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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.

References

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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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Contents :

Letter from the Editor:

Dear esteemed colleagues,

Very interesting subjects are included in this issue. L-carnitine as an adjuvant therapy to clomiphene citrate in the controlled ovarian stimulation was associated with higher follicle number, better endometrial thickness, and earlier days of HCG injection in PCO patients. The least invasive approach for extraction of perforated IUCD should always be considered as it allows an enhanced recovery. Ultrasound is an essential tool in the preoperative evaluation and can guide the surgical approach to be used. Preconception evaluation of hemoglobin concentrations (HC) is mandatory to define women with low or high HC. Women who had low HC must be managed cautiously using iron supplemental therapy (IST) till having HC and IST must be stopped. HC early in pregnancy could predict the oncoming development of insulin resistance (IR) and gestational diabetes mellitus (GDM).

Vitamin D deficiency (VDD), obesity, and deregulated immune milieu in direction of inflammation may predispose or aggravate poor ovarian response to ovarian stimulation (OS). Vit D supplementation therapy (VDST) improved ovarian function and quality of oocytes with subsequent improvement of embryo quality most probably through equalization of immune milieu to the direction of anti-inflammatory. The current study has demonstrated that carbetocin can be alternative to traditional oxytocin in the prevention of postpartum hemorrhage in high-risk women undergoing elective caesarean section.

The pre-operative assessment of the sliding sign is useful in the prediction of intra-abdominal adhesions, prior to caesarean section (CS) procedure, especially in normal weight and overweight cases. The negative sliding sign correlates with longer operative delivery time. Surgical management of PAS by CS proved that bladder dissection after delivery of the baby is much better in terms of decreasing blood loss, urinary tract injuries and emergent hysterectomy than if done earlier. Maternal DM is not associated with significant abnormalities in Doppler indices of placental or fetal circulation.

Supplementation of oestradiol to progesterone in luteal phase support confers no additional benefit to progesterone alone.Serum progesterone at the day of hCG is not a useful predictor of pregnancy outcome. Further studies with larger numbers are needed to confirm or refute this finding. There is a strong association between congenital uterine anomalies and adverse reproductive outcomes. The arcuate uterus was the commonest congenital uterine anomaly found in the study and this occurred mostly among women presenting with secondary infertility or recurrent pregnancy loss. Estimation of serum E2 is an unreliable marker for differentiating women according to several mature follicles.

Best regards.

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Effect of antioxidants as adjuvant therapy to clomiphene citrate in controlled ovarian stimulation: a comparative study

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Abstract

Aim of the work: This work aims to evaluate the value of adding L-carnitine to clomiphene citrate in ovulatory dysfunction according to ovulation and pregnancy rate.

Patient and method: This study was conducted at the obstetric and gynaecology clinic, Mansoura University Hospital, from May 2021 to August 2022. The study included 186 participants presenting with infertility; Participants were allocated into two groups. Group A: received clomiphene citrate(100mg) plus L-carnitine (2gm). Group B: received clomiphene citrate (100mg) alone. Patients were allocated to groups by case order. The outcomes include ovulation, estradiol level, endometrial thickness, and pregnancy rate.

Results: Group A had significantly higher follicle count, dominant follicle size, endometrial thickness, serum E2 and earlier day of HCG injection ($P < 0.001$) compared to group B. regarding the response to induction in both groups, there were non-significant differences between them regarding ovulation rate, pregnancy rate and early miscarriage rate ($P = 0.071,0.336,0.502$ respectively). Regarding the induction in Poly Cystic Ovary Syndrome (PCOS) patients in both groups, the patients with PCO in group (A) had an earlier day of HCG injection ($P = 0.035$). However, there were non-significant differences between PCO patients in both groups regarding other variants.

Conclusion: L-carnitine as an adjuvant therapy to clomiphene citrate in the controlled ovarian stimulation was associated with higher follicle number, better endometrial thickness, and earlier days of HCG injection in PCO patients.

Keywords: L-carnitine, induction of ovulation, infertility, clomiphene citrate.

INTRODUCTION

Globally, 15% of couples of reproductive age suffer from subfertility (1), and ovulatory dysfunction represents 30 to 40 % of infertility in women (1). According to the World Health Organization, ovulatory disorders are divided into four classes:

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1. Hypogonadotropic hypogonadal anovulation
2. Normogonadotropic normal-estrogenic anovulation
3. Hypergonadotropic hypoestrogenic anovulation
4. Hyperprolactinemic anovulation (2)

Anti-estrogenic drugs such as Clomiphene Citrate have been used for ovulation induction since the 1960s in 50–150 mg (3). The conception rate in clomiphene citrate-treated cycles is about 40% only; however, the ovulation rate is between 80-85% (4). Repeated ovulation stimulation affects the quantity of mitochondrial DNA in oocytes, leading to a decline in egg quality and the number of oocytes (5).

L-Carnitine (LC) is one of the conditionally essential nutrients called quasi-vitamins (6). L-carnitine intake can promote β-oxidation, endurance, burn fat and shorten post-workout recovery (7,8). LC increases the GnRH secretion from the hypothalamus by causing K+-induced depolarisation in the hypothalamic neuronal (9).

Treatment with LC increases serum levels of estradiol, progesterone, and LH and decreases prolactin levels (10). Its indirect endocrine effect prevents Poly Cystic Ovary Syndrome (PCOS), amenorrhea and other problems related to the female reproductive cycle (11).

From the previously mentioned benefits, we added L-carnitine to clomiphene citrate in ovulation induction to study the impact on ovulation pattern and pregnancy rates.

Patients and Methods :

The IRB of faculty medicine Mansoura University approved this study (MS.21.04.1447); it was done at the obstetrics and gynaecology clinic of Mansoura university from May 2021 to August 2022. patients were allocated to groups by order. Females with a history of infertility (primary or secondary) with ovulatory dysfunction (WHO class II & 1V), aged 20-35 years, have a body mass index(BMI) of 18-30 kg/m2. Hormonal profile (serum prolactin and TSH levels, FSH, LH levels) was investigated. A semen analysis of the husband and hysterosalpingogram should be okay.

Exclusion criteria:

Females over 35 years refuse to participate, with hypersensitivity to one of the used drugs cannot participate. Females with known causes of infertility (such as abnormal semen analyses - pelvic adhesions - endometriosis - large submucous fibroid, premature ovarian insufficiency and Hypogonadotropic hypogonadal anovulation) were excluded.

The entire history was taken, a general examination was done, and the study was explained to each participant. Transvaginal ultrasound (TVUS), abdominal ultrasound (TAUS), and hysterosalpingogram were done, and the husband's infertility was excluded from the semen analysis. This study included 186 cases, with 93 participants in each group.

Group A(n=93): received clomiphene citrate (Tecnovula 50mg oral tab Techno _ pharma Borg Al Arab algadida city Alexandria - Egypt) 100 mg every day in the form of two oral tablets, starting from day1 to day 5 of the menstrual bleeding for five days Plus L.carnitine 2gm (Carnivita Fort 1gram oral tab Eva pharma, 6th of October City Giza Egypt) every day starting in concomitant with Clomiphene citrate till the day of the positive pregnancy test.

Group B (n=93): received clomiphene citrate (100mg)) (Tecnovula 50mg oral tab Techno _ pharma Borg Al Arab algadida city Alexandria - Egypt) 100 mg every day in the form of two oral tablets, starting from day1 to day 5 of the menstrual bleeding for five days.

The first follow-up visit was on day ten of the menstrual bleeding. TVUS was used to measure the number and size of the growing follicles and the endometrial thickness. Serum estradiol level was measured on day 10. Then TVUS was done every other day until the mature follicle reached (18-24) mm, and ovulation was triggered by 5.000 IU of hCG (Choriomon 5000 I u amp IBSA Switzerland imported by the scientific habit office). Participants were advised to have Intercourse every other day and 36 hours of hCG administration. Ovulation was confirmed when the corpus luteum was seen (collapsed dominant follicle) then a pregnancy test (β-hCG level in blood) was done two weeks after ovulation to confirm or exclude pregnancy. TVUS was done 2-3weeks after a positive pregnancy test to ensure a gestational sac. In cases where the pregnancy was confirmed, participants were followed up till 13 weeks of pregnancy. The same protocols were repeated for three consecutive months in cases where the pregnancy was excluded. In instances where ovulation not occurred or menstruation was delayed, withdrawal bleeding by Dydrogesterone 20 mg was done for five days (Duphaston 10mg, Penta pharma_Egypt, Abu sultan industrial zone Ismailia Egypt), and ovulation induction was repeated.

Outcome Measures

The Primary outcome of this study was to include the ovulation response (Number and size of follicles), Pre-ovulatory endometrial thickness, and serum estradiol level. The pregnancy rate and early miscarriage rate were reported.

Sample size calculation:

The sample size was based on ovulation rate among females with clomiphene citrate only and females with clomiphene citrate with L.Carnitine retrieved from previous research (Kortam et al., 2019) Using G*power version 3.0.10 to calculate sample size based on the difference of 25.5%, 2-tailed test, α error = 0.05 and power = 90.0% the total calculated sample size was 154. By adding 20% to compensate for possible dropout then, the total estimated sample size was 186 (93 in each group).

The cumulative clinical data of the studied groups and results were collected, tabulated, and statistical analyses were done.

RESULTS

The participants were assigned into two groups; group (A) included 93 patients who received clomiphene citrate with a dose of 100 mg per day plus L-Carnitine with a dose of 2 grams per day. Group (B) included 93 patients who received clomiphene citrate alone with a dose of 100 mg per day.

