:** the uterus tilts backward at the cervix. CSN was considered large if the ratio between the depth and adjacent myometrial thickness is ≥ 0.50 (3).

Clinical symptoms of the patients were reviewed such as: Postmenstrual spotting, abnormal uterine bleeding, pain as dysmenorrhea, chronic pelvic pain or dyspareunia, and secondary infertility.

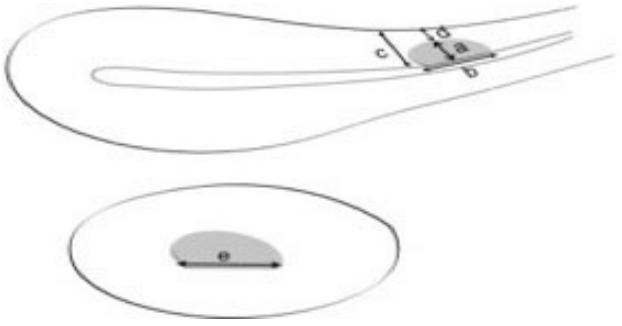

Figure1: Schematic presentation of Cesarean scar defect (CSD) measurements

In midsagittal plane: a: Depth and b: Width, thickness of c, adjacent and d residual myometrial thickness. In transverse plane: e, length of CSD (3).

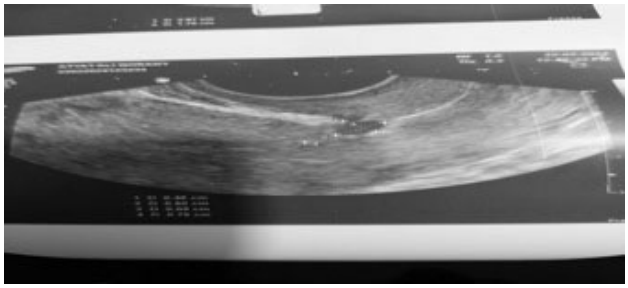

Figure2: Visualization of CS niche using TVUS, assessment of CS niche parameters. Depth, width, residual myometrial thickness, and adjacent myometrial thickness.

Sample Size: Using PASS 15 program for sample size calculation, setting confidence level at 95% and margin of error at 10%, it is estimated that sample size of 85 women will be needed to detect as expected 70% prevalence rate of CS scar niche (11).

Outcome measures: Primary outcome: The association between the position of the uterus (Anteverted/Retroverted) and large CSN (the ratio between the depth and adjacent myometrial thickness ≥ 0.50.). **Secondary outcomes:** The association between position of the uterus (Anteverted/Retroverted) and: Residual myometrial thickness (RMT). Depth, Width, Length of CSN. The association to the clinical symptoms as: Postmenstrual spotting: ≥ 2 days of intermenstrual spotting, or ≥ 2 days of brownish discharge after the end of menstruation if bleeding duration exceeds 7 days (discharge is considered normal if bleeding duration is < 7 days) (12). Abnormal uterine bleeding (AUB) as per FIGO guideline. Pain as dysmenorrhea, chronic pelvic pain or dyspareunia. If present will be assed using Visual Analogue Scale (VAS). Secondary infertility: inability of a couple to conceive after one year of regular, unprotected intercourse.

Ethical Considerations: The patient data were anonymous. Data presentation were not be by the patient's name but by diagnosis and patient confidentiality was protected. An informed consent was taken from all participants, it was in Arabic language and confirmed by date and time. confidentiality was preserved by assigning a number to patients initials and only the investigator knew it

Conflict of interest: the candidate declared that there is no conflict of interest and the cost of the study was paid by the candidate.

Statistical analysis: Analysis is to be performed using SPSS for windows v20.0, Data to be presented in terms of range, mean and standard deviation (for numeric parametric variables); range, median and inter-quartile range (for numeric non-parametric variables); or number and percentage (for categorical variables). Difference between two independent groups is to be analyzed using independent student's t-test as well as the mean difference and its 95% CI (for numeric parametric variables); or chi-squared test as well as the risk ratio and its 95% CI (for categorical variables). Binary logistic regression analysis is to be performed for estimating the association between good/poor response and the measured variables ROC curves are to be constructed for estimating the validity of measured variables as predictors of good or poor response validity is to be presented in terms of sensitivity, specificity, positive and negative predictive values and their corresponding 95% Cis significance level is set at 0.05.

RESULTS

During this study, 148 patients were assessed for eligibility. Of all eligible patients, 26 patients were excluded from the study based on the inclusion criteria, 8 patients refused to participate in the study and 28 patients were excluded due to other uterine pathologies. Ultimately, the analysis was based on the data of 86 women with a history of cesarean section.

Our study results demonstrated that on TVUS assessment, 66 (76.7%) women had AVF uterus and 20 (23.3%) women had RVF uterus. The mean length of Cesarean Scar Niche (CSN) was 6.17±2.19 mm, the width of CS

niche was 4.75±1.98 mm, the depth of CSN was 6.34±2.70 mm, the residual myometrial thickness was 4.07±1.24 mm, and the adjacent myometrium was 15.00±3.43 mm. Furthermore, the 51 patients (59.3%) had small CS niche and 35 patients (40.7%) had large CS niche, while the ratio between depth and adjacent myometrial thickness was 0.1 to 0.9 with mean 0.42±0.19.

In terms of clinical symptoms, the current research study revealed a range of presentations among patients. Postmenstrual spotting was observed in 42 patients (48.8%), while 55 patients (64%) experienced abnormal uterine bleeding (AUB). Dysmenorrhea was prevalent in 83 patients (96.5%), and chronic pelvic pain affected 54 patients (62.8%). Dyspareunia was reported by 39 patients (45.3%), and secondary infertility was noted in 21 patients (24.4%).

Our results revealed that large CS niche was significantly higher in RVF uterus (80%), compared to (28.8%) in the AVF uterus (p value= 0.001). Thus, there is a 2.78-fold increased risk in the cases with large cesarean scar niche in RVF uterus patients with statistically significant highest mean value of ratio between depth and adjacent myometrial thickness in RVF group compared to AVF group, (P=0.001). (Table 1, 2)

Degree of cesarean scar niche	AVF (n=66)	RVF (n=20)	RR (95% C.I.)	p-value	Sig.
Small CSN	47 (71.2%)	4 (20.0%)	2.78 (1.37-3.43)	0.001	HS
Large CSN	19 (28.8%)	16 (80.0%)			

Table (1): Comparison between AVF and RVF according to Degree of cesarean scar niche

Ratio between depth and adjacent myometrial thickness	AVF (n=66)	RVF (n=20)	Test value	p-value	Sig.
Mean±SD	0.38±0.19	0.55±0.12	15.500	0.001	HS
Range	0.1-0.9	0.28-0.75			

Table (2): Comparison between AVF and RVF according to ratio between depth and adjacent myometrial thickness.

In addition, our study results revealed a statistically significant higher mean value of the width of Cesarean Scar Niche (CSN) in the RVF group compared to the AVF group, (p-value= 0.038). Similarly, the depth of CSN was significantly greater in the RVF group than in the AVF group, (p-value= 0.001). Conversely, the mean value of residual myometrial thickness was significantly lower in the RVF group compared to the AVF group, (p-value= 0.013). However, no statistically significant differences were observed in the number of CSNs, the length of CSN, or the adjacent myometrium between the two groups, with p-values greater than 0.05. (Table 3)

TVUS	AVF (n=66)	RVF (n=20)	Test value	p-value	Sig.
Number of CSN					
1	65 (98.5%)	20 (100.0%)	0.307	0.580	NS
2	1 (1.5%)	0 (0.0%)			
Length of CSN (mm)					
Mean±SD	6.00±2.10	6.75±2.43	1.814	0.182	NS
Range	4-12	4-14			
Width of CSN (mm)					
Mean±SD	4.51±1.83	5.55±2.28	4.438	0.038	S
Range	2-10	3-12			
Depth of CSN (mm)					
Mean±SD	5.83±2.52	8.03±2.65	11.421	0.001	HS
Range	3-13	4-16			
Residual myometrial thickness (mm)					
Mean±SD	4.25±1.24	3.48±1.04	6.479	0.013	S
Range	1-7	1-5			
Adjacent myometrium (mm)					
Mean±SD	15.29±3.43	14.05±3.32	2.027	0.158	NS
Range	6-23	10-24			

Table (3): Comparison between AVF and RVF according to TVUS.

Regarding the basic data, our study results revealed that there was a statistically significant higher severe chronic pelvic pain in large CS niche group was (8.6%) compared to small CS niche group was (3.9%) **(p= 0.022)**, with no statistically significant difference in post-menstrual spotting, AUB, Dysmenorrhea, Dyspareunia and Secondary infertility, with p-value (p>0.05). **(Table 4)**

Association to the clinical symptoms	Small CSN (n=51)	Large CSN (n=35)	Test value	p-value	Sig.
PostMenstrual spotting					
No	25 (49.0%)	19 (54.3%)	0.230	0.631	NS
Yes	26 (51.0%)	16 (45.7%)			
AUB					
No	20 (39.2%)	11 (31.4%)	0.260	0.619	NS
Yes	31 (60.8%)	24 (68.6%)			
Frequent	2 (3.9%)	0 (0.0%)			
Heavy Menses	25 (49.0%)	21 (60.0%)			
Irregular Menses	13 (25.5%)	7 (20.0%)			
Prololged Menses	16 (31.4%)	11 (31.4%)			

Dysmenorrhea					
No	1 (2.0%)	2 (5.7%)	1.004	0.800	NS
Mild	17 (33.3%)	11 (31.4%)			
Moderate	24 (47.1%)	15 (42.9%)			
Sever	9 (17.6%)	7 (20.0%)			
Chronic pelvic pain					
No	23 (45.1%)	9 (25.7%)	9.593	0.022	S
Mild	6 (11.8%)	13 (37.1%)			
Moderate	20 (39.2%)	10 (28.6%)			
Sever	2 (3.9%)	3 (8.6%)			
Dyspareunia					
No	33 (64.7%)	14 (40.0%)	7.415	0.060	NS
Mild	12 (23.5%)	14 (40.0%)			
Moderate	5 (9.8%)	3 (8.6%)			
Sever	1 (2.0%)	4 (11.4%)			
Secondary infertility					
No	41 (80.4%)	24 (68.6%)	1.571	0.210	NS
Yes	10 (19.6%)	11 (31.4%)			

Table (4): Comparison between Small CSN and Large CSN according to Association to the clinical symptoms

DISCUSSION

Uterine niche is one of the emerging complications of caesarean section. With rising caesarean rates, the caesarean-related iatrogenic complications are also on the rise. These include placenta accreta, scar ectopic pregnancy and uterine niche. (12). Since risk factors for development of CS niche represents major conflict and often associated with complications, evaluating the correlation between uterine position and occurrence of CS niche was highlighted as a main point of interest (13). Consequently, this study was conducted and aimed to determine the association between the size of caesarean scar niche and uterine position in childbearing women.

The prevalence of scars with a large defect in our study was 40.7% which was similar to that reported by Menada Valenzano et al., at 42% (14) and Armstrong et al., at 43% (15). On contrary to Ofili-Yebovi et al., who reported a prevalence of 19% (9). The discrepancy is likely to be explained not only to different definitions of defect used in the studies but also to differences in stage of labor during Cesarean section, the indications for Cesarean delivery, and operative complications, among other factors.

Our results demonstrated that the large cesarean scar niche (CSN) was significantly higher in RVF uterus compared to the AVF uterus indicating a 2.78 folds increased risk of large CSN in RVF uterus. Additionally, there were statistically significant highest mean value of ratio between depth and adjacent myometrial thickness, depth and width of CSN in the RVF uterus. On the other hand, the mean value of residual myometrial thickness was significantly lower in the RVF group compared to the AVF group. However, no statistically significant differences were observed in the number of cesarean scar niche, its length, or the adjacent myometrium between the two group.

These findings are in agreement with previous studies, Galal et al., conducted a prospective cross-sectional study involving 300

women to assess the prevalence of scar niche following cesarean sections. They reported that RVF position of the uterus is a significant risk factor for CS niche. Compared to AVF, RVF position is associated with 2.26 times higher risk of developing a scar niche (p-value=0.026) (16). But they used a different criterion, cases had been reviewed after 12 weeks from last CS. while, in our study, cases were enrolled at least six months post CS.

Additionally, Olimy et al., conducted a prospective cohort study that enrolled 30 non-pregnant women. They revealed that the prevalence of RVF uterus was 36.67% while AVF uterus was 80%. RVF uterus was significantly associated with CS niche in 70%. However, they used two methods (TVUS, pelvic MRI) for evaluation of CS niche. (17).

Furthermore, Tang et al., and Antila- Langsjo et al., reported that a RVF uterus increased the risk for CS niche development (18, 3). In addition, Lumbanraja et al., conducted a prospective cohort study that enrolled 280 women to assesses the risk factors for niche development after cesarean section. This study revealed that the retroflexed uterus was 9.2 times more likely to have a niche than the anteflexed uterus ($P<0.001$; RR: 9.2). But they assessed the niche six weeks after cesarean section. (13)

Several other studies in the literature support these findings. In agreement to this, Osser et al. and Bij de Vaate et al. reported that symptomatic patients who underwent laparoscopic niche repair were mostly in the retroflexion position (19, 11). While Osser et al. classified the scar defects subjectively by the ultrasound examiner as a small or a large defect.

Our study agrees with all previous studies, which revealed that RVF uterus is a significant risk factor for CS niche. But those studies differ in the method used for detection of CS niche. Antila-Langsjo et al., used TVUS and sonohysterography for evaluating the CS niche. Tang et al., used TVUS and MRI. However, Lumbanraja et al., and our study assessed CS niche by using transvaginal ultrasonography.

Ofili-Yebovi et al., reported that uterine retroflexion may be a risk factor associated with deficient scars. A suggested explanation for this association is that retroflexed uterus exerts a high degree of tension on the lower uterine segment resulting in reduced vascular perfusion. This compromises the healing capacity of the Cesarean scar. Therefore, mechanical tension on the lower uterine segment in a retroflexed uterus might impair blood perfusion and oxygenation of the healing tissues, and that this could affect wound healing negatively (9). Tissue oxygenation is an important factor for wound healing (19). These findings align with our research results, which strengthens and augments these findings and results reliability.

In terms of clinical symptoms, the current research study revealed a range of presentations among patients. The most common symptom was dysmenorrhea was prevalent in 83 patients (96.5%), followed by abnormal uterine bleeding (AUB) affected 55 patients (64%), and chronic pelvic pain affected 54 patients (62.8%). Postmenstrual spotting was observed in 42 patients (48.8%), Dyspareunia was reported by 39 patients (45.3%), and secondary infertility was noted in 21 patients (24.4%). These findings highlight the diverse and significant symptomatology associated with the patients studied.

In contrast to our study WANG et al., conducted a cross-sectional study involving 293 women, they revealed that prolonged postmenstrual spotting was the most common symptom (63.8%), followed by dysmenorrhea (53.1%), chronic pelvic pain (39.6%) and dyspareunia (18.3%). (20)

In addition, Tang et al., conducted a case–control study that enrolled 189 women to determine the risk factors for development of caesarean scar niche. They reported that the most common complaints related to CS niche are prolonged menstrual bleeding, and

postmenstrual spotting (in up to three- quarters of women with CS niche), followed by pelvic pain (39.6%), dysmenorrhea (53.1%), dyspareunia (18.3%), and secondary infertility (all P < 0.01) (18).

Regarding the basic data, our study results revealed that there was a statistically significant higher prevalence of severe chronic pelvic pain in large CS niche group (8.6%) compared to small CS niche group (3.9%) (p= 0.022). In agreement with WANG et al., the mean defect width was significantly greater in patients with chronic pelvic pain compared to those without (P <0.001) (20).

Our results revealed that there was no statistically significant difference between small CS niche and large CS niche regarding obstetric history (Age, Term, Preterm, abortion, living, Number of previous CS, BMI), medical and surgical history, with no statistically significant difference in post-menstrual spotting, AUB, Dysmenorrhea, Dyspareunia and Secondary infertility.