Table (1): Demographic characteristics of the studied groups:

		Group A (n= 93)	Group B (n= 93)	95% CI	P
Age		28.20 ± 4.434	28.90 ± 4.884	-2.05, 0.65	0.308
BMI		26.31 ± 2.882	26.03 ± 2.680	-0.53, 1.08	0.494
Residence	Rural	38 (40.9%)	25 (26.9%)	-	0.044
	Urban	55 (59.1%)	68 (73.1%)		
Gravidity		2.13 ± 1.469	2.12 ± 1.265	-0.39, 0.41	0.963
Parity		1.57 ± 1.067	1.87 ± 1.154	-0.62, 0.02	0.066

Data are expressed as mean and standard deviation or as percentage and frequency—95% CI: 95% confidence interval of the mean difference between both groups. P is significant when < 0.05.

There were non-significant differences between both groups regarding age, BMI, gravidity and parity (P = 0.308, 0.494, 0.963 and 0.066, respectively). There was a statistically significant difference between both groups regarding the residence (P = 0.044), as shown in table (1).

Table (2): Response to induction in both groups:

		Group A (n= 93)	Group B (n= 93)	95% CI	P
Infertility type	Primary	16 (17.2%)	15 (16.1%)	-	0.844
	Secondary	77 (82.8%)	78 (83.9%)		
Infertility duration (month)		21.78 ± 11.505	22.34 ± 9.341	-3.61, 2.50	0.720
Follicle Count		3.44 ± 2.184	1.65 ± 1.515	1.24, 2.33	< 0.001
Dominant follicle size day 10		15.05 ± 2.637	13.58 ± 1.702	0.69, 2.25	< 0.001
Endometrial thickness day 10		8.26 ± 2.110	6.59 ± 1.562	1.13, 2.20	< 0.001
Serum E2 day 10		210.75 ± 109.375	119.84 ± 75.689	63.70, 118.13	< 0.001
Day of HCG injection		11.31 ± 1.071	13.89 ± 1.573	-3.12, -2.06	< 0.001
Ovulation rate		63 (67.7%)	51 (54.8%)	-	0.071
Side effects	None	90 (96.8%)	93 (100.0%)	-	0.218
	Gastric upset	2 (2.2%)	0 (0.0%)		
	Dizziness	1 (1.1%)	0 (0.0%)		
Pregnancy rate		30 (32.3%)	20 (21.5%)		
Number of sacs by US	1	26 (86.7%)	14 (93.3%)	-	0.502
	>1	4 (13.3%)	1 (6.7%)		
Early miscarriage		2 (6.7%)	3 (15.0%)	-	0.336

Regarding the response to induction in both groups, there were non-significant differences between them in infertility type, duration, history of ovulation induction cycles, ovulation, side effects, outcome, number of sacs by US and early miscarriage (P = 0.844, 0.720, 0.648, 0.071, 0.218, 0.098, 0.502 and 0.336 respectively) as shown in table (2).

Group (A), who received clomiphene citrate plus L-Carnitine, had significantly higher follicle count, dominant follicle size day 10, endometrial thickness day 10, serum E2 day ten and earlier day of HCG injection (P < 0.001) as shown in table (2).

Table (3): Demographic characteristics of PCO patients (WHO class II) in the studied groups:

		PCO in Group A (n= 13)	PCO in Group A (n= 13)	95% CI	P
Age		29.77 ± 4.419	28.36 ± 5.988	-3.00, 5.82	0.516
BMI		27.38 ± 2.873	26.00 ± 2.966	-1.09, 3.86	0.259
Residence	Rural	9 (69.2%)	4 (36.4%	-	0.107
	Urban	4 (30.8%)	7 (63.6%		
Gravidity		1.77 ± 1.166	1.45 ± 1.293	-0.73, 1.36	0.537
Parity		1.38 ± 0.870	1.45 ± 1.293	-0.99, 0.85	0.876

Data are expressed as mean and standard deviation or as percentage and frequency. 95% CI: 95% confidence interval of the mean difference between both groups. P is significant when < 0.05.

Table (3) shows that regarding the comparison between the PCO patients in both groups, there were non-significant differences regarding age, BMI, residence, gravidity and parity (P = 0.516, 0.259, 0.107, 0.537 and 0.876, respectively).

Table (4): Response to induction in PCO patients in both groups:

		PCO in Group A (n= 13)	PCO in Group B (n= 11)	95% CI	P
Infertility type	Primary	2 (15.4%)	4 (36.4%	-	0.237
	Secondary	11 (84.6%)	7 (63.6%		
Infertility duration (month)		16.69 ± 5.559	23.27 ± 13.454	-15.04, 1.88	
Follicle Count		1.46 ± 1.808	0.73 ± 1.009	-0.54, 2.01	
Dominant follicle size day 10		14.42 ± 2.417	14.13 ± 0.479	-2.59, 3.17	
Endometrial thickness day 10		8.38 ± 3.283	6.27 ± 1.489	-0.15, 4.35	
Serum E2 day 10		121.31 ± 89.100	83.00 ± 27.695	- 19.81, 96.42	
Day of HCG injection		11.60 ± 0.894	14.00 ± 1.826	-4.58, -0.22	
Ovulation rate		5 (38.5%	6 (54.5%	-	
Side effects	None	12 (92.3%)	11 (100.0%	-	0.347
	Gastric upset	-	-		
	Dizziness	1 (7.7%)	0 (0.0%		
Outcome	Failure	9 (69.2%	9 (81.8%	-	
	Pregnancy	4 (30.8%	2 (18.2%		
Number of sacs by US	1	3 (100.0%)	2 (100.0%)	-	
	2	-	-		
Early miscarriage		0 (0.0%)	0 (0.0%)	-	-

Data are expressed as mean and standard deviation or as percentage and frequency—95% CI: 95% confidence interval of the mean difference between both groups. P is significant when < 0.05.

Table (4) shows that regarding the response to induction in PCO patients in both groups, the patients with PCO in group (A) had an earlier day of HCG injection (P = 0.035). However, there were non-significant differences between PCO patients in both groups regarding other variants.

Discussion

Adding antioxidants to clomiphene citrate in ovulatory dysfunction will improve ovulation and pregnancy rate. The present study has demonstrated that administration of L-carnitine as an adjuvant therapy to the controlled ovarian stimulation by clomiphene citrate was associated with higher follicle count, dominant follicle size, endometrial thickness, serum E2 and earlier day of HCG injection. However, all these improvements in cycle parameters did not lead to a significant increase in the ovulation and pregnancy rates, which may be explained by the small sample size and the diversity of factors that affect the occurrence of pregnancy.

CC has been used to induce ovulation by blocking estrogen receptors, thus increasing follicle-stimulating hormone and ovulation (12).

Carnitine may be either the L-carnitine, the biologically active form, or the biologically

inactive D-carnitine (13). L-Carnitine is essential in energy production, oxidative stress and glucose metabolism; it can stabilise mitochondrial membranes, increasing the organelle's energy supply, and protect the cell from apoptotic death(10,14,15).

Miyamoto et al. 2010 stated that repeated ovulation induction decreased the amounts of mitochondrial DNA and increased 8-hydroxydeoxyguanosine in oocytes (5).

In our study, Group (A), who received clomiphene citrate plus L-Carnitine, had significantly higher follicle count, dominant follicle size, endometrial thickness, serum E2 day and earlier day of HCG injection ($P < 0.001$). Consistent with our results, El Sharkwy et al. (16) 2019 compared N-acetylcysteine and l-carnitine in ovulation induction; after three months of treatment, there was a significant improvement in menstrual pattern, FSH, LH, free testosterone, and insulin resistance markers.

Gaafar et al. 2007 (17) evaluated the Effect of N-Acetyl-Cysteine (NAC) in the ICSI cycle; the NAC group showed a non-significant increase in retrieved oocytes. The study group showed a significant increase in mature oocytes, a higher fertilisation rate, and an insignificant drop in follicular testosterone. However, these positive effects of NAC did not lead to a significant change in the pregnancy rate.

Sheida et al. (18) 2021 added L-Carnitine to the GnRH-antagonist protocol on assisted reproductive technology in women with polycystic ovarian syndrome. They stated that the duration of stimulation and endometrial thickness were comparable in both groups ($p > .05$). The Serum estradiol level on the day of HCG triggering was significantly higher in the L-Carnitine group compared to the control group ($p < .05$). However, chemical (26.8 vs 30.7%) and clinical (24.3 vs 25.6%) pregnancy rates, were non-significant between L-Carnitine and control groups respectively.

Levels of serum L-carnitine in women with polycystic ovarian syndrome are much less due to hyperandrogenism and hyperinsulinemia (11). The combination of L-carnitine and CC in clomiphene-resistant PCOS significantly improves ovulation and the cumulative pregnancy rates in clomiphene-resistant PCOS (64.4% vs 17.4% and 51.5 % vs 5.8 %)(19).

In the current study, regarding the comparison between the PCO patients in both groups, there were non-significant differences between them regarding the age, BMI, residence, gravidity and parity ($P = 0.516$, 0.259, 0.107, 0.537 and 0.876 respectively).

In the current study, regarding the response to induction in PCO patients in both groups, the patients with PCO in the group (A) had an earlier day of HCG injection ($P = 0.035$). However, there were non-significant differences between PCO patients in both groups regarding other variants, including Infertility duration, Follicle Count, Dominant follicle size at day 10, Endometrial thickness at day 10, Serum E2 at day 10, Ovulation, Side effects, Outcome, Early miscarriage and Number of sacs by TVUS.

Finally, in the present study, L-carnitine was a well-tolerated drug by all patients, with no manifest side effects reported from any case in the study group.

Conclusion

L-carnitine is a well-tolerated drug with minor side effects. Combining L-carnitine and clomiphene citrate in ovulatory dysfunction will significantly improve the follicular count, endometrial thickness and pregnancy rate.

No Conflicts of interest

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Perforated Intrauterine Contraceptive Device: Single Institution Experience

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Abstract

Background: Intrauterine contraceptive device (IUCD) is the most commonly used contraceptive method, especially in developing countries given its cost-effectiveness. However, its use is not without complications, the most important of which is uterine perforation.

Objective: To evaluate the clinical presentation, management and outcome of women with perforating IUCDs referred to a single tertiary centre, especially with a nationwide campaign to increase the utilization of contraceptive methods.

Methodology: This is a cross-sectional study conducted at a University Hospital in the time period from January 2017 to June 2018, with the aim to evaluate cases presenting with a confirmed diagnosis of perforated IUCD.

Results: We managed 32 women with perforated IUCD. All had copper IUCD. The most common presentation was pelvic pain (43.75%), however, 37.5% were asymptomatic. Ultrasound was valuable in the evaluation of surgical risk in most cases. Extraction of IUCD using minimal invasive approach was possible in 90.6 % of cases.

Conclusion: The least invasive approach for extraction of perforated IUCD should always be considered as it allows an enhanced recovery. Ultrasound is an essential tool in the preoperative evaluation and can guide the surgical approach to be used.

Keywords: Intrauterine contraceptive device, perforation, contraception, hysteroscopy, laparoscopy, cystoscopy.

INTRODUCTION

In developing countries, intra-uterine contraceptive devices (IUCDs) are considered one of the contraceptive methods of choice, being cheap, readily available, reversible & long-acting (1,2,3). However, despite its wide safety profile, still, its use is not free of complications. Uterine perforation by the IUCD and its dislodgement in an inappropriate site is considered a serious complication (4) that ranges from 0.05 to 13 per 1000 insertions (5).

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Case reports of uterine perforation reveal that it is no longer a rare complication, and its occurrence is increasing relative to the prevalent use of IUCD (6-9).

To our knowledge, few studies have reported the diversity of risk factors, presentation and diagnosis of such complication (6,8,10,11). So, the aim of our study is to highlight the clinical course of reported cases with perforated IUCD, starting from the history, clinical presentation and finally, the diagnosis and management.

Materials and Methods

This was a cross sectional study conducted at the Department of Obstetrics and Gynecology, Cairo University Hospital in the period from January 2017 to June 2018. The study was approved by the Research and Scientific committee of the Department of Obstetrics and Gynecology with ethical approval number (I16009). All cases with a confirmed diagnosis of perforated IUCD referred to the outpatient clinic of the Department of Obstetrics and Gynecology at Cairo University Hospital were included. All cases had detailed history. Diagnosis of all cases was confirmed by ultrasound showing an empty uterus and an X-ray confirming the intra-abdominal or intra-pelvic location of the device (Figure 1). All cases underwent careful preoperative evaluation using both transvaginal and transabdominal ultrasound with the aim to locate the perforated device and assess possible difficulties during the surgery such as involvement of viscera and expected adhesions. Demographic data of the patients were described including age, body mass index (BMI), parity and mode of delivery. Presenting complaints, duration of symptoms, time interval from last delivery to IUCD insertion and time interval from IUCD insertion to diagnosis were reported. A minimal invasive approach was always thought for removal of the perforated IUCD, unless otherwise indicated. We reported the surgical management and intraoperative findings including type and location of IUCD as well as presence of adhesions or pus formation. We evaluated the ability of ultrasound to locate the perforated IUCD as well as the surgical difficulty expected. We excluded cases with malposition, or partial embedment that were managed hysteroscopically at the outpatient clinic. Ease of IUCD placement was also evaluated by questioning the women about their experience at time of insertion.

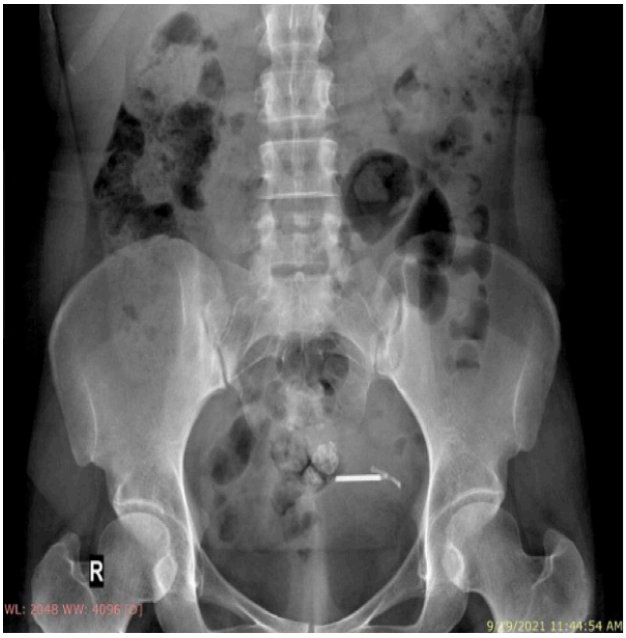

Figure 1: Plain X-ray showing abnormal position of IUCD, seen at left side of the pelvis.

Statistical Analysis

The statistical analysis was done using Microsoft Excel 2016. Data were statistically described in terms of mean ± standard deviation (±SD), or frequencies (number of cases) and percentages when appropriate.

RESULTS

We managed 32 cases with perforated IUCD who were referred to the Department of Obstetrics and Gynecology - Cairo University Hospital in the period from January 2017 to June 2018. The mean age of cases was 28.38 years ranging from (19 to 42 years) with a

mean body mass index (BMI) of 28.75 kg/m^2. The majority of women lactated (81.25%). The baseline characteristics of women with cases are summarized in **Table 1**. Majority of women had IUCD inserted at the end of the puerperium (71.9%). The average time from insertion to diagnosis was 680.8 days (ranging from 10 to 3285 days). In our study, two thirds of the cases were diagnosed during the first year of insertion with one third within the first month and another one third after 1 year.

The most common presenting complaint was pain (43.75%) followed by being asymptomatic (37.5%). **Figure 2** Pie Chart showing presenting complaints for women with perforated IUCD in our studied population.

Table 1: Baseline characteristics of the women who had perforated IUCD

Variable	Mean ± SD (Range)	N	%
Age (years)	28.38 ± 5 (19-42)	32	100
BMI (kg/m^2)	28.75± 4.63 (22-40.2)	32	100
Gravidity	2.71± 1.48 (1-6)	32	100
Parity	2.34± 1.06 (1-4)	32	100
• **P1**		9	28.1
• **P2**		8	25
• **P3 or more**		15	46.9
Mode of last delivery			
• **NVD**		18	56.3
• **CS**		13	40.6
• **VBAC**		1	3.1
Breastfeeding			
• **Yes**		26	81.3
• **No**		6	18.7
Time interval from delivery to insertion of IUCD (days)	78.125± 65.3 (30-330)		
• 6 weeks or less		23	71.9
• > 6 weeks- 6 months		7	21.9
• 6 months- 1 year		2	6.2
Time interval from insertion to diagnosis (days)	680.8± 1006.2 (10-3285)	32	100
• Less than or equal 1 month		10	31.25
• More than1 month – 1 year		10	31.25
• More than 1 year		12	37.5

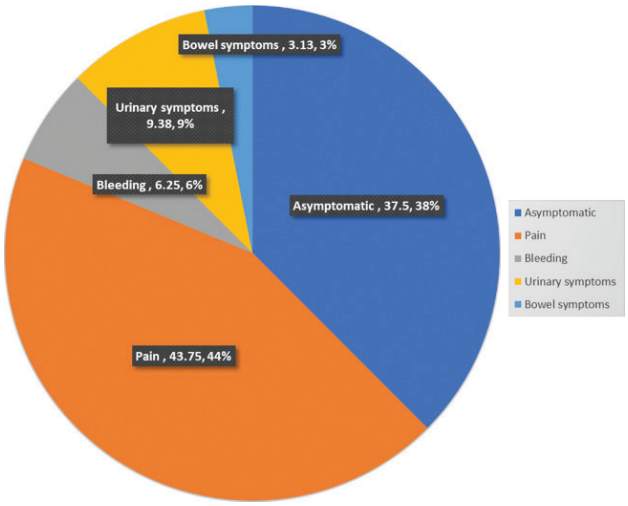

Figure 2: Pie Chart showing presenting complaints for women with perforated IUCD in our studied population.

We questioned women about their experience during the insertion of the IUCD. Only 22 were able to report on that. Out of the 22 women, 16 described insertion as being painful or painful with bleeding while 6 cases described the insertion as being painless and uneventful (27%). None of the cases confirmed having ultrasound immediately after the insertion.

All cases had combined transvaginal and trans-abdominal ultrasound with the aim to locate the IUCD by relating it to the pelvic organs. It was possible to identify the location of the IUCD in 30 cases, and this failed in 2 cases. Figure 3 demonstrates an example for localization of the IUCD using transvaginal scan which showed IUCD perforated into the left ovary with no visible bowel seen around predicting uncomplicated procedure, which was confirmed on laparoscopy.

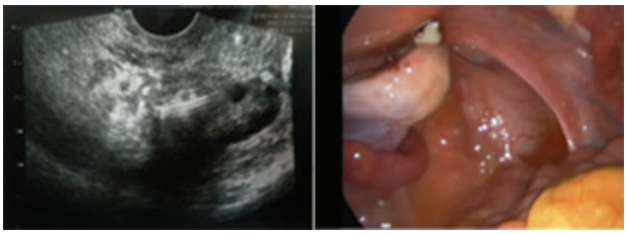

Figure 3 (a) **Figure 3 (b)**

Figure 3: IUCD perforating into left ovary
Figure 3a: TVS showing IUCD perforating into the left ovary , no evidence of bowel surrounding the ovary which was mobile during ultrasound examination
Figure 3b : Laparoscopic view confirming the ultrasound findings

Management of Perforated IUCD:
Four cases had partial perforation, while 28 cases had complete perforation of the uterine wall. One case was managed by laparotomy from the beginning due to high suspicion of bowel involvement. One case was extracted by cystoscopy due to perforation into the urinary bladder. Three cases were partially embedded in the uterine wall and were removed by hysteroscopy under anesthesia due to failed office hysteroscopy. The management of the remaining 27 cases was attempted by laparoscopy, however, two cases were converted to laparotomy; one due to bowel involvement and the other one was due to inability to locate the perforated IUCD, which was found during laparotomy hidden at the ileocecal fold between ileum and cecum. Table 2 showed surgical approach used for management of perforated IUCD. All cases went uneventfully with no complications. Hospital stay was least with cases managed with hysteroscopy, cystoscopy and laparoscopy.