Our study results disagreed with Antila RM et al., who reported that the prevalence of post-menstrual spotting was higher in large niche with 3.34 OR. (21)

The strength points of this study

The strength points of this study are that its setting at a single tertiary care center and it assessed the relation between the position of the uterus and the occurrence of a large CS niche that could help in counselling of the patients. In addition, it provided additional causes of secondary infertility to be minded in cases with recurrent implantation failures, post-menstrual bleeding and chronic pelvic pain in women with RVF uterus.

The limitations of the study

First, the study is not multicentric, which presents a significant risk of publication bias. Secondly, the lack of detailed information on how the Cesarean sections were performed, as many of the women were referrals, and the surgeries were carried out by different physicians at various institutions. Thirdly, using of TVUS only as a method for assessment of CS niche. Also, details regarding the suturing techniques, indications for Cesarean sections, and post-Cesarean conditions were not provided, leaving the impact of these factors on Cesarean Scar niche formation unknown. Moreover, the timing of ultrasound screenings was not uniform, preventing the collection of information on the onset of symptoms after the most recent Cesarean section.

CONCLUSION

The retroverted position of the uterus has been associated with an increased risk of developing more significant and larger CS niche in terms of depth and width. This could be due to the altered angle and mechanical stress on the cesarean scar site, potentially affecting healing. These results may help in the counseling of patients regarding their subsequent pregnancies when a decision involving a Cesarean section is being considered.

Gynecologists should consider the possibility of a niche when evaluating individuals with RVF uterus and have postmenstrual spotting and secondary infertility and have a history of CS.

Acknowledgements:

The authors appreciate the support from Ain Shams University Maternity Hospital Ultrasound for the outstanding support during the entire study.

Funding:

No funding.

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The Relation between Serum Pentraxin 3 Concentrations on Day of Embryo Transfer and Prediction of Pregnancy in Intracytoplasmic Sperm Injection (ICSI) Cycles

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Abstract

Background: Prediction of clinical pregnancy is rapidly becoming an important objective in assisted reproduction technology (ART); on the other hand, most patients are successfully fertilized and get embryos transferred (ET), but not all of transferred embryos actually implant and develop into viable pregnancies.

Aim of the Work: The aim of the present study was to assess the relationship between serum Pentraxin 3 concentrations on day of embryo transfer and prediction of pregnancy in intracytoplasmic sperm injection (ICSI) cycles.

Patients and Methods: This was a prospective observational cohort study, was conducted at Assisted Reproductive Technology Unit (ART Unit) at Ain Shams University Maternity Hospitals on 30 Women who underwent intracytoplasmic sperm injection (ICSI). The study started on February 2023 and ended on March 2024.

Results: Median age of the study population is 30 years, No statistically significant difference (p value >0.05) between pregnant & non pregnant groups as regards demographic data, obstetric history, biochemical assay, endometrial thickness, days of stimulation, number of gonadotropins ampoules and oocyte quality. No statistically significant difference between pregnant & non-pregnant groups as regards S.Pentraxin3($p > 0.05$) and has poor predictive value Using ROC curve (AUC =. 609, 95% CI =. 414 to. 781, p =. 350). Best cutoff criterion is serum pentraxin 3 > 3.1 ng/ml (sensitivity [95% CI] = 85.7% [42.1% to 99.6%], specificity = [95% CI] = 47.8% (26.8% to 69.4%) Using ROC curve for prediction of biochemical pregnancy using E2, E2 has good predictive value (AUC =. 817, 95% CI =. 634 to. 933, p <.001). Best cutoff criterion is E2 ≤41.0 pg/ml (sensitivity [95% CI] = 100% [59.0% - 100.0%], specificity = [95% CI] = 60.9% [38.5% - 80.3%]. Based on these results PTX3 doesn't seem to be correlated with subsequent pregnancy in ICSI cycles.

Conclusion: The measurement of PTX3 on day of embryo transfer is not predictive of subsequent pregnancy as there was no statistically significant difference between

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pregnant & non pregnant groups as regards PTX3 and there is only weak negative correlation between serum pentraxin 3 and BMI.

Keywords: Serum Pentraxin 3, Embryo Transfer, Intracytoplasmic Sperm Injection (ICSI).

INTRODUCTION

Despite extensive research in the field of assisted reproduction technology (ART), implantation of an embryo is the rate-limiting step in ART cycles [(1)].

The interaction between a good-quality embryo and a receptive endometrium at the time of implantation along with good embryo transfer technique determines the success of ART cycles [(2)].

Many noninvasive methods have been studied to assess endometrial receptivity clinically such as ultrasound measurement of endometrial thickness, pattern, volume, and endometrial blood flow. An endometrial thickness of >7 mm has been generally considered as a reliable sign of optimal implantation potential [(3)].

Various methods have been tried to increase endometrial thickness, its receptivity, and pregnancy rates in ART cycle such as supplementation of sildenafil, aspirin, and low-molecular-weight heparin [(4)].

Most important factors that influence achievement of pregnancy are good transferred embro quality and receptive endometrium so a marker that could predict endometrial repectivity will be beneficial whether to proceed with embryo transfer or embryo vitrification [(5)].

PTX3 is a member of the pentraxin protein family, which also includes inflammatory proteins like C-reactive protein (CRP) and serum amyloid P component (SAP) [(6)].

The Pentraxin 3 gene is located on chromosome 3; the protein has a molecular weight of 344 kDa and consists of multimeric glycoproteins. It is released by innate immune cells or stromal cells as a reaction on various pro-inflammatory cytokines or Toll-like-receptor activation and regulates complement activation [(7)].

Average PTX3 serum-values in healthy individuals have been published to be 2 ng/mL [1.95, 2.04] [(8)].

The mRNA expression of PTX3 in human endometrial stromal cells is also influenced by immunological factors as well as by progesterone, estrogen, and interleukin-1, but not by human chorionic gonadotropin [(9)].

On the other hand, obstetrical complications like pre-eclampsia and intrauterine growth restriction (IUGR), that are believed to result from defective trophoblast invasion and insufficient vascular remodeling are followed by an excessive maternal inflammatory response with higher serum levels of PTX. Additionally, Ibrahim et al. found that PTX3 serum levels were significantly increased in women with recurrent pregnancy loss compared to controls at the time of miscarriage, most likely caused by an abnormally exaggerated intrauterine inflammatory or innate immune response [(10)].

The importance of PTX3 in infections and inflammatory diseases reveals a possible role of PTX3 as a biomarker for implantation, especially as the exact necessary balance of immunological reactions and vascular changes necessary for adequate implantation is still discussed controversially [(11)].

AIM OF THE WORK

Aim of this study is to assess the relationship between serum Pentraxin 3 concentrations on day of embryo transfer and prediction of pregnancy in intracytoplasmic sperm injection (ICSI) cycles.

Research Question:

Could serum Pentraxin 3 concentration be a good biochmical marker for prediction of pregnancy in intracytoplasmic sperm injec-

tion (ICSI) cycles?

Research hypothesis:

Serum Pentraxin 3 concentration is a good biomarker for prediction of pregnancy in intracytoplasmic sperm injection (ICSI) cycles.

PATIENTS AND METHODS

Study Setting:

This study is a prospective observational cohort study, the study was conducted at Assisted Reproductive Technology Unit (ART Unit) at Ain Shams University Maternity Hospitals by ART Unit team. A total number of 30 Women who underwent intracytoplasmic sperm injection (ICSI).

Main Outcome Measures:

The primary outcome: correlation between serum pentraxin 3 and occurrence of pregnancy (chemical). The secondary outcome: correlation between serum pentraxin 3 and oocyte maturation, embryo grading, number of HMG ampules, days of stimulation and endometerial thickness and cut off limit of pentraxin 3 for embryo transfer.

Inclusion Criteria:

- Women who are between 18 and 40 years of age.
- Couples with unexplained infertility: Both ovaries & uterus are apparently free from any abnormalities, normal semen parameters, normal hormonal profile (FSH, LH, TSH, prolactin)

Exclusion Criteria:

- Smoking women.
- Endocrinological diseases: DM, thyroid gland diseases and adernal gland diseases.
- Inflammatory diseases like: Autoimmune diseases: SLE and rheumatoid diseases
- Chronic illness: HTN and chronic heart diseases.
- Women with history of previous repeated implantation failure.
- Women with history of previous PET, IUGR or recurrent abortion.

Study procedure:

Full history taking include past medical history, surgical history, obstetric history. General and abdominal examination for signs of disturbed endocrinological function like hirsutism or for any pelvic masses. Hormonal profile for screening of ovarian reserve or endocrinal abnormalities FSH, LH, E2, AMH, TSH and prolactin was performed to all patients on day 3 of spontaneous cycles.

TV U/S was carried out on day 3 of non stimulated cycle for good assessment of AFC (antral follicular count) & to exclude any patient with uterine, tubal or ovarian pathology. Controlled ovarian hyperstimulation was carried out with long agonist protocol (as according to Assisted Reproductive Technology Unit (ART Unit) at Ain Shams University Maternity Hospitals) starting with pituitary desensitization by triptorelin acetate (Decapeptyl 0.1 Ferring, Kiel, Germany) on day 14 of the non stimulated cycle and maintained till HCG administration

Administration of Gonadotropins of a starting dose 150 IU up to 450 IU; FSH (Gonapure, Minapharm pharmaceutics) & HMG (Meriofert, Ibsa - Switzerland) was started on day (2-3) of stimulated cycle after confirmind down regulation by decapeptyl 0.1 for 10-12 days by serum E2 (less than 50 pg/ml), endometrial thickness (less than 5mm) & no functioning ovarian cyst. Adjustment of gonadotropins dosage was based on individual variations among women.

A serial transvaginal ultrasound was performed monitoring follicular response & growth. HCG 5000-1000IU (choriomon, Ibsa - Switzerland) was administered when majority of follicles reach a diameter of 18 mm-22mm & oocyte pick up were scheduled after 34-36 hrs. After visualization of the oocyte-cumulus complex (OCC) in the follicular fluid, each oocyte was maintained at 37°C in culture medium and proper pH was main-

tained using 7 percent CO2 in air through all steps.

Embryo development was assessed on the morning of day 3, including blastomere number, size, and regularity, and the presence and percentage of fragmentation. All embryos were graded on day 3 according to Son et al. (12) classification.

Embryos with equal-sized 6–8 cells and <10% fragmentation are defined as good embryos. Those embryos with ≤4 blastomeres are defined as arrested embryos, regardless of volume of fragmentation.

On Day5: embryo grading was assessed according to Gardner's embryo grading system assigning: Blastocyst development stage, expansion and hatching status, 2. Inner cell mass (ICM) 3. Trophectoderm (TE). Embryo transfer was scheduled in day 5 at least 2 good quality embryo, Maternal serum samples were drawn at the time of embryo transfer, serum samples were centrifuged for 20 min, 1200×g at 10 °C. PTX3 serum levels were analyzed using the Quantikine® Elisa kit for Human Pentraxin 3/TSG-14, catalog no. DSST00, Lot 316507, R&D Systems, Inc., Minneapolis, MN, USA. After blood withdrawal, serum samples were centrifuged for 20 min, 1200×g at 10 °C. ELISA reagent (Wash Buffer, Substrate solution, Calibrator Diluent RD5–25 (diluted 1:2) human Pentraxin 3 standard) and microplate preparations were performed as described by the manufacturer.

Progesterone was administered intramuscularly at a dose of 100mg starting from oocyte pick up day & will be continued intramuscularly following the transfer for luteal phase support.The presence of pregnancy was assessed by B-HCG test (chemical pregnancy test) on the 14th day after the transfer

Statistical Methods

Statistical analysis was done using IBM© SPSS© Statistics version 24 (IBM© Corp. Armonk, NY) and MedCalc© version 20.218 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2023). P-Values <. 05 are considered statistically significant.

RESULTS

Table 1: Characteristics of the study population.

Variable	Metric
Age (years), median (IQR)	30.5 (27 – 36)
Weight (kg), median (IQR)	74.5 (60 – 88)
Height (cm), median (IQR)	162.5 (157 – 164)
BMI (kg/m2), median (IQR)	29.0 (25 – 34.0)
Duration of marriage (years), median (IQR)	7 (3 – 10)
Duration of infertility (years), median (IQR)	4.5 (2.0 – 7.0)
Gravidity, n (%)	
G0	16 (53.3%)
G1	9 (30.0%)
G2	4 (13.3%)
G3	1 (3.3%)
Parity, n (%)	
P0	25 (83.3%)

P1	4 (13.3%)
P2	1 (3.3%)
Previous miscarriages, n (%)	
Nil	20 (66.7%)
One miscarriage	9 (30.0%)
Three miscarriages	1 (3.3%)
Previous CS, n (%)	3 (10.0%)
Biochemical profile	
FSH (mIU/ml)), median (IQR)	6.4 (5.6 – 7.9)
LH (mIU/ml)), median (IQR)	6.9 (4.95 – 8.5)
TSH (mIU/ml)), median (IQR)	2.1 (1.5 – 2.8)
PRL (ng/ml)), median (IQR)	14.4 (10.0 – 20.0)
Hemoglobin (g/dl)), median (IQR)	12.1 (11.8 – 13.0)
E2 (pg/ml)), median (IQR)	41.0 (35.0 – 54.0)
RBS (mg/dl)), median (IQR)	93 (85 – 98)
AMH (ng/ml)), median (IQR)	2.65 (1.20 – 5.30)
Serum pentraxin 3 (ng/ml)), median (IQR)	3.75 (2.80 – 4.00)
ICSI details	
Stimulation days), median (IQR)	13 (11 – 17)
Number of HCG ampoules), median (IQR)	37 (22 – 45)
Number of retrieved oocytes), median (IQR)	11 (7 – 17)
Number of M2 oocytes), median (IQR)	7 (3 – 10)
Number of M1 oocytes), median (IQR)	1 (0 – 2)
Number of GV oocytes), median (IQR)	2 (0 – 2)
Number of abnormal oocytes), median (IQR)	1 (0 – 3)
Endometrial thickness (mm)), median (IQR)	10 (10 – 10)
Number of produced embryos), median (IQR)	4 (3 – 7)
Number of class A embryos), median (IQR)	2 (2 – 3)
Number of non-class A embryos), median (IQR)	2 (0 – 3)
Number of verified embryos), median (IQR)	0 (0 – 0)
Number of transferred embryos), median (IQR)	3 (2 – 3)
Biochemical pregnancy, n (%)	
Negative	23 (76.7%)
Positive	7 (23.3%)

IQR = interquartile range, n = number.

Table 2: Demographic characteristics and past obstetric history in patients with positive or negative pregnancy

Variable	Negative pregnancy test (N=23)	Positive pregnancy test (N=7)	p-Value*
Age (years), median (IQR)	30.0 (27 – 35.8)	35.0 (28.3 – 37.8)	.269
Weight (kg), median (IQR)	76.0 (64.8 – 92.8)	63.0 (54.3 – 84.5)	.281
Height (cm), median (IQR)	161.0 (157.8 – 164.0)	164 (157.3 – 167.8)	.225
BMI (kg/m2), median (IQR)	31.2 (25.6 – 37.5)	26.2 (20.9 – 29.4)	.073
Duration of marriage (years), median (IQR)	6.0 (3.0 – 9.8)	8.0 (4.8 – 9.8)	.491
Duration of infertility (years), median (IQR)	4.0 (2.0 – 7.0)	6.0 (3.0 – 7.5)	.640
Gravidity, n (%)			
G0	14 (60.9%)	2 (28.6%)	.027†
G1	7 (30.4%)	2 (28.6%)	
G2	2 (8.7%)	2 (28.6%)	
G3	0 (0.0%)	1 (14.3%)	
Parity, n (%)			
P0	19 (82.6%)	6 (85.7%)	.721
P1	3 (13.0%)	1 (14.3%)	
P2	1 (4.3%)	0 (0.0%)	
Previous miscarriages, n (%)			
Nil	17 (73.9%)	3 (42.9%)	.041
One miscarriage	6 (26.1%)	3 (42.9%)	
Three miscarriages	0 (0.0%)	1 (14.3%)	
Previous CS, n (%)	2 (8.7%)	1 (14.3%)	>.999‡

†. Linear by linear association unless otherwise indicated.
‡. Fisher's exact test.
n = num
*. Mann-Whitney test.
IQR = interquartile range.