Table 2: Surgical approach used for management of perforated IUCD

	Laparotomy	Cystoscopy	Hysteroscopy	Laparoscopy
Number of cases (%)	1 (3%)	1 (3%)	3 (9%)	27 (85%)
Estimated blood loss (ml)	200 ml	Nil	Nil	61.85 ± 54.9
Hospital stay (days) (mean +/- SD)	6	2	1	2.22 ± 1.18
Complications	Nil	Nil	Nil	Conversion to laparotomy (2 cases)

Intraoperative findings:

The type of IUCD in all cases was Copper T device (100%) as this is the most commonly used nationwide. Three cases had partial perforation into the myometrium. One case perforated the bladder, two cases had bowel perforation. Most common intra-abdominal location of the perforated IUCD was the pouch of Douglas. Table 3 showed location of the perforated IUCD.

Table 3: Location of the perforated IUCD

Location of the IUCD:	Number	Percentage
• Anterior to the uterus	6	18.75
• Above the fundus	3	9.4
• Pouch of Douglas	8	25
• Broad ligament	2	6.25
• Left adnexa	4	12.5
• Right adnexa	2	6.25
• Perforating sigmoid colon	2	6.25
• Ileocecal fold	1	3.1
• Urinary bladder	1	3.1
• Myometrium	3	9.4

In cases with complete perforation of the IUCD into the peritoneal cavity (n=28); abscess formation was observed in 5 cases (4 managed early within 10 days, 12 days, 20 and 25 days of insertion, and one found to have encysted pus collection after 1.5 years). Adhesions were found in 20 out of 28 women (71.42 %) with complete perforation into the peritoneal cavity and was found involving most commonly the omentum (90%) as well as bowel (50%), adnexa (30%) and bladder (15%) as demonstrated in **Figure 4. Figure 5:** demonstrates laparoscopic view of perforated IUCD with adhesions involving the omentum.

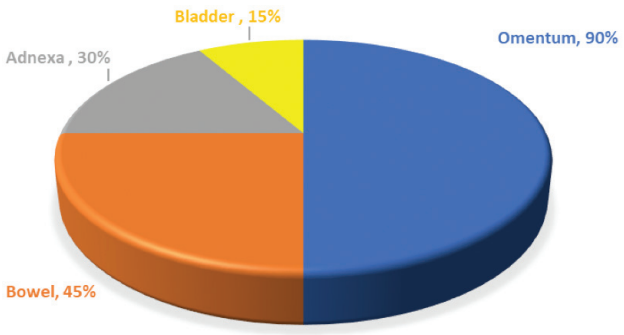

Figure 4: Structured involved in adhesions with perforated IUCD

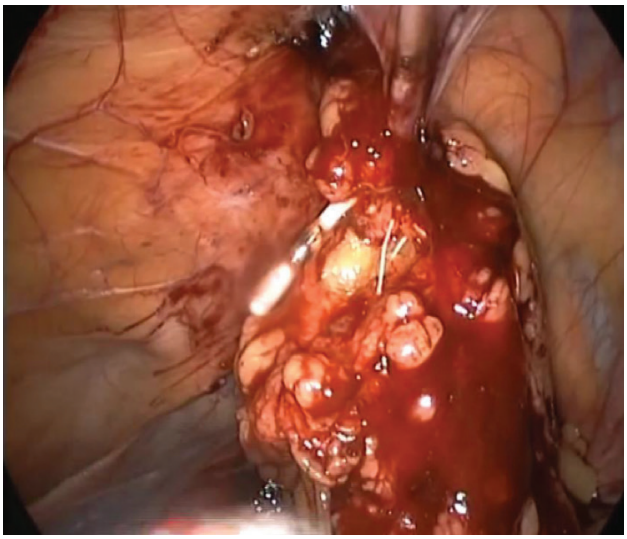

Figure 4: Laparoscopy showing IUCD with adhesions to omentum and anterior abdominal wall

DISCUSSION

IUCD is the most widely used contraceptive method, covering a large scale of the population, especially in developing countries. Though safe, but still uterine perforation by IUCD is considered one of the most serious complications following insertion. In face of under-reporting of the condition, the incidence documented in literature may not reflect the actual prevalence of its occurrence. In addition, there is diversity in risk factors, presentation, means of diagnosis and management.

In this study, we aimed to evaluate cases with perforated IUCD, including risk factors, clinical presentation, preoperative evaluation and management. We assessed 32 cases with confirmed uterine perforation by IUCD, who attended to Gynecology clinic in Kasr El-Aini Hospital, and were examined thoroughly by history taking, examination and investigations (plain X-ray and US).

In all cases (n=32), the type of IUCD was Copper-IUCD, being the cheapest and the most widely available in our community. Several studies have reported more cases of uterine perforation with Copper-IUCD in comparison to hormonal IUCD (known as the Levonorgestrel Intrauterine System or LNG-IUS), probably due to its availability, and patient's intention to use it for a longer time (9-13). In contrary, Turok et al (7), in evaluating 95 cases of uterine perforation; noted more prevalence with LNG-IUS (12), whereas, Jensen et al (14), didn't report such complication in 500 LNG-IUS insertions (9).

Lower parity, in the study by Caliskan et al., was found to be a risk factor for uterine perforation while increasing parity was found to reduce this risk (15). In our study, slightly more cases were encountered in women who were para 2 or less (53.1%) compared to 46.9% women who were para 3 or more. On the other hand, the expulsion rates were reported to be higher in multiparas (6, 8)

In the current study, 81.25% (n=26) of cases were lactating mothers, and the majority (71.9%) were inserted by the end of puerperium. Breast feeding and post-partum period have been proposed by many authors, as one of the risk factors of uterine perforation by IUCD, due to thin uterine wall, endometrial atrophy secondary to hypoestrogenic state, and accelerated uterine involution (10,12,16). Yet, Kho and Chamsy (6) stated that it is probably a reflection of the practice for the timing of insertion, rather than a definite causality. Supporting this is a multicenter 6-month follow-up study in which copper IUCD was inserted in 1,149 women with no perforations reported in any of the women whether breast-feeding or not (17).

It has been reported that most cases of uterine perforation by IUCD were inserted within 1 year after delivery, and the perforation has occurred during the insertion, whether partial or complete (5,6,9,12,13, 18).

In our study, 43.75% of cases had uterine scar due to previous cesarean delivery. Thus, the incidence did not increase with uterine scarring. This support the evidence in literature that previous history of cesarean delivery was not associated with the risk of uterine perforation (15, 19).

The time interval from insertion to diagnosis of a perforated IUCD is interestingly variable. In around 90% of cases, perforation was not recognized at the time of IUCD insertion (20, 21). In our study, almost equal number of cases were diagnosed within the first month, from one month to the first year of insertion and after one year of insertion with two thirds of cases diagnosed within the first year of insertion. This is somehow similar to previous data which reported that almost 50% of cases were diagnosed after 1 year of insertion (20).

Uterine perforation should be thought of in case of painful insertion associated with or without bleeding, however, painless insertion

does not rule out the possibility of uterine perforation. In our study, 27% of cases did not experience pain at time of insertion. Thus, routine ultrasound post-insertion is necessary to confirm correct placement. In a study by Chi et al., it was noted that the insertion of IUCD was less painful in lactating women which may be explained by higher levels of β-endorphins (17).

The most common presenting symptoms in our studied population was abdominal or pelvic pain (43.75%) followed by being asymptomatic (37.5%). Other symptoms included; urinary symptoms (9.38%), vaginal bleeding (6.25%) and bowel symptoms (3.31%). Similar data has been reported by many authors (4,10, 20,22,23). While others reported occurrence of pregnancy as one of the manifestation, or missing strings as the most common presentation (7,11, 24). Since only cases with confirmed uterine perforation were referred to our gynecology unit, we did not encounter cases presented with pregnancy on top of perforated IUCD.

It is of utmost importance to accurately localize the IUCD pre-operatively (25) as this will be of benefit to guide the surgical approach used. Ultrasonography was our first diagnostic tool, together with plain X-ray. We did not require CT scan in any of the cases. Most studies conquered with our diagnostic steps, and, preserved CT scan for complicated cases, in which bowel involvement, or surgical difficulty were suspected (4,6, 15, 22,25, 26, 27, 28). Other diagnostic modalities, such as; fluoroscopy (9,29,30), cystoscopy and proctoscopy (31) have been reported to be used in specific conditions.

The ability of the ultrasound to detect and localize the IUCD depends largely on the type of the IUCD. In the study by Kho and Chamsy, ultrasound could not detect the perforated device in more than 50% of the cases (6). In this study 46% of perforated devices were LNG-IUS. Copper IUCD are easier to detect by ultrasound scan than LNG-IUS as the visualization of the latter relies essentially on the observation of an acoustic shadow rather than visualization of the device itself (32)

A minimal invasive approach was considered in all cases except one case who required laparotomy from the start due to bowel involvement. Laparoscopy was used in 27 cases, however 2 of these cases were converted to laparotomy due to bowel perforation in one case and inability to locate the IUCD in the other case. One case required cystoscopy due to bladder perforation and 3 cases with partial perforation were removed using hysteroscopy. Thus, in our study, minimal invasive approach was successful in 90.6% of cases.