This table shows that: median age of the study population is 30 years, no statistically significant difference (p value >0.05) between pregnant & non pregnant groups as regards demographic data. There is no statistically significant difference (p value >0.05) between pregnant & non pregnant groups as regards obstetric history.

Table 3: Results of biochemical assay.

Variable	Negative pregnancy test (N=23)	Positive pregnancy test (N=7)	p-Value†
FSH (mIU/ml), median (IQR)	6.2 (5.0 – 7.3)	7.9 (6.3 – 10.4)	.053
LH (mIU/ml), median (IQR)	7.0 (5.3 – 8.9)	6.0 (4.2 – 7.9)	.315
TSH (mIU/ml), median (IQR)	2.6 (1.6 – 2.8)	1.8 (1.4 – 2.5)	.303
PRL (ng/ml), median (IQR)	14.0 (10.1 – 18.1)	19 (10.5 – 29.2)	.364
Hemoglobin (g/dl), median (IQR)	12.1 (11.8 – 12.8)	12 (11.3 – 13.5)	.731

E2 (pg/ml), median (IQR)	46.0 (38.5 – 59.2)	35.0 (26.0 – 37.5)	.012
RBS (mg/dl), median (IQR)	94.0 (86.5 – 98.8)	87.0 (83.5 – 93.0)	.326
AMH (ng/ml), median (IQR)	3.60 (1.62 – 6.12)	1.00 (0.64 – 3.82)	.111
Serum pentraxin 3 (ng/ml), median (IQR)	3.50 (2.58 – 4.00)	3.80 (3.55 – 4.75)	.388

†. Mann-Whitney test.

IQR = interquartile range.

The table shows that there is No statistically significant difference (p value >0.05) between pregnant & non pregnant groups as regards biochemical assay

Table 4: ICSI details

Variable	Negative pregnancy test (N=23)	Positive pregnancy test (N=7)	p-Value†
Endometrial thickness (mm), median (IQR)	10 (10 – 10)	10 (9 – 10)	.255
Stimulation days, median (IQR)	13 (12 – 17)	13 (10 – 15)	.374
Number of HCG ampoules, median (IQR)	33 (22 – 45)	42 (33 – 46)	.390
Number of retrieved oocytes, median (IQR)	13 (7 – 18)	7 (3 – 11)	.069
Number of M2 oocytes, median (IQR)	8 (3 – 11)	4 (3 – 6)	.065
Number of M1 oocytes, median (IQR)	1 (0 – 2)	1 (0 – 2)	.959
Number of GV oocytes, median (IQR)	2 (0 – 2)	1 (0 – 2)	.372
Number of abnormal oocytes, median (IQR)	1 (0 – 4)	0 (0 – 2)	.158
Number of produced embryos, median (IQR)	4 (3 – 7)	4 (2 – 5)	.534
Number of class A embryos, median (IQR)	2 (2 – 3)	2 (2 – 3)	.652
Number of non-class A embryos, median (IQR)	2 (0 – 4)	1 (0 – 3)	.652
Number of verified embryos, median (IQR)	0 (0 – 0)	0 (0 – 2)	.505
Number of transferred embryos, median (IQR)	3 (2 – 3)	3 (2 – 3)	.380

†. Mann-Whitney test.

IQR = interquartile range.

The table shows that there is no statistically significant difference (p value >0.05) between pregnant & non pregnant groups as regards endometrial thickness, induction of ovulation, oocyte quality and embryo grading.

Table 5: Receiver-operating characteristic (ROC) curve analysis for prediction of biochemical pregnancy using E2 or serum pentraxin 3

	Predictor	
	E2	Pentraxin 3
Sample size	30	30
Positive biochemical pregnancy	7 (23.3%)	7 (23.3%)
Negative biochemical pregnancy	23 (76.7%)	23 (76.7%)
Prevalence (%)	23.3	23.3
AUC	.817	.609
SE	.079	.116
95% CI	.634 to. 933	.414 to. 781

z statistic	4.030	0.935
p-Value (AUC =. 5)†	.0001	.350
Youden index (J)	.61	.34
Associated criterion	≤41 pg/ml	>3.1 ng/ml
Sensitivity (95% CI), %	100 (59.0 - 100.0)	85.71 (42.1 - 99.6)
Specificity (95% CI), %	60.87 (38.5 - 80.3)	47.83 (26.8 - 69.4)
+LR (95% CI)	2.56 (1.54 - 4.25)	1.64 (1.00 - 2.69)
-LR (95% CI)	0 (95% CI could not be estimated)	0.3 (0.046 - 1.93)
+PV (95% CI), %	43.8 (31.8 - 56.4)	33.3 (23.4 - 45.1)
-PV(95% CI), %	100 (95% CI could not be estimated)	91.7 (63.0 - 98.6)
Comparison of ROCs		
ΔAUC	.208	
SE	.156	
95% CI	-.097 to. 513	
z statistic	1.335	
p-Value (ΔAUC = 0)†	.182	

95% CI = 95% confidence interval, AUC = area under the curve, ΔAUC = difference between AUCs, +LR = positive likelihood ratio, -LR = negative likelihood ratio, +PV = positive predictive value, -PV = negative predictive value, ROC = receiver-operating characteristic curve, SE = standard error.

†. DeLong method.

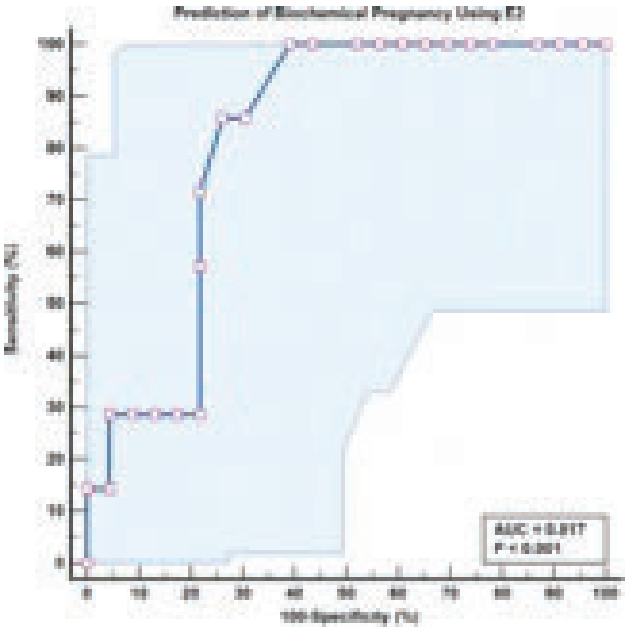

Fig. 1: Receiver-operating characteristic (ROC) curve for prediction of biochemical pregnancy using E2. Shaded area represents 95% confidence (95% CI) limits. E2 has good predicitve value (AUC =. 817, 95% CI =. 634 to. 933, p <.001). Best cutoff criterion is E2 ≤41.0 pg/ml (sensitivity [95% CI] = 100% [59.0% - 100.0%], specificity = [95% CI] = 60.9% [38.5% - 80.3%].

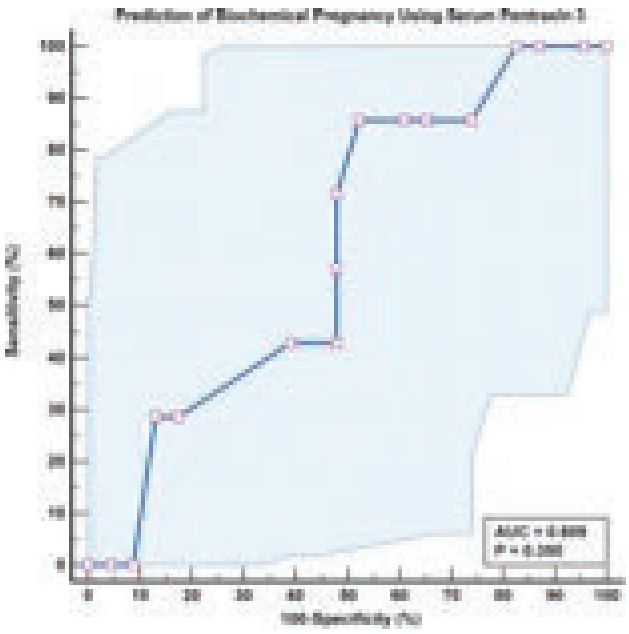

Fig. 2: Receiver-operating characteristic (ROC) curve for prediction of biochemical pregnancy using serum pentraxin 3. Shaded area represents 95% confidence (95% CI) limits. Serum pentraxin 3 has poor predicitve value (AUC =. 609, 95% CI =. 414 to. 781, p =. 350). Best cutoff criterion is serum pentraxin 3 > 3.1 ng/ml (sensitivity [95% CI] = 85.7% [42.1% to 99.6%], specificity = [95% CI] = 47.8% (26.8% to 69.4%).

Table 6: Correlations of serum pentraxin 3 with other variables

Variable	Serum pentraxin 3	
	Spearman's rho	**p-Value**
Age	-.324	.081
BMI	**-.376***	**.041**
Duration of marriage	-.236	.210
Duration of infertility	-.053	.781
Gravidity	-.175	.355
Parity	-.075	.693
Previous miscarriages	-.176	.353
FSH	.221	.240
LH	.139	.463
TSH	-.002	.990
PRL	-.113	.552
E2	-.066	.730
AMH	.184	.330
Hemoglobin	**.484****	**.007**
RBS	-.147	.439
Number of stimulation days	.319	.086
Number of HCG ampoules	.010	.960
Number of retrieved oocytes	-.103	.587
Number of M2 oocytes	-.096	.613
Number of M1 oocytes	.046	.808
Number of GV oocytes	.070	.715
Number of abnormal oocytes	-.111	.560
Endometrial thickness	-.246	.191
Number of produced embryos	-.026	.893
Number of class A embryos	.067	.724
Number of non-class A embryos	-.159	.400
Number of verified embryos	.224	.234
Number of transferred embryos	.256	.172

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

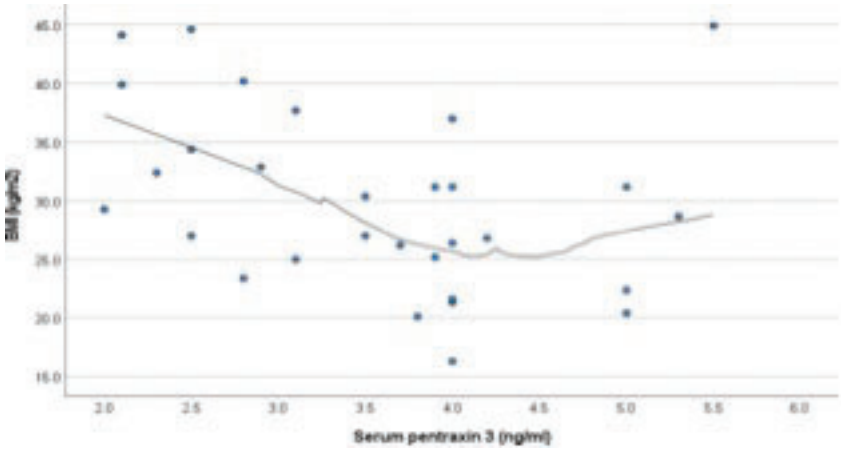

Fig. 3: Scatter plot illustrating correlation between serum pentraxin 3 and BMI. Fitted lines represent local regression smoothing trend lines. There is weak negative correlation between both variables (Spearman's rho = -.376, p =. 041).

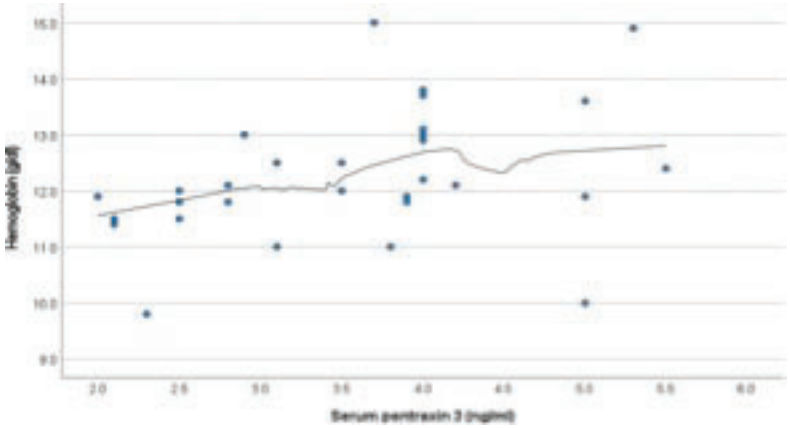

Fig. 4: Scatter plot illustrating correlation between serum pentraxin 3 and hemoglobin. Fitted lines represent local regression smoothing trend lines. There is moderate positive correlation between both variables (Spearman's rho =. 484, p =. 007).

Table 7: Correlations of E2 with other variables

Variable	E2	
	Spearman's rho	p-Value
Age	-.205	.276
BMI	.148	.436
Duration of marriage	-.059	.758
Duration of infertility	.035	.852
Gravidity	-.210	.266
Parity	.084	.658
Previous miscarriages	-.208	.270
Serum pentraxin 3	-.066	.730
FSH	-.190	.314
LH	.000	.999
TSH	.043	.820

PRL	-.102	.593
AMH	-.021	.912
Hemoglobin	-.326	.079
RBS	.053	.780
Number of stimulation days	-.071	.711
Number of HCG ampoules	-.116	.541
Number of retrieved oocytes	.171	.367
Number of M2 oocytes	.117	.539
Number of M1 oocytes	.052	.785
Number of GV oocytes	-.036	.851
Number of abnormal oocytes	.272	.146
Endometrial thickness	-.078	.681
Number of produced embryos	.043	.821
Number of class A embryos	.089	.642
Number of non-class A embryos	.009	.961
Number of verified embryos	-.051	.787
Number of transferred embryos	.085	.655

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

PTX3 is a member of the pentraxin protein family, which also includes inflammatory proteins like C-reactive protein (CRP) and serum amyloid P component (SAP) (6).

The mRNA expression of PTX3 in human endometrial stromal cells is also influenced by immunological factors as well as by progesterone, estrogen, and interleukin-1, but not by human chorionic gonadotropin (9).

The importance of PTX3 in infections and inflammatory diseases reveals a possible role of PTX3 as a biomarker for implantation, especially as the exact necessary balance of immunological reactions and vascular changes necessary for adequate implantation is still discussed controversially (11).

The current study showed that there were no statistically significant difference between pregnant & non-pregnant groups regarding demographic variables & obstetric history ($p > 0.05$).

Freis et al. (13) aimed to investigate if serum levels of PTX3 at the time of embryo transfer differ between women with an ongoing pregnancy compared to those without implantation. They did not observe any significant differences concerning age (mean± SD) with 35.69±4.39 years for women without implantation vs. 33.92±3.53 years in women with ongoing pregnancy, or BMI (mean± SD), respectively, with BMI of 23.03±3.28 vs. 25.03±4.58 kg/ m2.

Irez et al. (14) aimed to investigate the relationship of Pentraxin 3 level with embryo implantation in the cumulus culture fluid. They showed that no statistically significant difference (p value >0.05) between pregnant & non pregnant groups as regards demographic data.

The current study showed that there was no statistically significant difference between pregnant & non-pregnant groups as regards biochemical assay (FSH, LH, TSH, PRL, AMH,E2) ($p > 0.05$).

The present results were supported by studies of Hauzman et al. (15), Ozdemir et al. (16) & Brandenberger et al. (17) as they reported that no significant difference was observed regarding biochemical profile (FSH, LH, E2) ($p > 0.05$).

In the present study, there was no statistically significant difference between pregnant & non-pregnant groups as regard endometrial thickness ($p > 0.05$).

Ozdemir et al. (16) showed that endometrial thickness median values did not differ in terms of the pregnancy status. The median value was 9 for non-pregnant women and 9 for pregnant women.

On the other hand, Brandenberger et al. (17) showed that the endometrium thickness was higher in the pregnancy group ($P = 0.048$).

Zhao et al. (18) showed a significant difference in endometrial thickness on the day of HCG administration and the change in endometrial thickness from the third day of gonadotropin stimulation to the day of HCG administration between pregnant women and non-pregnant women ($p < 0.05$). This finding indicates that adequate endometrial development is favorable for improved pregnancy rate. They proposed that the endometrium of non-pregnant women may be associated with pathological abnormalities, such as lacking a normal proliferative response to the rising estradiol level. In a binary logistic regression model, the endometrial thickness on day 3 of gonadotropin stimulation, endometrial thickness on the day of HCG administration, and the change in endometrial thickness from the third day of gonadotropin stimulation to the day of HCG administration were independent predictive factors for pregnancy.

The present study reported that there was no statistically significant difference between pregnant & non-pregnant groups as regards days of ovarian stimulation nor the number of ampoules of gonadotropins ($p > 0.05$).

In agreement with the current study, Hauzman et al. (15) also showed that there was no statistically significant difference between pregnant & non-pregnant groups as regards days of stimulation or number of gonadotropins ampoules used in his study ($p > 0.05$).

Freis et al. (13) showed that no significant difference in progesterone levels was observed at the time of embryo transfer (135.74±78.73 ng/ml for women without implantation vs. 140.65±73.25 ng/ml, $p=0.87$ in women with ongoing pregnancy). Furthermore, estradiol levels at the time point of ovulation induction also did not differ significantly between the two groups (720.63±1297.95 vs. 649.95±712.17 pg/ ml, $p=0.19$).

The current study showed that there was no statistically significant difference between pregnant & non-pregnant groups as regards oocyte number or quality ($p > 0.05$).

Hauzman et al. (15), Tavmergen et al. (19), also reported that number of oocyte retrieved or oocyte quality didn't show any significant difference between pregnant & non-pregnant groups ($p > 0.05$).

The current study showed that there is No statistically significant difference (p value >0.05) between pregnant & non pregnant groups as regards embryo grading.

in the study of Hauzman et al. (15) showed that there was no statistically significant difference between pregnant & non-pregnant groups as regards number or quality of embryos ($P > 0.05$).

Our results showed that there was no statistically significant difference between pregnant & non-pregnant groups as regard S.Pentraxin 3 ($p > 0.05$).

In agreement with our study, Zhang et al. (20) showed that PTX3 levels in follicular fuid did not correlate with oocyte quality.

Irez et al. (14) showed that the rate of oocyte score 2 was significantly higher in pregnant compared to non-pregnant women.

By comparing mRNA expression of PTX3 in

cumulus cells of fertilized vs. unfertilized oocytes, Tranguch et al. (21) showed that PTX3 was found to be decreased in those who were not fertilized.

Our study showed that;. Serum pentraxin 3 has poor predicitve value Using ROC curve (AUC =. 609, 95% CI =. 414 to. 781, p =. 350). Best cutoff criterion is serum pentraxin 3 > 3.1 ng/ml (sensitivity [95% CI] = 85.7% [42.1% to 99.6%], specificity = [95% CI] = 47.8% (26.8% to 69.4%).

Using ROC curve for prediction of biochemical pregnancy using E2, E2 has good predicitve value (AUC =. 817, 95% CI =. 634 to. 933, p <.001). Best cutoff criterion is E2 ≤41.0 pg/ml (sensitivity [95% CI] = 100% [59.0% - 100.0%], specificity = [95% CI] = 60.9% [38.5% - 80.3%].

Our study showed that there is weak negative correlation between serum pentraxin 3 and BMI. (Spearman's rho = -.376, p =. 041).

In order to establish an optimum threshold, Freis et al. (13) conducted a study examining various PTX3 concentrations and their respective levels of specificity and sensitivity. Using a PTX3-concentration of 0.923 ng/ml as a potential threshold, it was found that 2 out of the 25 patients (8.00%) who had a successful embryo transfer in the study population would not have been able to have their embryo transfer in the fresh IVF/ICSI cycle. Additionally, it was determined that they could have prevented unsuccessful embryo transfer in 9 out of the 26 patients (34.62%) who did not have implantation.

Irez et al. (14) demonstrated that the levels of PTX3 in the culture media of cumulus cells were considerably elevated in the pregnant group compared to the non-pregnant group (98.9 ng/mL vs 53.2 ng/mL, respectively, p=0.005, Mann Whitney-U test). There were no notable disparities in patient ages, BMI, Gonadotrophin doses, days of follicle induction, the total number of oocytes, and the number of MI and GV oocytes between the two groups.

In 2013, Huang et al. (22) conducted a study to assess the correlation between the cumulus gene expression of seven specific genes, such as glutathione peroxidase 3 and PTX3, and the process of fertilization and embryo development in women diagnosed with polycystic ovaries. The researchers have demonstrated a correlation between reduced PTX3 expression and an elevated rate of multinucleation, as well as an aberrant rate of fertilization.

Saito et al. (23) demonstrated that there is a correlation between recurrent implantation failure and an elevation in Th1-dependent cytokines, specifically tumor necrosis factor alpha (TNF-alpha). According to Zhang et al. (20) and Han et al., TNF-alpha appears to promote the expression of PTX3. This could explain the elevated levels of PTX3 in the blood observed in our investigation. These findings suggest a connection between high levels of PTX3 and failure embryo transfer, possibly due to immunological alterations.

Qn the other hand, Irez et al. (14) shown through multivariate regression analysis that PTX3 had a significant association with pregnancy (ß coefficient = 0.385, p=0.009). The concentration of PTX3 had a substantial predictive capacity for pregnancy, as demonstrated by the receiver operating characteristic (ROC) analysis. The sensitivity and specificity of a level of 64.25 ng/mL were 86% and 80% respectively. The concentration of PTX3 was substantially greater in the grade 1 embryo group as compared to the grade 2 and grade 3 embryo groups.

CONCLUSION

The measurement of PTX3 on day of embryo transfer is not predictive of subsequent pregnancy as there was no statistically significant difference between pregnant & non pregnant groups as regard PTX3 and there is only weak negative correlation between serum pentraxin 3 and BMI. Therefore, determination of PTX3 doesn't appear to be useful in predicting the outcome of ICSI cycles.

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Vaginal versus sublingual misoprostol administration before intrauterine device insertion in women with previous failed trial

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Abstract

Background: Intrauterine device (IUD) is one of the most efficient contraceptive techniques, despite its low use rate due to the user's fear of discomfort and the provider's insertion issues. Misoprostol is a drug that softens and facilitates dilatation of the cervix.

Objectives: The aim of the current work was to compare the efficacy of using misoprostol (Vaginally versus sublingually) before IUD insertion in cases of previously failed trial.

Patients and methods: A prospective interventional comparative study included 80 women who had history of failed previous IUD insertion, attending at Outpatient Clinics, Menoufia university and El Hawamdia primary health care, from April 2022 till August 2023 .There were randomly divided into Group A which included 40 women who used misoprostol vaginally, 2-4 hours before IUD insertion and group B included 40 women who used misoprostol sublingually 2-4 hours before IUD insertion. Success of IUD insertion, participant pain and satisfaction were compared between the two groups.

Results: There was statistically significant difference between vaginal misoprostol group and sublingual group regarding IUD insertion with a P value of 0.034. There was also highly significant difference in the occurrence of pain with a P value of 0.001 according to visual analog scale and numeric rating scale.

Conclusion: usage of Vaginal misoprostol (400 μg) 2-4 h before IUD insertion increase the rate of IUD and reduces the incidence of pain during the procedure.

Keywords: intrauterine device, sublingual misoprostol, vaginal misoprostol, cervical ripening.

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INTRODUCTION

Intrauterine devices (IUD) are an effective and increasingly popular form of reversible contraception [1].

The IUD is the most used method of contraception in Egypt since 2000, 66.4% of currently married women aged 15-49 are using a contraceptive method, and about third of them according to Egypt Family Health Survey 2021 (EFHS) rely on IUD. However, the proportion using the IUD has dropped to 29% in 2021 compared with 30% in 2014, and 36% in 2008 according to EFHS-2021[2].

The low percentage of IUD use is mainly due to the user's fear of discomfort and the provider's difficulties during insertion [3].

Up to 14% and 20% of parous and nulliparous women, respectively, have failure of insertion [4].

Healthcare providers may be hesitant to place an IUD in women who have had a cesarean section previously and didn't have a vaginal delivery because this method appears to be risky and has the potential for complications like failure of insertion, discomfort during insertion, and perforation [5].

The insertion of a speculum, tenaculum traction on the cervix, uterine sounding, inserting the insertion tube through the cervix, and placing the device inside the uterine cavity are all associated with insertion-associated pain [6].

Misoprostol is a prostaglandin E1 analogue that is widely used in labor induction as a cervical ripening agent. It is also used to ripen the cervical tissue prior to transcervical procedures such as hysteroscopy, dilation and curettage, and dilation and evacuation. Misoprostol has also been recommended as a medication to lessen the pain associated with IUD implantation [7].

The effectiveness of misoprostol in aiding IUD insertion and reducing pain is debatable among practitioners, and there is variability in its use before IUD placement. The published research show significant variations in the misoprostol dosage, administration method, and timing of administration prior to operation. Furthermore, misoprostol was administered by a variety of methods in several investigations (sublingual, oral, rectal, and vaginal) [8].

Aim

The aim of this study was to compare the efficacy of using misoprostol (vaginally versus sublingually) before IUD insertion in cases of previously failed trial.

PATIENTS AND METHODS

This prospective comparative study performed from April 2022 to August 2023.

Ethical Consideration:

The registration and approval of the Ethical Committee of the Faculty of Medicine, Menoufia University of the trial ,recorded no 12/2021 OBSGN37.

The study population was divided into two groups:

Group A: Included 40 women who received 400 µg misoprostol vaginally (Misotac®, Sigma Pharmaceutical Industries, Egypt) each tablet contain misoprostol 200 µg).

Group B: Included 40 women who received 400 µg misoprostol sublingually.

Participants Inclusion criteria were Women aged between 18 and 45 years with a history of previous failed trial of IUD insertion. Exclusion criteria were women who had contraindications for either misoprostol or IUD use.

The participants' data of both groups were gathered and recorded included the women's age, parity, body mass index (BMI), axis of the uterus, number of C.S and regularity of cycles.

All women had US examination before insertion using DP 10 from Mindray,CHINA, to determine the uterine axis and any intra cavity pathology.

In both groups, 2-4hrs after receiving misoprostol participants return for the insertion of

IUD. Pain and bleeding during insertion was recorded; patients instructed to return after six weeks for follow up.

Participants who had failed IUD insertion in the first attempt were also instructed to return on the next menstrual cycle to perform a second attempt in the same pattern as the first attempt.

After the IUCD was inserted, each participant had a standard check-up six weeks later. Vaginal US and vaginal examination were done at this visit. Records of IUCD infections and expulsions were made.

The collected data were organized, tabulated, and analyzed using appropriate statistical tests.

Statistical consideration:

- Sample size: based on previous study of Rasheedy et al., [9]. sample size calculation rendered 80 participants for prospective comparative study.
- **Statistical analysis**

Data were fed to the computer and analyzed using IBM SPSS software package version 26 (IBM Corp., Armonk, New York, USA). Significant test results were quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level ($P > 0.05$). If P value is less than 0.05, the result will be significant. If P value is more than 0.05, the result will be nonsignificant. Qualitative data were presented as Number (N), percentage (%) and were compared using Chi-square test (Fisher's exact test). When comparing quantitative data, the Student t-test was used for normally distributed variables and the Mann Whitney test was used for non-normally distributed variables. The quantitative data were given as mean ($\overline{x}$), standard deviation (SD), and range (minimum-maximum).

RESULTS

Each participant who enrolled up for the study were had a history of previous CS, There were no significant differences between the studied groups regarding age (p=0.733), BMI (P=0.771), Parity (p=0.361), number of cesarean section (p=0.121) or uterine axis (p=1.000). (Table 1).

Table (1): Demographic data and uterine axis among the studied groups

Variable	Vaginal group (A) (N=40)	Sublingual group (B) (N=40)	Tests of significance	P- Value
Age(years) Mean ±SD	30.3±6.7	30.8±6.3	t= 0.34	0.733
BMI Mean ±SD	28.9 ±3.5	28.7 ±4.0	t= 0.29	0.771
Parity: Para 1 > 2	11 29	7 33	χ^2 =2.04	.361
Previous CS: 1 > 2	13 27	7 33	χ^2 =2.40	0.121
Uterine axis : AVV	**N (%)**	**N (%)**	FE =0.21	1.000
	37 (92.5%)	38 (95%)		
RVV	3 (7.5%)	2 (5%		

CS: caesarean section, **AVF:** Anteverted anteflexed uterus, **RVF:** Retroverted flexed uterus, **SD:** Standard deviation, **χ^2:** Chi square test, **t**=Student t test, **FE**; Fisher's exact test

There was statistically significant difference, as regards success of insertion between studied groups .In the 1st attempt, group A showed a significant higher success rate (n=37, 92.5%) than group B (n=30, 66.5%), with P<0.001. While the outcomes of the second attempt at IUD insertion (which occurred during the next cycle) and the reasons for failure did not significantly differ among the two groups (P=1.000). (Table 2, Fig 1)

Table (2): Comparison between studied groups regarding the Success rate of intrauterine device insertion.

			Vaginal group (A) (N=40)	Sublingual group (B) (N=40)	Chi-square test (χ^2)	P value
1st insertion attempt:	Success	NO.	37	30	4.50	0.034*
		%	92.5	75.0		
	Failed	NO.	3	10		
		%	7.5	25.0		
2nd insertion attempt	Success	NO.	2	7	FE 0.01	1.000
		%	66.7	70		
	Failed	NO.	1	3		
		%	33.3	30.0		
Causes of failure:	Inaccessible cervix	NO.	1	1	FE 1.33	0.248
		%	100	33.3		
	Cervical Stenosis	NO.	-	2		
		%	-	66.7		

FE; Fisher's exact test, *Significant

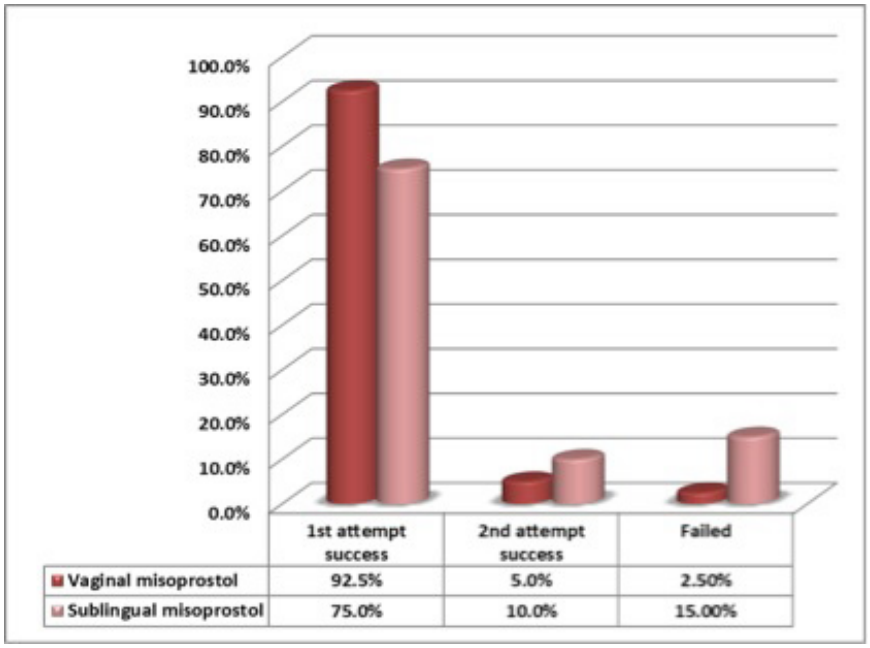

Figure (1): comparison between two studied groups according to success rate from first or second attempt.