Laparoscopy should be considered the first and mainstay management of cases with perforated IUCD, being both safe and effective (1,4,6,10). Mantoğlu et al (10), reported successful laparoscopic management in almost 99% of their cases, but their study was limited by the small number of cases (n= 10), and their data being retrieved from a single center. On the contrary, the systematic reviews proposed by Gill et al (8), and Mosley et al (11), reported lower percentage of laparoscopic intervention; 64.2% (out of 179 cases) and 72.1% (out of 129 cases) respectively. Still these data should be interpreted with caution; Gill et al (8), was subject of bias, being limited by the quality of the primary studies included. Mosley et al (11), was limited by the long-time span (from 1970 to 2010), during which dramatic changes have occurred in laparoscopic tools and techniques.

Intra-operatively, the missed IUCD was located in various sites, similar to those previously mentioned in the literature (8,10,11), however, the pouch of Douglas was the most common site in which the IUCD was found. This agrees with the study by Zakin et al who found that the pouch of Douglas is the most common location for a completely perforated IUCD (27)

In cases in which IUCD perforated into the peritoneal cavity, abscess formation was noted in 15.6 % of cases, and was seen as early as 10 days and up to 1.5 years following the insertion. Thus, findings from our study suggests that perforating IUCD may induce sterile inflammatory reaction with pus formation in early phases.

On the other hand, adhesions were found in 71.4 % of the cases, most commonly involving the omentum (90% of cases). This agrees with the findings from the study by Sengul et al., 2014 in which IUCD was found most commonly attached to the omentum (33). Rarely, adhesion formation caused by the perforated IUCD resulted in intestinal obstruction (34). Several studies found that adhesion formation was more common with IUCDs than the LNG-IUS (6,35)

In conclusion, physicians should be aware of the risk of IUCD perforation. Post-insertion ultrasonography is essential to confirm correct placement. Regular follow up allows early detection of perforated IUCD. The least invasive approach for extraction of perforated IUCD should always be considered as it allows an enhanced recovery with a more favorable outcome. Ultrasound is an essential tool in the preoperative evaluation and can guide the surgical approach to be used.

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The prevalence of Gestational Diabetes Mellitus among pregnant women with varied hemoglobin concentrations and in relation to the use of Iron Supplemental Therapy

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Abstract

Objectives: To determine the prevalence of gestational diabetes mellitus (GDM) among pregnant women with varied hemoglobin concentrations (HC).

Patients & Methods: 847 newly pregnant women underwent estimation of HC, evaluation of insulin resistance (IR) using the homeostasis model assessment of IR (HOMA-IR) score and glucose tolerance using 75-oral glucose tolerance test (OGTT) at the 6th, 13th, 24th, and 36th gestational week (GW). Women were categorized according to the 6th GW HC into Low (<11 g/dl), Normal (11-<13 g/dl), and High HC (>13 g/dl). Women who had LHC received iron supplemental therapy (IST) till HC was adjusted, and IST was stopped. The study outcome is the incidence of IR and/or GDM among the studied women, and the predictability of the 6th GW HC for the oncoming development of IR and/or GDM.

Results: This is a cross-sectional study conducted at a University Hospital in the time period from January 2017 to June 2018, with the aim to evaluate cases presenting with a confirmed diagnosis of perforated IUCD.

Results: At the 6th GW the frequency of LHC, NHC, and HHC was 22%, 64%, and 14%, respectively. During pregnancy, the frequency of NHC women decreased, while that of HHC women increased. The frequency IR and HOMA-IR score progressively increased during pregnancy in all women with significantly higher frequency and score among HHC women. At the 24th and the 36th GW, 75 and 71 women developed GDM with significantly higher frequency among HHC women than LHC and NHC women and in LHC women compared to NHC women. Statistical analyses defined high HC at the 6th GW as the significant predictor for the development of GDM at the 24th GW, while high BMI at the 6th GW and multiparity as predictors for high HOMA-IR score.

Conclusion: Preconception evaluation of HC is mandatory to define women with low or high HC. Women who had low HC must be managed cautiously using IST till having NHC and IST must be stopped. HHC early in pregnancy

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could predict the oncoming development of IR and GDM.

Keywords: Hemoglobin concentration, Pregnancy, Insulin resistance, Gestational Diabetes mellitus.

Introduction

Gestational glucose intolerance with onset or first recognition during pregnancy is an abnormal initial gestational diabetes mellitus (GDM) screening test (1). Glucose intolerance during pregnancy, even if it did not progress to GDM, is associated with a risk of obstetric and neonatal complications [(2)].

Increased rates of obesity among women of reproductive age, rising maternal age, and the implementation of diagnostic criteria and procedures for GDM lead to a continuously rising international prevalence of GDM (1). The screening of GDM depends mainly on the oral glucose tolerance test (OGTT) at 24-28 gestational weeks (GW); however, early diagnosis and intervention of GDM may limit the development of or improve the previously documented adverse pregnancy outcomes [(3)].

Anemia is a global health concern, and iron deficiency anemia (IDA) affects about 50% of cases especially pregnant women [(4)] due to depletion of body iron stores to cope with the high demand for iron to maintain fetal and placental iron metabolism [(5)]. Thus, maintaining adequate iron status during pregnancy through replenishment of maternal iron stores is mandatory for both the mother and the developing fetus [(6)].

The normal iron content in adults is about 60 g/dl; however, iron overload has deleterious effects and is involved in the development of cancer, type 2 diabetes, and cardiovascular conditions [(7)], and can affect the normal functioning of the innate and adaptive immune responses [(8)], promote the generation of reactive oxygen species and cell oxidative stress [(9)] and brain iron accumulation causes neurodegenerative diseases [(10)].

Gestational IDA and DM are two prevalent pregnancy-induced complications that affect maternal and neonatal outcomes. On the other side, the need for iron supplemental therapy (IST) to correct IDA is essential but as previously documented iron overload may induce the development of DM; such dilemma requires to be explored. Thus, the current study aimed to determine the prevalence of GDM among pregnant women with varied hemoglobin concentrations (HC).

Design

A prospective interventional comparative study.

Setting

Department of Obstetrics & Gynecology, Faculty of Medicine, Benha University.

Ethical consideration

The study protocol was approved by the Local Ethical Committee at Benha Faculty of Medicine by approval number: RC: 4-2-2022.

Patients

During the duration of the study since Jan 2019 till Oct 2021, women attended the Antenatal Care Unit, Benha University Hospital for assurance of being pregnant underwent history taking as regards the presence of a history of GDM or IDA during the previous pregnancies, current DM, family history of DM, hormonal disturbances, nutritional deficiencies, bleeding attacks, preconception menstrual disturbances especially polymenorrhagia and menorrhagia, drug intake, food hypersensitivity, previous treatment for any grade of dyspepsia, or maintenance on peptic ulcer treatment. Then, women underwent clinical examination including determination of body height and weight, and body mass index (BMI) was calculated as weight (kg)/ height (m^2) as a baseline BMI.

Exclusion criteria

Exclusion criteria included current DM, previous GDM, multiple pregnancies, fetal abnormalities, BMI of >35 kg/m^2, previous history of bleeding episodes, maintenance on IST, liver, or kidney diseases.

Inclusion criteria

Newly pregnant women with singleton fetuses, free of exclusion criteria and accepted to sign the written fully informed consent to attend the follow-up visits were enrolled in the study.

Evaluation tools

1. Hemoglobin (Hb) hemoglobin concentration was estimated to detect gestational IDA that was defined as HC <11 g/dl in early or late pregnancy or <10.5 g/dl in mid-pregnancy [11].
2. The homeostasis model assessment of IR (HOMA-IR) score for detection of IR women using >2 as diagnostic cutoff point [12].
3. Glucose intolerance was assessed using the 75-oral glucose tolerance test (75-OGTT) according to the previously documented levels [13] as follows: FBG ≥92 mg/dl and 2-hr PPBG ≥153 mg/dl indicated development of GDM.

Investigations

Four blood samples were obtained at the 6th, 13th, 24th and 36th GW and at the start of each trimester for estimation of blood glucose and hemoglobin contractions and ELISA estimation of serum human insulin (catalog no. ab200011, Abcam Inc., San Francisco, USA) [14].

Grouping

- According to HC estimated at the 6th GW, the enrolled women were categorized into three groups: low HC (LHC) if HC was <11.0 g/dl, Normal HC (NHC) if HC was in the range of 11-<13 g/dl, and high HC (HHC) if HC was ≥13 gm. %.
- According to the HOMA-IR score determined throughout the pregnancy course, women who had HOMA-IR scores of >2 were diagnosed as IR women.
- According to glucose intolerance as evaluated by 75-OGTT throughout the pregnancy course women were categorized as having GDM or no GDM.

Iron Supplemental therapy

During the duration of the study since Jan Iron supplemental therapy (IST) was provided as ferrous bisglycine sulfate complex (Ferrous glycine sulfate 487 mg equivalent to 80 mg elemental iron + Folic acid 1 mg; Minapharm, Egypt; Schwarz Pharma), which is composed of 2 glycine molecules bound to a ferrous cation to form a double heterocyclic ring compound that can protect the iron from dietary inhibitors of absorption of non-heme iron and intestinal interactions, so it has high bioavailability (15). In addition, vitamin C was given to enhance the bioavailability of iron from the bis-glycine chelate (16). Vitamin C was given as an effervescent tablet (Vitacid C effervescent tablets, Cid Co Egypt), a form that enhances the absorption and metabolism of vitamin C when taken after a meal. Ferrous bisglycine sulfate complex was preferred for the inverse relationship between the absorption of amino-chelated iron with body iron stores (17). However, it was prescribed only to women who had HC <11 g/dl to guard against iron overload, and once HC was adjusted, IST was stopped.