Regarding Insertion complications, there was no significant difference among the studied groups regarding Perforation, heavy bleeding and vasovagal like reaction (p=1.000). However, Pain during insertion was significantly higher in group B (n=26, 65%) than group A (n=5, 12.5%) with (p<0, 001). (Table 3)

Table (3): Comparison between the studied groups regarding complications during IUD insertion

			Vaginal group (A) (N=40)	Sublingual group(B) (N=40)	Tests of significance	P value
Vasovagal like reaction:	yes	NO	39	38	Fisher's exact test (FE) =4.50	1.000
		%	97.5	95.0		
	no	NO	1	2		
		%	2.5	5.0		
Perforation:	yes	NO	-	-	-	
		%	-	-		
	no	NO	40	40		
		%	100	100		
Heavy bleeding during insertion:	yes	NO	-	-	-	-
		%	-	-		
	no	NO	40	40		
		%	100	100		
Pain during insertion:	yes	NO	5	26	Chi square test =26.00	<0.001*
		%	12.5	65.0		
	no	NO	35	14		
		%	87.5	35.0		

Regarding VAS during insertion and patient self-reporting of pain by numeric rating scale (NRS), there was a significantly different outcome in the severity of pain between the two groups. (Table 4, Fig 2)

Regarding participant satisfaction, there was a statistically significant difference (P <0.05) between the groups under study.

Table (4): Comparison between studied groups regarding pain assessment and Participant satisfaction:

	Vaginal misoprostol (n=40)	Sublingual misoprostol (n=40)	Mann-whitney test (U)	P-value
Pain during insertion according to the health provider using visual analog scale (VAS): Mean ± SD Range	0.6±1.8 0-8	1.5±1.8 0-8	4.19	<0.001**
patient self-reporting of pain by numeric rating scale (NRS): Mean ± SD Range	0.6±1.9 0-8	2.0 ±2.1 1-10	6.39	<0.001**

Regarding 6 weeks follow-up results after IUD insertion there was no significant difference among the studied groups regarding 1st menstruation after insertion, post insertion US or infection (P >0.05).

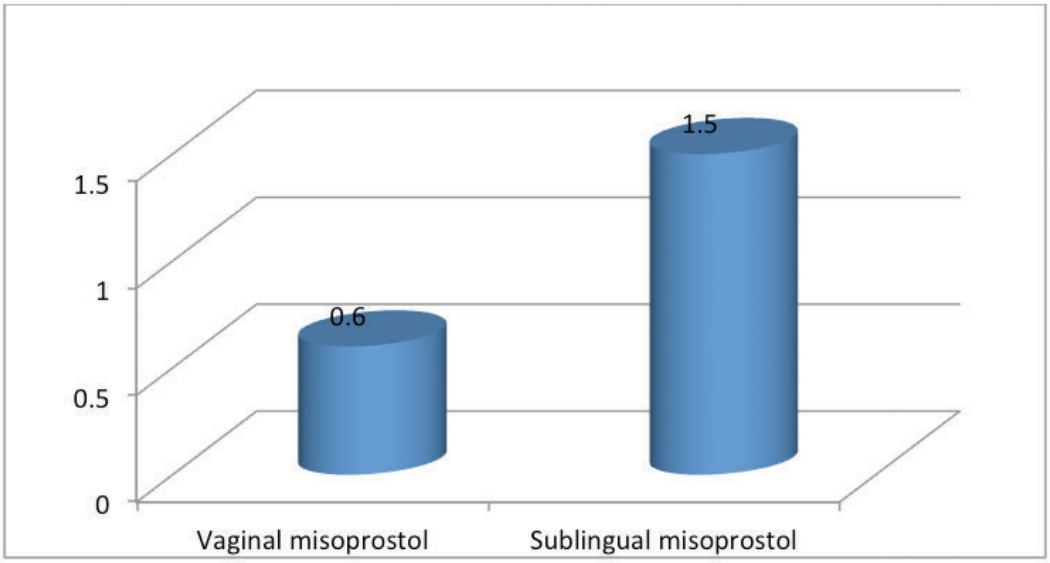

Figure2: means of VAS in studied groups

DISSCUSSION

The objective of misoprostol administration prior to IUD placement was to prime the internal cervical os to ease insertion or reduce the incidence of insertion failure and decrease the pain related to the operation. Lobez et al., [7]

This study compared the effectiveness of taking misoprostol sublingually versus vaginally before inserting an IUD in cases with a previous failed trial.

In the current study, the success rate in the first attempt was 92.5% in vaginally administrated group and 75% in sublingual group; with P value=0.034(<0.05), this means that there was a significant difference.

This contract with Emara et al., [10] who found in his comparative study including 249 women , that misoprostol is best administered vaginally as the success rate for the first trial was (96.4%) vaginally, (94%) rectally and (79.5%) sublingually as it has a higher chance of causing cervical ripening.

In contrast, Mohammed et al., [11] reported that using 400 µg of misoprostol sublingually or vaginally prior to IUD implantation associated with successful insertion on the first attempt (94% for vaginally administrated group) and (97% in sublingual group). There were no statistically significant differences between the two groups in terms of both the reasons for the success rate as well as the failure rate from the first and second tries (which occurred during the next cycle).

In the present study there were no significant difference between studied groups regarding results of 2nd attempt of IUD insertion (which was during next cycle) and causes of failure (P >0.05). These results come in accordance with Mohammed et al., [11] and Emara et al., [10].

The results agreed with Bahamondes et al., [12] who discovered that misoprostol was valuable in easing the placement of an IUD; approximately 4% of IUD insertions failed on the first try; one study that applied a similar tactic to their report was a case series with eight women whose cervical stenosis caused an IUD insertion to fail on the first try. 24 hours prior to the second attempt of inser-

tion, the authors gave 400 µg of misoprostol vaginally, and they reported that every insertion was successful. Li et al., [13]

According to age, BMI, parity, number of C.S. and axis of the uterus, there was no statistically significant difference between studied groups. Our findings concurred with a study by Mohammed et al. [11], which found no statistically significant variations between the two groups' baseline characteristics.

The current study revealed that pain during insertion was significantly increased in the sublingual group (65%) than vaginal group (12.5%) with p value <0.001.

We used two different scales for pain assessment comparing between the studied groups, patient self-reporting of pain by numeric rating scale (NRS) which was in Vaginal misoprostol [Mean ± SD (0.6±1.9)]Vs Sublingual misoprostol group [Mean ± SD (2.0 ±2.1) with p value <0.001] and visual analog scale (VAS) recorded by the health provider which was in Vaginal misoprostol group[Mean ± SD (0.6±1.8)] Vs Sublingual misoprostol group [Mean ± SD (1.5 ±1.8) with p value<0.001],thus there was highly significant difference in pain between vaginal and Sublingual misoprostol groups .

By asking participants about their satisfaction during the entire experience, 92.5% of women in the vaginal group were very satisfied in comparison to 70.3 % in the sublingual group; therefore the difference between the two groups was statistically significant.

This is in line with the results of Mohammed et al. [11], who observed that giving misoprostol prior to IUD insertion was substantially linked to nearly three times less pain during the insertion in the vaginal group (0.18%) than in the sublingual group (0.38%).

Unfortunately there were other studies didn't agree with our study results as they concluded that misoprostol does not help in decreasing pain before IUD insertion, for example:

Lathrop et al. [14] found that using misoprostol to induce cervix ripening before inserting an IUD does not make the procedure easier or reduce pain; on the contrary, it appears to be linked to an increase in pain reports.

Dijkhuizen et al. [15] found that using self-administered misoprostol to ripen the cervical tissue before inserting an IUD does not make the procedure easier for the healthcare practitioner or lessen the patient's reported pain.

There is no difference in statistical significance between the two groups as regard the complications that occurred after IUD insertion (such as perforation, heavy bleeding, insertion difficulty and vasovagal-like reaction).

Similar results were found by Emara et al., [10] who found that there is no statistically significant difference between both groups.

After six weeks of IUD placement, both groups' follow-ups showed no statistically significant difference regarding the results of US examination or infection incidence between the vaginal misoprostol and sublingual misoprostol groups.

These results were in agreement with the study done by Mohammed et al. [11] and Emara et al., [10].

By asking participants about their first menstruation after IUD insertion, there was no statistically significant difference between both groups regarding 1st menstruation after insertion. But this result was in disagreement with the study done by Emara et al., [10] who found that there was statistically significant difference between the studied groups regarding first menstruation after misoprostol insertion with more bleeding in sublingual group (9.6%) followed by vaginal group (8.4%) and then by rectal group (6%), and study done by Mohammed et al., [11] who found that there was statistically significant difference between both groups regarding amount of menstrual bleeding after IUD insertion (first menstruation).

CONCLUSION

For IUD insertion in women with previous insertion failure we concluded that misoprostol is best administered vaginally rather than sublingually since it has a higher chance of causing cervical ripening and reducing the level of pain experienced throughout the process. Repeated attempts of misoprostol in the next cycle may be beneficial when a previous insertion attempt has failed.

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Short Cervical Length and Marginal Venous Sinus as Predictors for Antepartum Hemorrhage in Cases of Placenta Previa

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Abstract

Background: Obstetric hemorrhage remains as one of the major causes of maternal death in developing countries and is the cause of up to 50% of estimated 500000 maternal deaths that accur. The incidence of placenta previa in term is 1 in 200. Antepartum hemorrhage [APH] is defined as bleeding from or in the genital tract occurring from 24 week of pregnancy and prior to the birth of the baby. APH complicates 3 – 5% of pregnancies and is the leading cause of perinatal and maternal mortality. The most important causes of APH are placenta praevia and placenta abruption.

Aim of the Work: This study aimed to assess the validity of short cervical length and venous sinus at the margin of the placenta as 3rd trimesteric ultrasound findings for prediction of hemorrhage of placenta previa by TVS and color Doppler.

Patients and Methods: This cross sectional study was conducted on 60 pregnant women attending Ain Shams

University Maternity Hospital with gestational age ≥ 28 weeks, singleton viable pregnancy and diagnosed placenta previa. The term placenta praevia should be used when the placenta lies directly over the internal os. For pregnancies at more than 16 weeks of gestation the term low-lying placenta should be used when the placental edge is less than 20 mm from the internal os on transabdominal or transvaginal scanning (TVS).

Results: Cases with antepartum hemorrhage non-significantly had lower cervical length and more frequent venous sinus. There was significant negative correlation between antepartum hemorrhage attacks and cervical length. Cervical length had significant low diagnostic performance in predicting antepartum hemorrhage, but had significant moderate diagnostic performance in predicting two or more antepartum hemorrhage attacks. Cervical length ≤39.0 mm had moderate diagnostic characteristics in predicting antepartum hemorrhage, while venous sinus had low characteristics. Cervical length ≤39.0 mm and venous

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sinus had high sensitivity and negative predictive value, but low other characteristics. Overall diagnostic characteristics of cervical length ≤39.0 was higher than that of venous sinus. Previous cesarean sections were significantly higher in cases that underwent hysterectomy.

Conclusion: There is sufficient evidence to show that shortened cervical length is a strong indicator for APH and that ultrasound measurements of the maternal cervix will become the preferred method due to reliability and objectivity.

Keywords: Short Cervical Length, Marginal Venous Sinus, Antepartum Hemorrhage, of Placenta Previa.

INTRODUCTION

Obstetric hemorrhage remains as one of the major causes of maternal death in developing countries and is the cause of up to 50% of estimated 500000 maternal deaths that accur. The incidence of placenta previa in term is 1 in 200 [(1)].

Antepartum hemorrhage [APH] is defined as bleeding from or in the genital tract occurring from 24 week of pregnancy and prior to the birth of the baby. APH complicates 3 – 5% of pregnancies and is the leading cause of perinatal and maternal mortality. The most important causes of APH are placenta praevia and placenta abruption [(1)].

Placenta previa is the complete or partial covering of the internal os of the cervix with the placenta. It is a major risk factor for antepartum hemorrhage and postpartum hemorrhage and can lead to maternal morbidity and mortality worldwide [(2)].

APH has a heterogeneous pathophysiology and cannot reliably be predicted. The rates of placenta praevia and accreta have increased and will continue to do so as result of rising rates of caesarean deliveries, increased maternal age and use of assisted reproductive technology [ART] placing greater demands on maternity -related resources. The highest rates of complication for both mother and newborn are observed when these conditions are only diagnosed at delivery [(1)].

The literature contains numerous reports addressing antenatal sonographic prediction of placental adherence, only one of which analyzed predictors for massive bleeding at Cesarean section [(1)].

The presence of lacunae in the placenta, sponge-like findings in the cervix and a lack of a clear zone were also associated with massive bleeding during the Cesarean operation. Adherence of the placenta diagnosed at Cesarean was also associated with massive bleeding, and had the highest odds ratio among these variables [(3)].

The hypothesis of this study is that short cervical length and marginal venous sinus in pregnant women with placenta previa might offer advantages in predicting women at risk of APH.

AIM OF THE WORK

This study aims to assess the validity of short cervical length and venous sinus at the margin of the placenta as 3rd trimesteric ultrasound findings for prediction of hemorrhage of placenta previa by TVS and color Doppler.

Research hypothesis: Short uterine cervical length in the third trimester and venous sinus at the margin of the placenta may predict an increased risk of antepartum hemorrhage in pregnant women demonstrating placenta previa.

Research question: Do 3rd trimester short uterine cervical length and venous sinus at the margin of the placenta predict an increased risk of antenatal bleeding in pregnant women demonstrating placenta previa?

PATIENTS AND METHODS

This case-control study was conducted at Obstetrics and Gynecology Department,

Faculty of Medicine, Ain shams University Hospitals from April 2023 till July 2024. A total of 60 women were enrolled.

Study population:

Pregnant women attended Fetal Medicine Unit and antenatal care clinic with the following criteria:

Inclusion criteria:

1. Gestational age ≥ 28 weeks.
2. Singleton viable pregnancy.
3. Diagnosed placenta previa: The term placenta praevia should be used when the placenta lies directly over the internal os. For pregnancies at more than 16 weeks of gestation the term low-lying placenta should be used when the placental edge is less than 20 mm from the internal os on transabdominal or transvaginal scanning (TVS) [(2)].

Exclusion criteria:

1. Multifetal pregnancy.
2. Known causes of bleeding tendency as severe anemia, coagulopathies, anticoagulants, etc.
3. Known fetal abnormalities.
4. Presence of cervical cerclage.
5. Polyhydramnios.

Study interventions and procedures:

After approval of study protocol, women were enrolled into the study according to inclusion and exclusion criteria. The demographic, maternal characteristics were extracted during their antenatal health care visit. According to inclusion and exclusion criteria; patients were subjected to:

Complete history taking of clinical importance including: Personal history: age, residence, occupation, marital status and special habits as smoking, alcohol, etc. **Menstrual history:** day of last menstrual period and regularity. Obstetric history: gravidity, parity, previous miscarriages or obstetric complications. Contraceptive history: type, duration of use before pregnancy. Medical history: medical comorbidities with pregnancy as hepatic, renal, endocrinal, psychosocial condition, cardiovascular, diabetes, chronic hypertension. Surgical history: Previous cesarean sections and its neonatal outcomes. Family history of maternal or fetal complications with pregnancy.

General examination with special emphasis on: Obstetric abdominal examination "Leopold maneuvers". The Leopold maneuvers are used to palpate the gravid uterus systematically. It is used to determine the position, presentation, and engagement of the fetus in utero.

Investigation: Routine investigations as complete blood picture, liver and kidney function tests, coagulation profile "prothrombin time (PT), partial thromboplastin time (PTT) and international normalized ratio (INR)", viral hepatitis markers: hepatitis B and C viruses, bBlood group (ABO) and Rh.