Study outcomes

1. The primary outcome is the incidence of IR and/or GDM among studied women.
2. The secondary outcomes include:

- The relation between HC estimated at the

6th GW and the disturbed glucose homeostasis variables at the 24th GW.

- The predictability of HC was estimated at the 6th GW and preconception patients' data for the disturbed glucose homeostasis variables at the 24th GW.

Statistical analysis

Results were analyzed using paired t-test, One-way ANOVA, Chi-square (X2 test) and Mann-Whitney tests by IBM® SPSS® software (Version 22, 2015; Armonk, USA). Spearman's correlation analysis was applied to evaluate correlations between patients' data and HC estimated at the time of pregnancy diagnosis (the 6th GW) and the disturbed glucose homeostasis variables at the 24th GW. The ROC curve was used to determine the predictors of the development of GDM among patients' data and the 6th GW HC. The Regression analysis using the Stepwise method was applied to determine the predictability of the correlated variables for the disturbed glucose homeostasis variables. Significance was documented if P-value was <0.05.

Results

During the duration of the study, the data of 847 women were analyzed. Estimated HC at the 6th GW defined 186 women with low HC (LHC), 542 women had normal HC (NHC) and 119 women had high HC (HHC; Fig. 1); demographic and clinical data that were obtained at the 6th GW were shown in table 1.

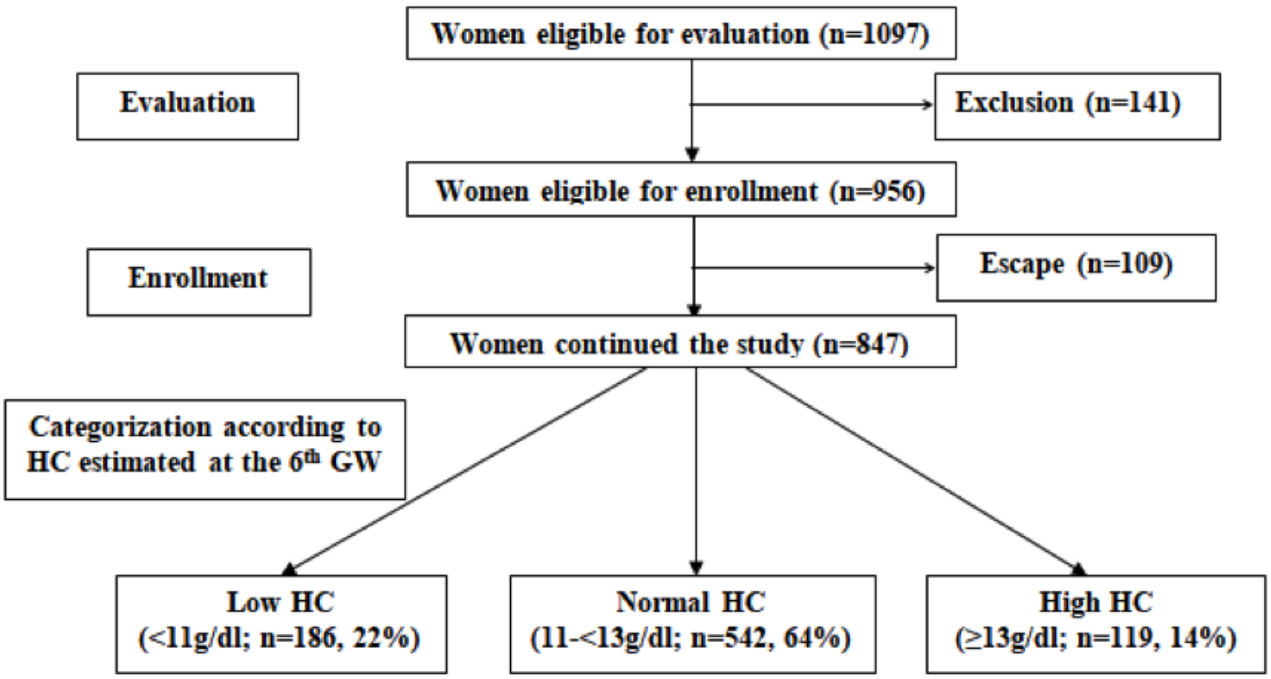

Fig. (1): Study Flow Chart

Table 1: Demographic and clinical data of studied women

Data	Findings
Age (years)	28 (2.8)
Weight (kg)	82.9 (6.4)
Height (cm)	169.7 (3.4)
Body mass index (kg/m^2)	28.8 (2.2)
Gravidity	2 [1-2]
Parity	1 [1-2]
Systolic blood pressure (mmHg)	114.7 (7.7)
Diastolic blood pressure (mmHg)	75.9 (5.7)

Pregnancy affected hemoglobin concentration (HC) in various directions, but the frequency of women who had NHC decreased with the progress of pregnancy with a significantly lower frequency of women who had NHC at the 13th, 24th, and 36th GW in comparison to the frequency at the 6th GW. The frequency of women who had LHC fluctuates during pregnancy reaching a summit at the 13th GW. As a consequence of IST, the frequency of women had HHC increased progressively reaching a summit at the 24th GW (Table 2).

Table 2: Women distribution according to estimated levels of HC during the pregnancy course

		Time of estimation				Actual P-value for differences					
		6th GW	13th GW	24th GW	36th GW	6th vs. 13th	6th vs. 24th	6th vs. 36th	13th vs. 24th	13th vs. 36th	24th vs. 36th
LHC	Frequency	186 (22%)	217 (25.6%)	177 (20.9%)	195 (23%)	0.077	0.594	0.601	0.021	0.213	0.291
	HC (g/dl)	10 (0.37)	10.1 (0.49)	9.97 (0.6)	10.02 (0.52)	0.275	0.855	0.997	0.047	0.375	0.754
NHC	Frequency	542 (64%)	488 (57.6%)	501 (59.1%)	497 (58.7%)	0.0072	0.041	0.025	0.522	0.886	0.658
	HC (g/dl)	12 (0.54)	12 (0.59)	12.05 (0.56)	12.03 (0.54)	0.766	0.220	0.391	0.392	0.712	0.664
HHC	Frequency	119 (14%)	142 (16.8%)	169 (20%)	155 (18.3%)	0.122	0.0012	0.0175	0.091	0.406	0.387
	HC (g/dl)	14 (0.58)	13.9 (0.62)	14.11 (0.6)	13.99 (0.58)	0.834	0.171	0.985	0.0208	0.633	0.324

HC: Hemoglobin concentration; LHC, NHC, HHC: Low, Normal, and High HC

The calculated HOMA-IR score showed a progressive increase in the frequency of IR women and a score with the progress of pregnancy in all studied women. Interestingly, at the 6th GW, there were 77 IR women (9.1%); 11 LHC women (5.9%), 35 NHC women (6.5%), and 31 HHC women (26.1%) with significantly (P<0.001) higher frequency of IR women among HHC women at the time of diagnosis of pregnancy in comparison to frequency among LHC and NHC women, despite the non-significantly higher score in comparison to score determined for LHC (P1=0.083) and NHC (P2=0.823). At the 13th GW, 140 women were IR (16.5%) with significantly (P<0.001) higher frequency among HHC women in comparison to LHC and NHC women and significantly higher score in comparison to LHC (P1=0.031) and NHC (P2=0.021) scores. At the 24th and the 36th GW, 262 (30.9%) and 299 women (35.3%),

respectively, became IR with significantly (P<0.001) higher frequency and score among HHC women in comparison to women of other groups and significantly (P1<0.001) higher score among NHC women compared to LHC women (Table 3).

At the 6th and 13th GW, no case of GDM was reported, while at the 24th GW, 75 women (8.85%) developed GDM with significantly higher frequency among HHC women in comparison to LHC women (P1=0.0022) and NHC women (P2<0.001) and significantly (P1=0.015) higher frequency of women who developed GDM among LHC women compared to NHC women. At the 36th GW, 146 women (17.2%) became diabetic with significantly higher frequency among HHC women compared to the frequency among LHC (P1=0.0001) and NHC women (P2<0.001) and significantly (P1=0.037) higher frequency among LHC women compared to NHC women. At the 6th GW, the estimated levels of FBG and 2-hr PPBG showed non-significant differences between the studied women. At the 13th and 24th GW, both FBG and PPBG measures were significantly lower in LHC women in comparison to NHC and HHC women, with non-significantly higher FBG, but significantly higher PPBG measures in HHC women compared to NHC women. At the 36th GW, both FBG and PPBG measures were significantly higher in HHC women in comparison to LHC and NHC women with significantly higher BG measures in NHC women compared to LHC women (Table 3).

Table 3: Insulin resistance and glucose intolerance data reported during pregnancy course of studied women.