Antenatal ultrasound examination, which included ultrasound measurements of classical fetal biometric parameters that included biparietal diameter **(BPD)**, head circumference **(HC)**, abdominal circumference **(AC)** and femur length **(FL)** that were taken using MindrayDP-15 Digital Ultrasonic Diagnostic Imaging System and GE Logiq E9 ultrasound machine, 2–5 MHz wide band convex, curved array transducer.

All ultrasound examinations were done by an expert and professional medical personnel to ensure the accuracy of examination results. Ultrasound was done using US H60 machine with abdominal probe. Obstetric US was performed to measure fetal biomatrices, amniotic fluid index (AFI), site of placenta and diagnosis of placenta previa using GTG [(2)], then using TV probe frequency 5-7.5 MHz to detect the cervical length and the marginal sinus. Marginal sinus will be confirmed by color Doppler.

Transvaginal ultrasound was performed with

the patient in the lithotomy position with empty bladder. The cervical canal was measured from the external cervical os to the internal cervical os and placenta was clearly visualized. The placental edge near the internal cervical os was determined as the outer edge of the placenta parenchyma or marginal sinus with color Doppler. Any attack of bleeding was recorded in details (gestational age, duration, amount of bleeding). CBC was done after any attack of bleeding and immediately before delivery.

Statistical methods

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 28.0, IBM Corp., Chicago, USA, 2021. Quantitative data tested for normality using Kolmogorov-Smirnov test, then if normally distributed described as mean±SD (standard deviation) as well as minimum and maximum of the range, and then compared using independent t-test, while if not normally distributed described as Median (1st−3rd Interquartiles) as well as minimum and maximum and then compared using Mann Whitney test, while Spearman test was used for correlations. Qualitative data described as number and percentage and compared using Chi square test as well as Fisher's Exact test. ROC curve was used to evaluate the performance of cervical length in predicting antepartum hemorrhage. The level of significance was taken at p-value ≤0.050 was significant, otherwise was non-significant.

RESULTS

Table 1: Comparison according to antepartum hemorrhage regarding demographic characteristics.

Characteristics	Antepartum hemorrhage		p-value
	Present (Total=34)	Absent (Total=26)	
Age (years)	32.3±5.8	31.2±5.3	^0.456
Baseline gestational age (week)	32.7±2.5	33.6±2.5	^0.152
Gravidity	4.5 (3.0–6.0)	5.0 (2.8–5.0)	⌂0.354
Parity	3.0 (2.0–4.0)	3.0 (1.0–3.3)	⌂0.344
Previous cesarean section	2.0 (1.8–3.0)	2.5 (1.0–3.0)	⌂0.976

^Independent t-test. ⌂Mann−Whitney test.

Table (1) showed that: No significant difference according to antepartum hemorrhage regarding demographic characteristics.

Table 2: Comparison according to antepartum hemorrhage regarding baseline ultrasound findings

Characteristics		Antepartum hemorrhage		p-value
		Present (Total=34)	Absent (Total=26)	
Cervical length (mm)		38.5±5.9	41.8±6.8	^0.051
Type of placenta	**Anterior**	14 (41.2%)	13 (50.0%)	§0.595
	Posterior	14 (41.2%)	11 (42.3%)	
	Central	6 (17.6%)	2 (7.7%)	
Presence of venous sinus		26 (76.5%)	16 (61.5%)	#0.211
Intrauterine growth restriction		3 (8.8%)	3 (11.5%)	§0.999

^Independent t-test. NA: Not applicable.

Table (2) showed that: Cases with antepartum hemorrhage non-significantly had lower cervical length and more frequent venous sinus.

Table 3: Comparison according to antepartum hemorrhage regarding fetal findings

Characteristics	Antepartum hemorrhage		p-value
	Present (Total=34)	Absent (Total=26)	
Fetal weight (kg)	2.6±0.4	2.8±0.4	^0.087
Intrauterine fetal death	0 (0.0%)	0 (0.0%)	NA

^Independent t-test. NA: Not applicable.

Table (3) showed that: No significant difference according to antepartum hemorrhage regarding fetal findings.

Table 4: Correlations of antepartum hemorrhage attacks among the studied cases

Variables	Antepartum hemorrhage attacks	
	r	p-value
Age (years)	0.114	0.386
Gravidity	0.185	0.156
Parity	0.181	0.165
Previous cesarean section	-0.015	0.908
Cervical length (mm)	-0.374	**0.003***
Neonatal weight (kg)	-0.155	0.236

Spearman correlation test. *Significant.

Table (4) showed that: There was significant negative correlation between antepartum hemorrhage attacks and cervical length.

Table 5: Diagnostic performance of cervical length in predicting antepartum hemorrhage

Attacks	AUC	SE	p-value	95% CI	Cut point
One or more	0.676	0.071	**0.020***	0.536−0.816	≤39.0 mm
Two or more	0.754	0.065	**0.009***	0.627−0.882	≤39.0 mm

AUC: Area under curve. SE: Standard error. CI: Confidence interval, *significant

Table (5) showed that: Cervical length had significant low diagnostic performance in predicting antepartum hemorrhage, but had significant moderate diagnostic performance in predicting two or more antepartum hemorrhage attacks.

Table 6: Diagnostic characteristics of cervical length and venous sinus in predicting antepartum hemorrhage

Characters	Cervical length ≤39.0 mm		Venous sinus	
	Value	95% CI	Value	95% CI
Sensitivity	67.6%	49.5%–82.6%	76.5%	58.8%–89.3%
Specificity	69.2%	48.2%–85.7%	38.5%	20.2%–59.4%
Diagnostic accuracy	68.3%	55.0%–79.7%	60.0%	46.5%–72.4%
Youden's index	36.9%	13.2%–60.6%	14.9%	-8.6%–38.4%
Positive predictive value	74.2%	55.4%–88.1%	61.9%	45.6%–76.4%
Negative predictive value	67.6%	49.5%–82.6%	55.6%	30.8%–78.5%

CI: Confidence interval

Table (6) showed that: Cervical length ≤39.0 mm had moderate diagnostic characteristics in predicting antepartum hemorrhage, while venous sinus had low characteristics.

Table 7: Diagnostic characteristics of cervical length and venous sinus in predicting two or more antepartum hemorrhage attacks

Characters	Cervical length ≤39.0 mm		Venous sinus	
	Value	95% CI	Value	95% CI
Sensitivity	90.9%	58.7%–99.8%	90.9%	58.7%–99.8%
Specificity	57.1%	42.2%–71.2%	34.7%	21.7%–49.6%
Diagnostic accuracy	63.3%	49.9%–75.4%	45.0%	32.1%–58.4%
Youden's index	48.1%	26.1%–70.0%	25.6%	4.0%–47.2%
Positive predictive value	32.3%	16.7%–51.4%	23.8%	12.1%–39.5%
Negative predictive value	96.6%	82.2%–99.9%	94.4%	72.7%–99.9%

CI: Confidence interval

Table (7) showed that: Cervical length ≤39.0 mm and venous sinus had high sensitivity and negative

predictive value, but low other characteristics. Overall diagnostic characteristics of cervical length ≤39.0 was higher than that of venous sinus.

Table 8: Comparison according to hysterectomy regarding demographic characteristics

Characteristics	Hysterectomy		p-value
	Present (Total=7)	Absent (Total=53)	
Age (years)	31.3±4.7	31.9±5.7	^0.778
Baseline gestational age (week)	34.1±2.3	32.9±2.5	^0.237
Gravidity	5.0 (5.0–6.0)	4.0 (3.0–5.0)	⌂0.246
Parity	4.0 (2.0–5.0)	3.0 (2.0–3.0)	⌂0.108
Previous cesarean section	4.0 (2.0–5.0)	2.0 (1.0–3.0)	⌂**0.016***

^Independent t-test. ⌂Mann−Whitney test.

Table (8) showed that: Previous cesarean sections were significantly higher in cases that underwent hysterectomy.

Table 9: Comparison according to hysterectomy regarding baseline ultrasound findings

Characteristics		Hysterectomy		p-value
		Present (Total=7)	Absent(Total=53)	
Cervical length (mm)		36.5±5.6	0.4±6.5	^0.131
Type of placenta	**Anterior**	5 (71.4%)	22 (41.5%)	§0.226
	Posterior	1 (14.3%)	24 (45.3%)	
	Central	1 (14.3%)	7 (13.2%)	
Presence of venous sinus		6 (85.7%)	36 (67.9%)	#0.663
Intrauterine growth restriction		0 (0.0%)	6 (11.3%)	§0.999

^Independent t-test. NA: Not applicable.

Table (9) showed that: No significant difference according to hysterectomy regarding baseline ultrasound findings.

Table 10: Comparison according to hysterectomy regarding fetal findings

Characteristics	Hysterectomy		p-value
	Present (Total=7)	Absent (Total=53)	
Fetal weight (kg)	2.6±0.3	2.7±0.4	^0.620
Intrauterine fetal death	0 (0.0%)	0 (0.0%)	NA

^Indepednent t-test. NA: Not applicable.

Table (10) showed that: No significant difference according to hysterectomy regarding fetal findings.

DISCUSSION

Obstetric hemorrhage remains as one of the major causes of maternal death in developing countries and is the cause of up to 50% of estimated 500000 maternal deaths that occur [(1)].

Antepartum hemorrhage [APH] is defined as bleeding from or in the genital tract occurring from 24 week of pregnancy and prior to the birth of the baby. APH complicates 3 – 5% of pregnancies and is the leading cause of perinatal and maternal mortality. The most important causes of APH are placenta praevia and placenta abruption [(1)].

Placenta previa is the complete or partial covering of the internal os of the cervix with the placenta. It is a major risk factor for antepartum hemorrhage and postpartum hemorrhage and can lead to maternal morbidity and mortality worldwide [(2)].

APH has a heterogeneous pathophysiology and cannot reliably be predicted. The rates of placenta praevia and accreta have increased and will continue to do so as result of rising rates of caesarean deliveries, increased maternal age and use of assisted reproductive technology [ART] placing greater demands on maternity -related resources. The highest rates of complication for both mother and newborn are observed when these conditions are only diagnosed at delivery [(4)].

The presence of lacunae in the placenta, sponge-like findings in the cervix and a lack of a clear zone were also associated with massive bleeding during the Cesarean operation. Adherence of the placenta diagnosed at Cesarean was also associated with massive bleeding, and had the highest odds ratio among these variables [(3)].

This study aimed to assess the validity of short cervical length and venous sinus at the margin of the placenta as 3rd trimesteric ultrasound findings for prediction of antepartum hemorrhage of placenta previa by TVS and color Doppler.

This cross sectional study was conducted on 60 pregnant women attending Ain Shams University Maternity Hospital with gestational age ≥ 28 weeks, singleton viable pregnancy and diagnosed placenta Previa. The term placenta praevia should be used when the placenta lies directly over the internal os.

Our study showed demographic characteristics of the studied cases show mean±SD of maternal age and gestational age at enrollment was 31.9±5.6 years and 33.1±2.5 weeks respectively.

Regarding the baseline ultrasound findings of the studied cases, we found that, Mean±SD of cervical length was 39.9±6.4 mm, placenta was anterior (45.0%), posterior (41.7%) or central (13.3%). Venous sinus was in (70.0%) of cases.

Antepartum hemorrhage occurred in more than half of our cases (56.7%). Less than quarter of cases needed blood transfusion (21.7%) and (11.7%) of cases needed hysterectomy.

In this study, the first antepartum hemorrhage

attack findings was Mean±SD 31.9±2.1 week of gestational age affecting 34 women. The second antepartum hemorrhage attack findings was at 32.3±2.1 week and affecting 12 women. While the third attack was 31.5±2.1 week and affect only 2 women.

In our result, no significant difference according to antepartum hemorrhage regarding demographic characteristics (maternal age, parity and gestational age) and fetal findings.

Regarding baseline ultrasound findings, Cases with antepartum hemorrhage non-significantly had lower cervical length and more frequent venous sinus.

In disagreement with our result, Long et al. (5) in a study involved 233 women with APH and 302 women without APH in the cohort where the clinical and ultrasound features in patients with or without APH were compared.

In this study, we found that, there was significant negative correlation between antepartum hemorrhage attacks and cervical length.

In agreement with our result, Long et al. [5] revealed that, a binary logistic regression model was constructed to determine the independent risk factors for PP with APH. The model revealed that type of placenta, antepartum cervical length, placental location, partially absent over lying myometrium, and previous history of UAE remained as the significant factors associated with APH, while other parameters, such as number of pregnancies, parity, number of cesarean deliveries, type of placenta accrete, loss of the retro placental clear zone, number of placental lacunae, and utero-vesical hyper vascularity Doppler signal grading were excluded. Cervical length was inversely correlated to APH (OR: 0.972, 95% CI: 0.952~0.993).

Regarding diagnostic performance of cervical length in predicting antepartum hemorrhage, cervical length had significant low diagnostic performance in predicting antepartum hemorrhage, but had significant moderate diagnostic performance in predicting two or more antepartum hemorrhage attacks.

In agreement with our result, Eid et al. [6] in his study as transvaginal measurement of cervical length was done for 30 cases of complete placenta Previa identified between 28 weeks and 36 weeks of gestation and this was correlated to the clinical outcome of pregnancy with recordings for the gestational age at time of delivery, occurrence if any complications as antepartum hemorrhage, blood transfusion, caesarean hysterectomy, preterm delivery and the need for NICU admission and whether the caesarean section was elective or emergency caesarean section was needed before 36 weeks due to massive hemorrhage. They revealed that, the increased risk for peripartum complications as emergency caesarean section, massive antepartum haemorrhage and caesarean hysterectomy can be predicted by ultrasonographic detection of shortened cervical canal.

This study also agreed with Alessandra and his colleagues, in their study which was performed including pregnancies with low-lying placenta for which third trimester TVU CL was available. Multiple pregnancies were excluded. Short cervix was defined as TVU CL ≤25 mm. Outcomes of interest were compared with respect to the TVU CL. They found that women with placenta previa in the 3rd trimester and found that short cervix <25mm correlated strongly with the increased incidence for APH similar to us they reported these cases to have higher incidence for blood transfusion more NICU admission [7].

On the contrary Hasegawa and his colleagues who conducted astudy on 182 cases of singleton pregnancies with placenta previa were reviewed. The US findings including the type of placenta previa, placental location, presence of placenta lacunae, lack of clear zone, sinus venosus at the margin of the placenta. they did not find that CL in cases of placenta previa has any statistical significance with or without APH [8].

Hessami et al. [9] in meta-analysis study aimed to examine the association between cervical length and the risk of adverse outcomes in placenta previa pregnancies. In addition, the diagnostic accuracy of cervical length in predicting emergency cesarean delivery due to hemorrhage was evaluated. They revealed

that, Short cervical length (≤30 mm) measured at 28 to 34 weeks of gestation can assist in predicting the risk of emergency cesarean delivery due to hemorrhage in pregnancies with placenta previa. Furthermore, short cervical length is significantly associated with the risk of antenatal bleeding, preterm birth, and postpartum hemorrhage in pregnancies with placenta previa.

However, in a previous retrospective study which demonstrated that in ultrasonographic findings, the frequency of a marginal sinus was slightly higher (16%) in cases with bleeding episodes compared to those without (0%). Although the pathophysiological changes associated with the marginal sinus have not been well clarified, they hypothesized that an expanded marginal sinus indicates the retention of maternal blood flow in the intervillous space and decidual tissue, which may collapse occasionally due to uterine contraction, thus resulting in a large amount of hemorrhage during pregnancy [10].

The correlation between sponge-like findings and perinatal maternal massive hemorrhage has been reported previously. Both the incidence of preterm delivery owing to sudden massive hemorrhage and the mount of bleeding during Cesarean section have been found to be significantly higher in cases in which two-thirds of the placenta covered the internal os and there was a sponge-like echo., the same correlation was found. Hurton et al. reported that the areas with sponge-like echoes were most likely composed of clusters of richly developed blood vessels (presumably varices with various degrees and patterns of dilatation). Two recent reports presumed that the sponge-like findings were cervical varices [11].

Saitoh et al. demonstrated that the risk of antepartum massive hemorrhage was higher in cases with an echo-free space at the placental edge overlying the internal os (similar to the marginal sinus defined in this study). They suggested that the echo-free space in the marginal areas of placenta (an area associated with turbulent blood flow) was either a marginal sinus of the placenta or varices developing in the decidual tissue [12].

Also, cervical length ≤39.0 mm and venous sinus had high sensitivity and negative predictive value, but low other characteristics. Overall diagnostic characteristics of cervical length ≤39.0 was higher than that of venous sinus.

Mitsuzuka et al. (13) in a study included 129 singleton pregnant women with placenta previa. They stated that, the shortest CL measured throughout gestation was used for analysis, and they defined CL less or more than 30 mm as short or normal CL, respectively. They performed univariate and multivariate analyses, and a receiver-operating characteristics (ROC) curve was plotted to determine the cut-off CL value to predict APH. APH occurred in 26 patients. The adjusted odds ratio for APH was 3.80 (95% CI, 1.36-10.65) in patients with short CL. ROC analysis was performed to determine a cut-off CL value of 35 mm to predict APH, with a sensitivity of 80.7% and a specificity of 60.2%.

In our study, previous cesarean sections were significantly higher in cases that underwent hysterectomy.

In agreement with our result, a study was conducted by Giambattista et al. (14) on Two-hundred and forty-seven women were selected and reviewed all singleton pregnancies with a diagnosis of placenta previa, with the distance between the lower placenta edge and the internal cervical os is ≤2 cm. They found that, previous cesarean sections were significantly higher in cases that underwent hysterectomy. 92% of women who had hysterectomy had a previous history of at least one CS with highly statistically significant difference (P <0.001).

Similarly, Liu et al. (15) in study included 219 pregnant women with placenta previa and scarred uterus complicated with pregnancy. They found that, the hysterectomy group had higher incidence for those with a previous history of two or more prior cesarean deliveries (20.0% vs 7.2%, P=0.049).

Also, they found that, there was no significant difference according to hysterectomy regarding baseline ultrasound findings.

In dis agreement with our result, Liu et al.

(15) revealed that, since ultrasonic examination plays an important role in the prediction of surgery risks, they analyzed the characteristics of ultrasound images in detail. Four signs indicating placenta invasion including vascular lacunae, loss of normal hypoechoic retroplacental zone, retroplacental myometrial thinness, and placental thickness, were all more prevalent in cases with hysterectomy. In addition, the incidence of central placenta previa and anterior placenta previa were higher in patients that received hysterectomy with highly statistically significant difference ($P < 0.001$).

In this study, they found that, there was no significant difference according to hysterectomy regarding fetal findings.

In agreement with this result, Liu et al. (15) who stated that, basal maternal and neonatal information for hysterectomy and control cases are shown and there were no statistically significant differences in neonatal birthweight and Apgar scores.

CONCLUSION

There is sufficient evidence to show that shortened cervical length is a strong indicator for APH and that ultrasound measurements of the maternal cervix will become the preferred method due to reliability and objectivity.

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CD49 in Optimization the Cologenicity of Mesenchymal Stem Cell Culture Passage

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Abstract

Background: Stem Cell Populations Expressing Integrin a6 (CD49f) expression has been founded in bone marrow-derived mesenchymal stem cells (BM-MSCs), fetal urinary bladder obtained MSCs, and UCB-MSCs. It is viable that the expression of CD49f might affect the stemness of MSC culture. The goal of this work was to assess the best passage of MSC culture by CD 49 surface marker.

Methods: This comparative study was performed on 15 umbilical cords (UC) collected at Gynecology and Obstetrics Department of Sohag University Hospitals, fresh samples was taken immediately after caesarean section, maternal age within 20-36 years old, newborn weight >1300:3500 gm. The UC is gathered and kept in a sterile specimen cup comprising 0.9% normal saline at 4C° till processing (for best result within 12 –24 h of birth).

Results: CD49F and CD 73 were significantly increased in Day 12 than Days 3,6, and 9. There was a significant relation between CD73 and blood grouping at 6 days. There was no significant relation between CD 49F and CD73 and fetal sex. There was a notable negative correlation between CD 49F and the time with maternal age at 9 days. There was no significant correlation between CD49F and CD73 and time with GA. There was no significant correlation between CD49F and time with last delivery. a significant positive association existed between CD 73 and time with delivery at 6 days.

Conclusion: Blood group and maternal age may influence MSC culture outcomes, with time-dependent variations in surface marker expression.

Keywords: CD49, Optimization, Cologenicity, Mesenchymal Stem Cell Culture.

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Introduction

Human mesenchymal stem cells (MSCs), owing to their regenerative and immunomodulatory properties, are widely employed in the management of cartilage and

bone injuries, cardiovascular disorders, gastrointestinal ailments, autoimmune illnesses, neurological conditions, and malignancy. The application of MSCs in treatment has been shown to be safe and potentially effective [1].

MSCs were first discovered from bone marrow mononuclear cells (BM-MNCs) as fibroblastic colony-forming units (CFU-Fs). Owing to their paracrine and multipotency effects, MSCs are well-suited for regenerative therapy. Nowadays, there is no agreement on an exclusive surface molecule to distinguish MSCs from diverse origins [2].

Research conducted in the late 1960s primarily identified MSCs in the BM; however, subsequent research has shown that these cells may be isolated from other tissues, including adipose tissue, cardiac tissue, Wharton's jelly, peripheral blood, dental pulp, cord blood, and more recently, menstrual blood and chorionic villi [3].

MSCs have been shown to differentiate into mesodermal lineages, including bone, adipose tissue, and cartilage. They possess the ability to decrease T-cell proliferation and activation, and their reduced immunogenicity suggests potential for allogeneic applications [4]. Research in regenerative medicine has gained remarkable speed during the last decade, mostly owing to advancements in the area of stem cells. Cell therapy has been approved for only a limited number of therapeutic indications; yet, it has the potential to provide innovative remedies for presently untreatable illnesses. Cell therapy primarily relies on the utilization of stem cells as therapeutic agents to facilitate tissue regeneration, cell replacement, or immunoregulation. MSCs were first identified in bone marrow [4].

The confluence of culture was shown to be favorably connected with the expression of stem cell populations exhibiting CD49d, CD200, and CD106, while showing a negative correlation with CD49f. The expression of CD49f and the knockdown of CD49f in embryonic stem cells (ESCs) lead to differentiation into three germ layers, indicating that CD49f is involved in the preservation of pluripotency and serves as an indicator for ESCs. CD49f is recognized as a unique HSC marker and has been demonstrated to enrich cells able to producing long-term multilineage grafts. CD49f expression has been identified in BM-MSCs, fetal urine bladder-derived mesenchymal stem cells, and UCB-MSCs. The expression of CD49f might reflect the stemness of MSC culture [2].

The aim of this work was to reach the best passage of MSC culture by CD 49 surface marker.

Materials and Methods

This comparative work had been conducted on 15 umbilical cords (UC) collected at Gynecology and Obstetrics Department of Sohag University Hospitals, fresh samples as taken immediately after Caesarean Section (CS), maternal age within 20-36 years old, newborn weight >1300:3500 gm. The study was done from January 2019 to December 2023 following approval from the Ethics Committee Sohag University Hospitals, Sohag, Egypt. Each subject provided informed written consent.

Criteria for exclusion had been any case with maternal age (<18 or >36 years old), newborn weight (<1000 gm), serologic positive results (Hepatitis B virus, Hepatitis C virus and HIV) and any ultrasound abnormalities are being noted through the pregnancy.

Isolation, primary culture of human UC MSCs from Wharton's jelly.

All protocols were used in class II, Type A2 laminar flow hood Always be dressed in double gloves, protective eyewear, and a lab coat throughout isolation procedures Using a universal precaution when holding human tissue and dispose of contaminated materials appropriately.

Materials Needed: Dulbecco modifica-

tion of minimum essential media with stable glutamine (DMEM) (Cat no:S-RX0066-500, Lot no:S18028L0066), fetal Bovine Serum (FBS), Penicillin/streptomycin (Lot:0000490305)(M-R-P on bottle/pack (India only)country of origin India, trypsin/EDTA solution (Lot:0000370979) country of origin India, HU CD49F FITC MAB (Cat:561893, Lot:8026520), HU CD73 RE MAB (Lot:5190613107), phosphate buffer saline PBS (Ca++ and Mg++ free), sterile forceps, scalpel, and scissors, 15 mL and 50 mL conical centrifuge tubes, sterile 100 mm and T-75 plastic culture dishes and flasks, sterile pipettes and sterile pasteur pipettes.

Preparation of supplemented tissue sample:

The UC is gathered and kept in a sterile specimen cup containing 0.9% normal saline at 4C°until processing (for best result within 12 –24 h of birth). The cord's surface is washed with sterile phosphate-buffered saline to remove as much blood as possible. Blood vessels are separated from each segment after longitudinally incising the cord. The residual tissue is washed. The cord is manipulated inside a sterile 10 cm petri dish using sterile forceps; it is then sectioned into 3–5 cm segments using a sterile blade Then every cord tissue piece sliced into very small pieces.

After cord tissue piece cut into very small pieces put in flask. 5-3 ml of DMEM (low glucose), 10-20%FBS and 1% AB (complete media) are added to the cell pieces in flask (25cm2). The tissue culture flasks were incubated at 37°C in a 5% CO2 incubator (Binder15-03520, Germany) for 2 - 3 days with no disturbance to allow the adherence of tissue pieces. Following three days of incubation, the CM was replaced and after 7 - 10 days of incubation, tissues were discarded.

Fig. (1): Very small pieces of umbilical cord in flasks

Primary Culture

After initial collecting of the primary culture, change the medium every 3 – 4days. After 12 – 14 days, remove the tissue and exchange the medium every 3 days. Once the cells are 80 -85 % confluent, subculture to next passage. Total cells are counted using a hemocytometer or automated cell counter (Cells are counted by trypan blue) and subculture according to cell count.

Fig. (1): Showing culture media was changed and tissue were removed

Subculture of MSC Cells

That protocol was planned for the subculture of one 25 cm2 culture flask of actively multiplying cells near confluence. All of the culture medium was eliminated from the flask. A 3 mL PBS was put in flask. The flask was shaken to achieve complete surface coverage. The PBS solution had been removed from the flask, and 1 mL of trypsin solution was introduced. The flask was shaken to provide a uniform coating. The flask was incubated at 37°C for 3 to 5 minutes. When the cells are partly separated and rounded, gently tap the flask to remove the cells from its surface. Four to five milliliters of complete media were added to the flask, and the detached cells were transferred to a sterile 15 milliliter conical tube. The cells underwent centrifugation at 500 × g for 3 minutes. Examine the cell pellet.

Fig. (3): Cell pellet after Centrifugation

Take care while aspirating medium from cell pellets: The supernatant from the tube was carefully eliminated without detaching the cell pellet, the cell pellet was Suspend in 1 mL complete media. Pipetting the cells up and down then detect the concentration of cells in the suspension by a hemocytometer or automated cell counter, the cells in supplemented media were diluted and seeded in the new culture flask and the cultures were incubated in a 37°C, 5% CO2 and humidified cell culture incubator.

Principles of flowcytometry (FCM):

Flowcytometry was reported as a diagnostic technique used for measurement of numerous cell properties to recognize cells or particles suspended in fluid. It could recognize size, granularity and the fluorescence characters of As, cells were labeled with specific fluorescent antibodies that attach to specific cellular molecules. So, when laser beam hit the fluorescent antibodies it sends light that was transformed to electric signal in percentage to the amount of light [5].

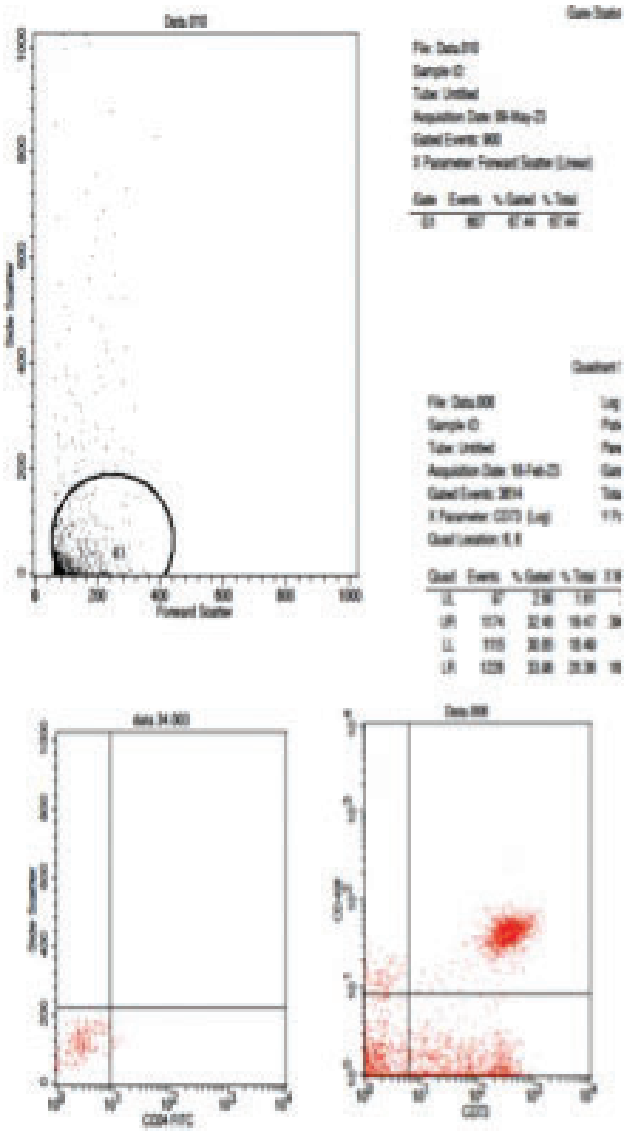

Fig. (4): Passage number three (mother 34years, p3+0,last delivary 3 years, pregnancy 37w5d, Blood group A+,Fetal weight 2850gm)

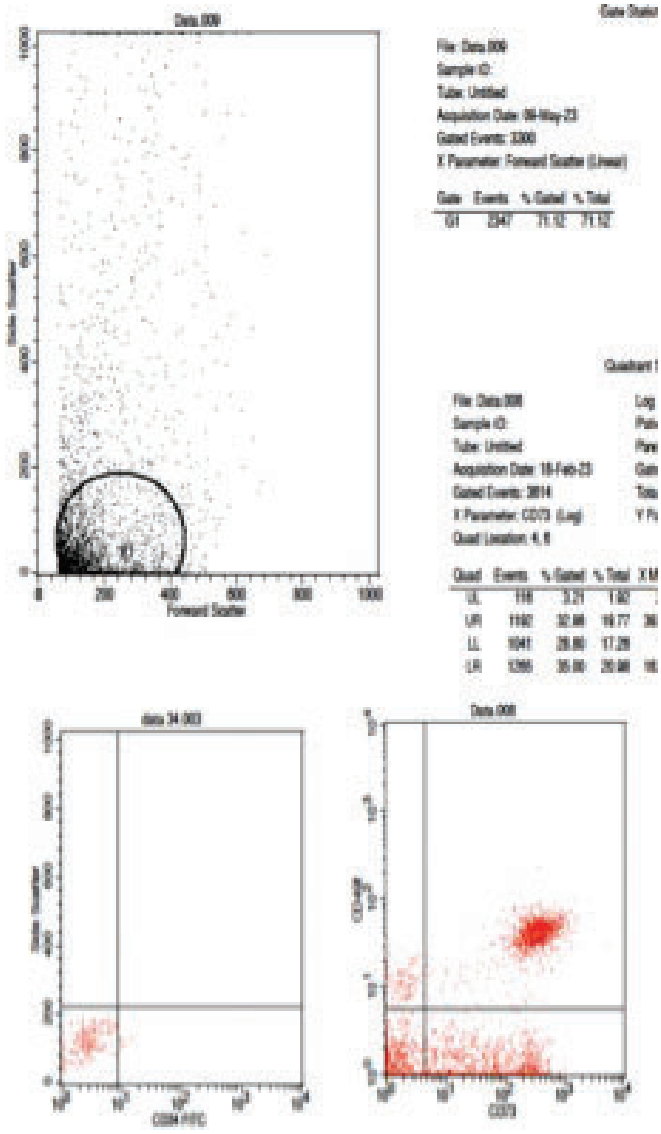

Fig. (5): Passage number four (mother 34years, p3+0,last delivary 3 years, pregnancy 37w5d, Blood group A+,Fetal weight 2850gm)