		6th GW	13th GW	24th GW	36th GW
Insulin resistance as judged by HOMA-IR score					
LHC	Frequency	11 (5.9%)	34 (15.7%)	44 (24.9%)	47 (24.1%)
	Score	0.96 (0.4)	1.18 (0.48)	1.29 (0.6)	1.55 (0.65)
NHC	Frequency	35 (6.5%)	57 (9.7%)	115 (23%)	133 (26.8%)
	P1 value	0.732	0.334	0.607	0.884
	Score	1 (0.32)	1.2 (0.4)	1.57 (0.53)	1.87 (0.51)
	P1 value	0.271	0.988	0.0001	0.0001
HHC	Frequency	31 (26.1%)	49 (34.5%)	103 (60.9%)	119 (76.8%)
	P1 value	0.00022	0.0005	0.00001	0.00014
	P2 value	0.00015	0.00026	0.00001	0.00019
	Score	1.11 (0.62)	1.3 (0.66)	1.81 (0.78)	2.28 (0.74)
	P1 value	0.083	0.031	0.0008	0.00001
	P2 value	0.823	0.021	0.061	0.00011
Total frequency of IR		77 (9.1%)	140 (16.5%)	262 (30.9%)	295 (34.8%)
Glucose intolerance and development of GDM as judged by 75-OGTT					
LHC	Frequency*	0	0	16 (9%)	34 (17.4%)
	FBG	77.8 (7.8)	79 (6.7)	83.1 (7.4)	87.5 (8.2)
	2hr PPBG	118.5 (5.4)	119 (8.1)	127.6 (13.1)	135.1 (15.5)

NHC	Frequency*	0	0	24 (4.8%)	57 (11.5%)
	P1 value	-	-	0.015	0.037
	FBG	79.2 (4.6)	82.5 (4.3)	85.8 (5.8)	88.6 (6.7)
	P1 value	0.063	<0.001	<0.001	0.094
	2hr PPBG	117.7 (7.7)	125.7 (7.8)	133.3 (9.7)	141.4 (12)
	P1 value	0.982	0.00085	0.00072	0.0009
HHC	Frequency*	0	0	35 (20.7%)	55 (35.5%)
	P1 value	-	-	0.0022	0.0001
	P2 value	-	-	0.0001	0.00001
	FBG	78.8 (5.4)	82.1 (5)	88.8 (7.5)	92.6 (8.9)
	P1 value	0.274	0.0009	0.00016	0.00001
	P2 value	0.615	0.886	0.521	0.00001
	2hr PPBG	118 (6.8)	127.5 (7.6)	138.1 (15.8)	152.1 (16.4)
	P1 value	0.722	0.0007	0.00012	0.00001
	P2 value	0.827	0.032	0.0096	0.00001
Total frequency of GDM		0	0	75 (8.85%)	146 (17.2%)

P1 value indicates the significance of difference versus LHC women; P2 value indicates the significance of difference versus NHC women

Spearman's correlation analysis showed a positive significant correlation between the development of GDM and women's age, BMI, parity, and the 6th GW HC, in increasing order of significance. The 24th GW FBG and PPBG levels and the calculated HOMA-IR score showed a positive significant correlation with BMI and the 6th GW HC, while the calculated HOMA-IR score also showed a positive significant correlation with parity (Table 4). ROC curve analysis defined high 6th GW HC as the highly significant predictor for the development of GDM at the 24th GW with AUC= 0.653 (SE= 0.039, P-value <0.001; 95% CI: 0.576-0.729), followed by high BMI with AUC= 0.584 (SE= 0.036, P-value =0.015; 95% CI: 0.513-0.655), high parity rate with AUC= 0.419 (SE= 0.036, P-value =0.019; 95% CI: 0.347-0.490), and finally higher age with AUC=0.420 (SE= 0.033, P-value =0.021; 95% CI: 0.354-0.485), (Fig. 2). Regression analysis defined high HC estimated at the 6th GW as a significant predictor for high FBG, PPBG, and HOMA-IR score at the 24th GW, while high BMI at the 6th GW was also a predictor for high PPBG and HOMA-IR score, and multiparity as a predictor for high HOMA-IR score (Table 4).

Table (4): Statistical analyses for variables determined at the 6th GW as predictors for disturbed glucose homeostasis at the 24th GW.

Variables	Development of Gestational diabetes		24th GW fasting blood glucose		24th GW Post-prandial blood glucose		24th GW Insulin resistance score	
Spearman's correlation analysis								
	Rho.	P-value	Rho.	P-value	Rho.	P-value	Rho.	P-value
Age	0.080	0.019	0.042	0.217	0.043	0.214	0.011	0.869
BMI	0.083	0.015	0.080	0.020	0.072	0.035	0.105	0.002
Gravidity	0.027	0.435	0.019	0.591	0.013	0.845	0.067	0.051
Parity	0.086	0.012	0.053	0.124	0.023	0.508	0.115	0.001
6th GW HC	0.151	0.00087	0.197	0.0009	0.307	0.00014	0.230	0.0001
Regression analysis								
	24th GW FBG				24th GW PPBG		24th GW HOMA-IR score	
	β		P-value		β	P-value	β	P-value
Age	0.062		0.067		0.033	0.312	0.002	0.962
BMI	0.061		0.073		0.079	0.018	0.102	0.001
Gravidity	0.315		0.010		0.762	0.021	0.710	0.315
Parity	0.022		0.514		0.018	0.587	0.084	0.013
6th GW HC	0.196		0.0005		0.263	0.00034	0.231	0.00041

GW: Gestational week; BMI: Body mass index; HC: Hemoglobin concentration

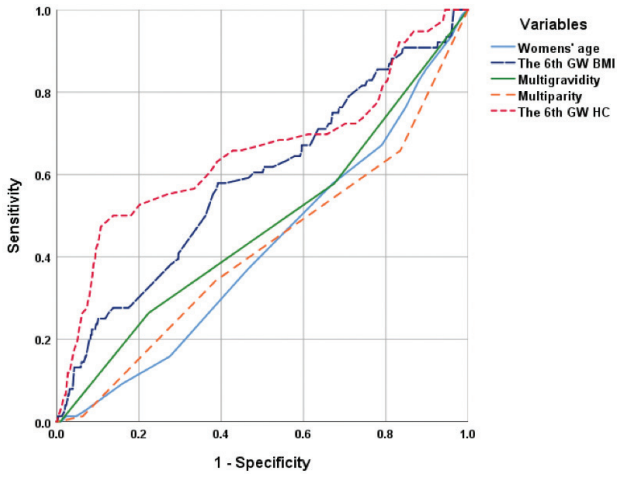

Fig. (2): ROC curve analysis of women's data determined at the 6th GW as predictors for oncoming GDM.

Discussion

Estimation of hemoglobin concentration (HC) at the time of diagnosis of pregnancy (the 6th GW) defined a prevalence of women who had low HC of 22%. This figure is coincident with that recently detected in multiple surveys in varied countries all over the world (18-20) and indicated the necessity of preconception evaluation of HC to allow correction of anemia before getting pregnant. On the other side, the current study detected a prevalence of high hemoglobin concentration (HHB) of 14%; a figure that is in agreement with that recently detected by Amarasinghe et al. [20].

Evaluation of insulin resistance (IR) at the 6th GW detected an incidence of IR of 9.1% despite the blood glucose (BG) measures being within normal range and lower than that defined by IADPSG for diagnosis of diabetes. Moreover, the incidence of IR early in pregnancy was positively correlated with body mass index (BMI) and increased progressively during the pregnancy course, so there were 299 IR women (35.3%) at the 36th GW. Also, the obtained results of the 75-OGTT at the 24th and 36th GW defined 75 and 71 women had developed GDM for a total incidence of 17.2%.

These findings spotlight a fact that pregnancy per se is a diabetogenic state of the type-2 category because it is associated with IR as evidenced by the positive relation between FBG levels estimated at the 24th and 36th GW and HOMA-IR score and with at the 6th GW BMI. In line with these findings and assumptions, Gorkem et al. [21] reported significantly higher serum insulin levels and HOMA-IR values in GDM women than in control pregnant women. Thereafter, Psoinos et al. [22] detected a high incidence of pre-pregnancy subclinical IR with a positive association between HOMA-IR and BMI, amount of subcutaneous fat, high serum inflammatory markers, and high possibility of complicated pregnancy. Also, Bano et al. [23] detected a prevalence of IR, gestational glucose intolerance, and GDM among their series of pregnant women of 27.9%, 22.05%, and 52.2% and detected a significant correlation between IR, BMI, and GDM.

Moreover, the detection of 71 GDM women at the 36th GW indicated the necessity of continuous follow-up of pregnant women for the disturbed glucose homeostasis variables till the end of pregnancy without reliance only on estimations determined at the 24th GW. In hand with this finding and suggestion, multiple recent studies [24-26] indicated the necessity of the detection of late-pregnancy GDM because it is associated with adverse fetomaternal outcomes.

Multiple studies tried to explore the underlying mechanisms for the development of gestational IR and DM, whereas Gorkem et al. [21] attributed the development of gestational IR and DM to the detected higher levels of serum placental growth factor that was positively correlated with serum insulin levels and HOMA-IR scores. Recently, gestational IR was attributed to adipose tissue inflammation and endoplasmic reticulum stress which promote adiponectin degradation [27]. Another recent study attributed IR during pregnancy to the development of non-alcoholic fatty liver secondary to mitochondrial dysfunction causing oxidative stress and to epigenetic mechanisms related to alterations in genes involved in lipid metabolism, and inflammation [28].

Interestingly, women with at the 6th GW HHC showed the highest prevalence of GDM that was significantly higher than its prevalence in women with LHC or NHC. Moreover, there was a positive significant correlation between 6th GW-HC and FBG estimated at the 24th GW and statistical analyses defined HHC early in pregnancy as a significant predictor for oncoming development of GDM. In support of these results, an early study suggested that HHC during early pregnancy can predict the risk of GDM [29] and a meta-analysis detected that women with LHC are 39% less likely to develop GDM than women with NHC or HHC [30]. In line with the obtained results, Abumohsen et al. [31] found women who had HHC in the 1st trimester were at higher risk of high FBS, and Li et al. [32] documented that HHC is a risk factor for developing GDM than NHC with increased risk of GDM with higher HBC.

Recently, Sissala et al. [33] found mothers with GDM had higher HBC than controls, and HBC is positively associated with preconception BMI, FBG, and glucose levels at the time of diagnosis of GDM and using multivariable regression analysis HBC remained an independently associated parameter for GDM. Also, Eidgahi et al. [34] found hematocrit value, HBC, and FBG estimated in the 1st and 2nd pregnancy trimesters were significantly higher in GDM women in comparison to non-GDM women and the concurrent use of these variables could predict GDM with AUCs of 87%, 70%, and 83%.