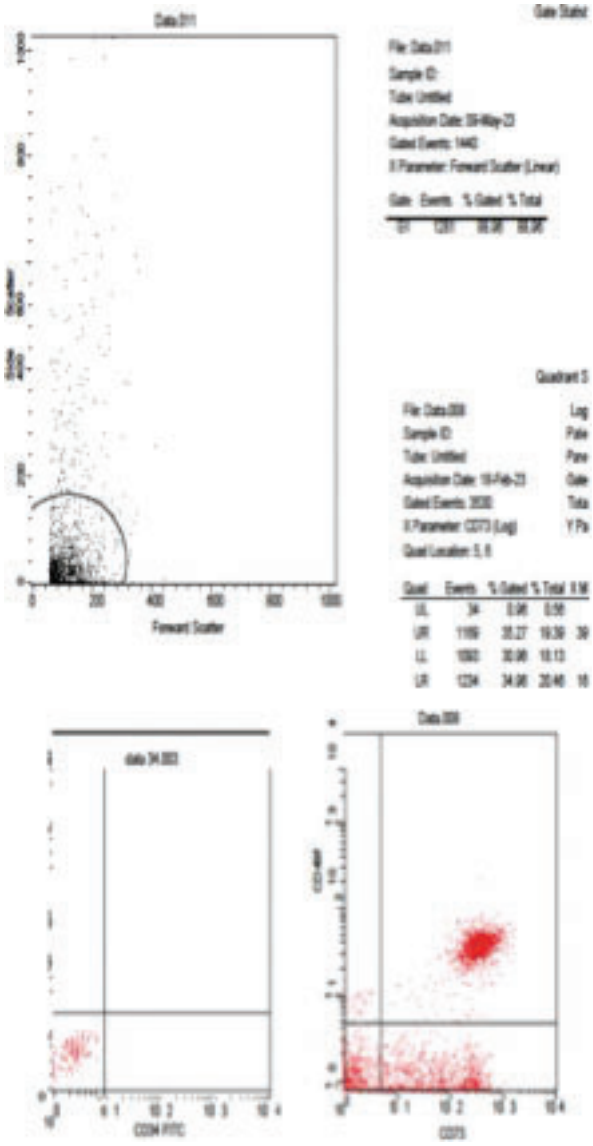

Fig. (6): Passage number Five (mother 34years, p3+0,last delivery 3 years, pregnancy 37w5d, Blood group A+, Fetal weight 2850gm)

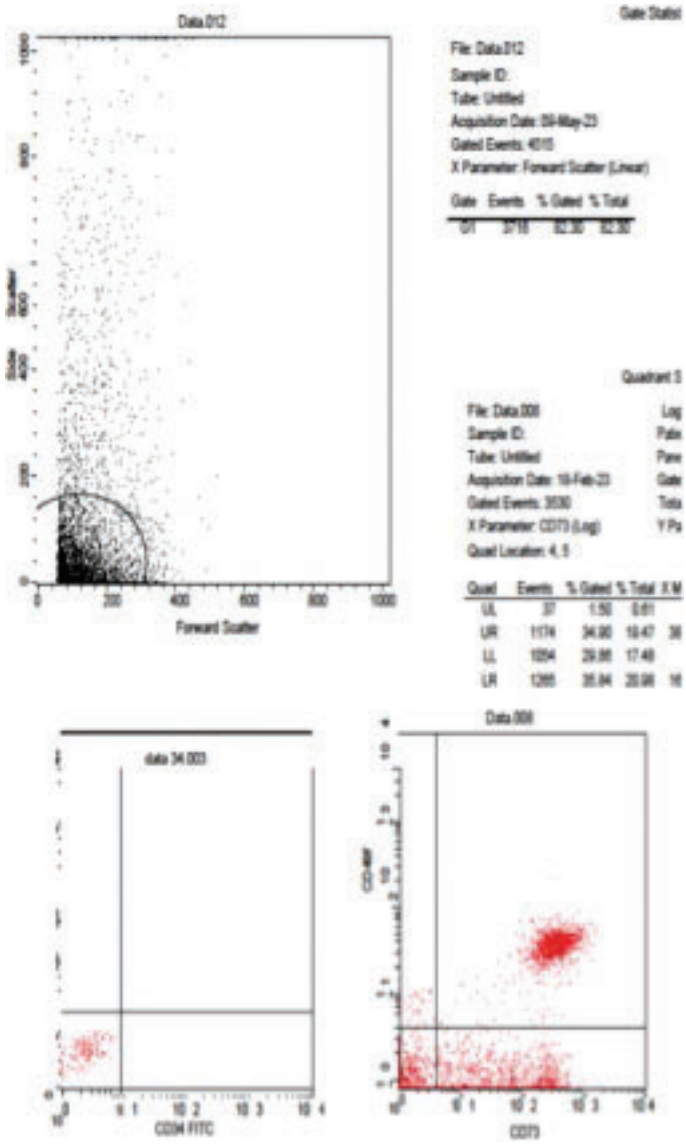

Fig. (7): Passage number six (mother 34years, p3+0, last delivery 3 years, pregnancy 37w5d, Blood group A+, Fetal weight 2850gm)

Statistical analysis

Statistical analysis had been conducted utilising SPSS v26 (IBM Inc., Chicago, IL, USA). Quantitative parameters had been reported as mean and standard deviation (SD) and contrasted between all groups utilising ANOVA (F) test with post hoc test (Tukey). Qualitative parameters had been displayed as frequencies and percentages (%) and had been analysed utilising the Chi-square test.

Relation between various variables was conducted by Pearson moment correlation equation. A two tailed P value < 0.05 was considered statistically significant.

Results

Descriptive data of mother (age, parity, abortion, fetal GA, last delivery) and fetal (sex, blood group and fetal weight) were explained in this table. Table 1

Table 1: Descriptive data of mother (age, parity, abortion, fetal GA, last delivery) and fetal (sex, blood group and fetal weight)

		N=15
Descriptive data of mother		
Age (years)		31.200 ± 4.887
Parity	**P0**	1(6.67%)
	P1	1(6.67%)
	P2	3(20.0%)
	P3	5(33.33%)
	P4	3(20.0%)
	P6	1(6.67%)
	P7	1(6.67%)
Abortion	**A0**	11(73.33%)
	A1	2(13.33%)
	A2	1(6.67%)
	A3	1(6.67%)
GA (weeks)		36.134±2.512
Last delivery (years)		4.143±2.797
Descriptive data of Fetal		
Sex	**Male**	5(33.33%)
	Female	10(66.67%)
Fetal Blood group	**A+**	4(26.67%)
	B+	5(33.3%)
	O+	3(20.0%)
	AB+	3(20.0%)
Fetal Weight (gm)		2730.000±478.166

Data are displayed as mean ± SD or frequency (%). GA: Gestational age.

CD49F and CD 73 were significant increase in Day 12 than day 3,6 and 9. Table 2

Table 2: Comparison Of CD49F and CD 73 as regard 3day, 6Day, 9Day and 12Days

	CD 49F (%)	COMP.	Differences	Paired Test	P
3 Days	28.435±8.755	**--**	--	--	--
6 Days	34.699±2.555	**3-6D**	-6.264±8.284	-2.929	**0.011***
9 Days	36.481±2.229	**3-9D**	-8.046±9.714	-3.208	**0.006***
12 Days	37.526±2.669	**3-12D**	-9.091±10.559	-3.335	**0.005***
CD 73 (%)					
3 Days	59.977±8.036	**--**	--	--	**--**
6 Days	67.620±3.699	**3-6D**	-7.643±9.304	-3.182	**0.007***
9 Days	70.873±4.163	**3-9D**	-10.896±10.607	-3.978	**0.001***
12 Days	72.434±4.956	**3-12D**	-12.457±11.195	-4.310	**0.001***

Data are displayed as mean ± SD. * Significant p value <0.05, CD49f: Stem Cell Populations Expressing Integrin a6.

There was no significant relation between CD 49F and blood grouping at all times. There was a significant relation between CD73 and blood grouping at 6 days while other times without significant. Table 3

Table 3: Relation between CD 49F and CD73 and blood grouping

	Fetal Blood group				F	P
	A+	B+	O+	AB+		
CD 49F (%)						
3 Days	34.043±0.952	26.048±8.873	30.733±9.759	22.637±12.123	1.239	0.342
6 Days	34.838±1.127	35.720±1.635	34.670±3.869	32.840±3.954	0.758	0.540
9 Days	35.235±1.005	36.666±1.590	38.513±3.480	35.800±2.453	1.497	0.270
12 Days	35.788±0.891	37.710±1.758	38.737±3.467	38.327±4.602	0.840	0.500
CD 73 (%)						
3 Days	65.680±1.629	58.278±9.426	60.333±7.792	54.847±9.886	1.207	0.353
6 Days	67.403±1.837	71.052±2.923	67.020±2.643	62.790±1.256	7.771	**0.005***
9 Days	69.068±2.668	73.580±3.159	69.227±3.500	70.413±7.016	1.163	0.368
12 Days	70.020±2.487	74.802±3.337	71.377±4.658	72.763±9.550	0.698	0.572

Data are displayed as mean ± SD. * Significant p value <0.05, CD49f: Stem Cell Populations Expressing Integrin a6.

There was no significant relation between CD 49F and CD73 and fetal sex. Table 4

Table 4: Relation between CD 49F and CD73 and fetal sex

	Fetal Sex		t	P
	Male	Female		
CD 49F (%)				
3 Days	29.160±8.775	28.072±9.196	0.219	0.830
6 Days	35.882±1.633	34.107±2.793	1.299	0.216
9 Days	36.544±1.597	36.449±2.568	0.075	0.941
12 Days	37.368±1.826	37.605±3.095	-0.156	0.878
CD 73 (%)				
3 Days	61.286±9.083	59.322±7.897	0.433	0.672
6 Days	69.836±2.606	66.512±3.765	1.759	0.102
9 Days	72.310±2.919	70.154±4.632	0.942	0.364
12 Days	73.460±3.355	71.921±5.686	0.553	0.590

Data are presented as mean ± SD. CD49f: Stem Cell Populations Expressing Integrin a6.

There was no significant correlation between CD73 and the time with maternal age and fetal weight. There was a notable negative correlation between CD 49F and the time with maternal age at 9 days, while other timed without significant. Table 5

Table 5: Correlation between CD 49F and CD73 regarding the time with maternal age and fetal weight

	Age (years)		Fetal Weight (gm)	
	r	P	r	P
CD 49F				
CD 49F (%) 3 Days	-0.042	0.882	0.362	0.185
CD 49F (%) 6 Days	-0.071	0.801	0.043	0.879
CD 49F (%) 9 Days	-0.575	0.025*	-0.146	0.603
CD 49F (%) 12 Days	-0.365	0.181	-0.266	0.339
	CD73			
CD 73 (%) 3 Days	-0.053	0.853	0.288	0.298
CD 73 (%) 6 Days	-0.170	0.545	-0.019	0.946
CD 73 (%) 9 Days	-0.097	0.730	-0.319	0.247
CD 73 (%) 12 Days	-0.183	0.514	-0.395	0.145

r: Pearson Coefficients. * Significant p value <0.05, CD49f: Stem Cell Populations Expressing Integrin a6.

There was no significant correlation between CD49F and CD73 and time with GA. There was no significant correlation between CD49F and time with last delivery. There was a notable positive correlation between CD 73 and time with delivery at 6 days, while other timed without significant. Table 6

Table 6: Correlation between CD49F and CD73 and time with GA and last delivery

	GA (weeks)		Last delivery (years)	
	r	P	r	P
CD 49F				
CD 49F (%) 3 Days	0.342	0.213	-0.237	0.415
CD 49F (%) 6 Days	0.114	0.686	0.195	0.505
CD 49F (%) 9 Days	-0.121	0.667	0.093	0.751
CD 49F (%) 12 Days	-0.147	0.602	0.068	0.818
CD73				
CD 73 (%) 3 Days	0.384	0.158	-0.278	0.336
CD 73 (%) 6 Days	-0.198	0.479	0.641	**0.014***
CD 73 (%) 9 Days	-0.298	0.281	0.419	0.136
CD 73 (%) 12 Days	-0.330	0.230	0.312	0.277

r: Pearson Coefficients. * Significant p value <0.05, CD49f: Stem Cell Populations Expressing Integrin a6., GA: Gestational age.

Discussion

Umbilical cords have the ability to be used as a source of MSCs for several causes; they are regarded as medical wastes, and thus their utilization in researches has minor ethical consideration; they can rapidly proliferate in culture and are considered to be immune privileged [6].

These results agreed with Beeravolu et al. [7] who assured that explant- trypsin method is a easy approach for the dissection of the umbilical cord for the isolation of MSCs and said that the insufficient digestion of tissue pieces 1-2 mm in size by using a commercial trypsin solution allowed outgrowth of cells without causing massive cell damage, maintaining viability cells from the tissues and collect significant quantities of MSCs.

However, Trivanovic et al. [8] reported those significant tissue explants of umbilical cord about 10 mm in size were top for cell isolation. In our study, we assessed the efficacy of MSCs isolation protocol from human umbilical cord-Wharton's jelly and detected the number of obtainable cells and their viability from passage 3 to passage 6 with increasing in count.

In our study, we acquired plenty number of live MSCs from cord segment of 8- 10 cm. the cultured cells from explant trypsin method were assessed by flowcytometry and the results revealed that the cells had been positive for MSCs surface markers, CD73 and CD49f with 83.33% and 43.55% respectively and negative for hematopoietic cell surface markers CD34.These results agreed with Campard et al. [9] who revealed that the cells were positive for CD73 with elevated expression level 81.6%.

Also our study agreed with Suto et al. [10] found that CD73 (cluster of differenetiation73) is regarded one of surface markers that recognize MSCs.

Although, CD49f might lack use as a single

unique marker for MSCs, since it is also extensively expressed in epithelial cells, monocytes, endothelial cells, platelets, and thymocytes [2].

The human umbilical cord and placenta are noninvasive, primitive, and abundant sources of MSCs that have garnered attention due to the absence of ethical or moral implications. Cellular proliferation was noted within 3 to 4 days of explants from the cord-placenta junction, while growth was noticed following 7 to 10 days and 11 to 14 days from the cord lining. Wharton's jelly Cells derived from the cord lining, Wharton's jelly, and cord-placenta junction were analyzed for the expression of particular cellular markers. P3-5 cells exhibited strong expression of MSC markers, including CD73 [11].

Also, our study agreed with Nekanti et al. [12] who assumed that the clinical-scale expanded Wharton's jelly -MSCs were positive for mesenchymal markers CD 73, 6 and negative for HSC CD 34.

Limitations of the study involve that the sample size was relatively small. The work had been in a single centre.

Conclusions

Day 12 is the optimal passage for MSC culture, with significant increases in CD49F and CD73 compared to Days 3, 6, and 9. CD49F showed a negative correlation with maternal age on Day 9, while CD73 was significantly related to blood group on Day 6 and positively correlated with time from delivery at Day 6. No significant relationships were found between MSC markers and fetal sex, fetal weight, gestational age, or time since last delivery. These results suggest that certain factors, like blood group and maternal age, may influence MSC culture outcomes, with time-dependent variations in surface marker expression.

Financial support and sponsorship: Nil

Conflict of Interest: Nil

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