The reported progressively increasing prevalence of IR from 5.9% at the 6th GW (5.9%) to 24.1% at the 36th GW and of GDM from 9% at the 24th GW to 17.4% at the 36th GW among LHC women, who received iron supplemental therapy (IST),

points to a diabetogenic effect of IST and support the relation between HHC and GDM. Similarly, Zhang et al. [35] documented that long-duration peri-conception IST for anemic pregnant women is associated with increased GDM risk. Recently, Rajendran et al. [36] found IST in anemic pregnant women improves hematological status and decreased inflammation, but once these women became non-anemic, continued IST increases oxidative stress and inflammation.

The pathogenic mechanisms for the relation between HHC and development of IR and type-2 diabetes mellitus was still a matter of debate; however, an earlier study attributed this coincidence to the impaired function of pancreatic islet β cells that affects insulin secretion [37]. Another study attributed the IR to the altering effect of iron on the expression of insulin receptors in hepatocytes [38]. A more recent study had focused on the relationship between iron overload and increased oxidative stress which inhibits insulin internalization and function leading to hyperinsulinemia and IR with HHC [41]. Recently, genetic evidence was provided to support a causal link between increased systemic iron status and increased risk of type-2 diabetes [40].

Conclusion

Variability of HC among newly pregnant women is evident and necessitates preconception evaluation to define women with low or high HC. Women who had low HC must be managed cautiously using IST till having NHC and IST must be stopped. HHC early in pregnancy could predict the oncoming development of IR and GDM even in normoglycemic women.

Limitation

Evaluation of pregnancy outcome and the effect of hemoglobin variability, IR, and GDM on maternal and fetal welfare

Recommendation

Judicious use of IST during pregnancy is mandatory to reduce the incidence of IR and/or GDM and improve maternal and fetal outcomes. Evaluation of glucose homeostasis parameters must be continued till the end of pregnancy to detect the development of late GDM.

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Vitamin D Supplemental Therapy might improve the outcome of Intracytoplasmic Sperm Injection for Women had Diminished Ovarian Reserve

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Abstract

Objectives: Evaluation of the relation between serum levels of 25-hydroxy vitamin D (25-OHVD), tumor necrosis factor-α (TNF-α) and interleukin (IL)-4 and outcome of intracytoplasmic sperm injection (ICSI) for infertile women secondary to diminished ovarian reserve (DOR).

Patients & Methods: 82 infertile DOR women, defined according to Bologna Criteria, were evaluated and gave blood samples for estimation laboratory parameters before and after receiving 12-w VD supplemental therapy (VDST). All women received the ovarian stimulation flexible antagonist protocol, ICSI was performed, and the day-3 embryos were graded. All women who had no fertilization or had embryos of grade P-G2 underwent another session of ICSI after VDST.

Results: Pre-VDST, 77 had VD deficiency (VDD) and 5 had insufficiency, Post-VDST 65 women had VDD, 14 had insufficiency and 3 women had sufficient level. Post-VDST AFC was increased by 85.3% and serum levels of 25-OHVD, IL-4, and anti-Müllerian hormone were increased by 31.7%, 18.66%, and 6.18%, respectively, while TNF-α levels were decreased by 12.8% with significantly lower frequency of embryos of poor and fair grades. ROC curve analysis defined pre-VDST high serum levels of 25-OHVD and IL-4, and high AFC as positive, while high levels of TNF-α and high BMI as negative predictors for the high quality embryo.

Conclusion: VDD, obesity, and deregulated immune milieu in direction of inflammation may predispose or aggravate poor ovarian response to OS. VDST improved ovarian function and quality of oocytes with subsequent improvement of embryo quality most probably through equalization of immune milieu to the direction of anti-inflammatory.

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INTRODUCTION

Menopause represents the definite end of a woman's reproductive life and the onset of a persistent hypoestrogenic state and despite the increased mean menopausal age, a significant individual variation in age at natural menopause is evident [1].

The term diminished ovarian reserve (DOR) is applied clinically to infertile women who were predicted or thought to have a poor ovarian response (POR) to ovarian stimulation (OS) [2]. POR is accepted as a manifestation of DOR and early ovarian aging and a woman was diagnosed to have POR if she fulfilled at least two of the Bologna Criteria [3] and retrieval of ≤3 oocytes was applied as the cutoff to discriminate women with POR [3].

The balance between T helper 1 (Th1) and T helper 2 (Th2) cytokines plays a critical role in the immune response and multiple clinical processes [4]. The shift of the ratio between the pro-inflammatory (Th1) cytokines, as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, and anti-inflammatory (Th2) cytokines as IL-10 and IL-4 toward a predominance of Th1 may represent more severe inflammatory status [5].

Vitamin D (VD) is a fat-soluble vitamin involved primarily in calcium metabolism [6]. However, experimental studies assured that VD is a promising agent with a remarkable ability to decrease the severity of inflammation [7]. Moreover, based on growing evidence, a role of VD was postulated in reproductive health in both animals and humans [8].

Vitamin D deficiency (VDD) is a significant public health concern all over the world especially for being associated with many immune-mediated diseases [9]. VD supplemental therapy (VDST) was found to improve general and orthopedic health after bariatric surgeries [10], to have significant effects on blood pressure, abdominal obesity, and insulin and glucose metabolism in patients with metabolic syndrome [11], and of potential importance in women with or at a high risk of uterine fibroid development [12].

Objectives

This study targets to evaluate the relation between VD sufficiency status and serum inflammatory milieu, and the outcome of assisted reproductive technologies (ART) for infertile women secondary to DOR.

Design

Prospective interventional double-blinded study.

Setting

Departments of Obstetrics and Gynecology, and Medical Biochemistry, Faculty of Medicine, Benha University, and multiple private centers in Benha and Cairo; Egypt.

Ethical consideration

The study protocol was approved by the Local Ethical Committee at Benha Faculty of Medicine; RC2-4-2021. The enrolled women must sign their consent to participate in the study, give blood samples for required investigations and undergo receive the prescribed therapies. Blindness means that enrolled women will be blinded about the type of investigations and the biochemist will be blinded about the indication for studying these parameters and about the demographic data of studied women.

Patients & Methods

All women attending the Infertility Units seeking assisted pregnancy due to primary or secondary infertility were eligible for evaluation. All women underwent clinical and US evaluation to determine the possible cause of infertility and gave blood samples for routine and study parameters' evaluation.

Exclusion criteria

Age older than 40 years, male factor infertility, infertility secondary to endocrinopathy, congenital malformation, exposure to radio- or chemotherapy, ovarian or uterine diseases, obesity grade II with body mass index >35 kg/m2, patients had serum 25-hydroxy vitamin D (25-OHVD) level >75 nmol/l, or refusal to participate in the study.

Inclusion criteria

Infertile women aged <40 years, had BMI <35 kg/m^2, free of causes of infertility other than DOR, and signed the written fully informed consent to participate in the study and undergo the assigned investigation and receive the appropriate therapies were included in the study. Ten fertile women with age- and BMI cross-matched with DOR women and free of inclusion and exclusion criteria were enrolled as a control group for lab parameters.

Evaluation tools

1. Evaluation of ovarian reserve (OR) according to Bologna criteria including antral follicle count (AFC) and anti-Müllerian hormone (AMH) levels within ranges of <5–7 follicles &/or <0.5–1.1 ng/ml, respectively [13].
2. Evaluation of VD sufficiency status: women who had serum 25-OHVD level of ≥75 nmol/L were considered to have sufficient VD level and were excluded from the study, while women who had serum levels in the range of 50-75 nmol/L were considered to have insufficient VD level and women had serum level of <50 nmol/L were considered to have VDD and were categorized as mild, moderate and severe VDD if 25-OHD serum levels were 25-50 nmol/L, 12.5-25 nmol/L and <12.5 nmol/L, respectively [14].

Investigations

1. **US evaluation:** AFC was determined on the day 3 of the cycle by trans-vaginal ultrasound (TVU)
2. **Laboratory investigations**

   Two blood samples were obtained before (Pre-VDST) and after VDST (Post-VDST); 5 ml blood were withdrawn under complete aseptic conditions, allowed to clot, and then centrifuged at 3000 rpm for 10 minutes to separate serum that was collected in a sterile Eppindorff tube and stored at -80oC till be assayed. Blood samples were collected and numbered by an assistant who was blinded about the indication for investigations.

Studied lab parameters

Serum levels of anti-Müllerian hormone (AMH), 25-hydroxy vitamin D (25-OHVD), human tumor necrosis factor-α (TNF-α), and interleukin 4 (IL-4) were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions and were read using a 96 well microplate ELISA reader (Dynatech. MR 7000)

1. Human serum AMH level was measured with an ELISA kit (catalog no. ab267629 Abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique [15].
2. Human serum 25-OHVD level was measured with an ELISA kit (catalog no. ab213966 Abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique [16].
3. Human serum TNF-α level was measured with an ELISA kit (catalog no. ab46087 Abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique [17].
4. Human serum IL-4 level was measured with an ELISA kit (catalogue no.

ab100570 Abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique [18].

Study protocol

ll women who fulfilled the inclusion criteria received the ovarian stimulation protocol according to hospital guidelines using the gonadotrophin-releasing hormone (GnRH) flexible antagonist in the form of subcutaneous injection of Gonal F in a dose ranging between 300-650 IU (75 IU; 5.5µg, Merck Serono Ltd, UK) daily from the 2nd day of the cycle in conjunction with cetrorelix (Cetrotide®, Merck, Germany) 250 µg daily starting when the dominant follicle reached 14 mm till the day of Human chorionic gonadotrophin (hCG) injection. The hCG injections using choriomon, (10000 units; IBSA; Switzerland) was injected in addition to two ampoule triptoreline acetate (Decapeptyl, Ferring Pharmaceuticals Ltd., Wittland, Germany; 0.1 mg, subcutaneous injection) as co-triggering when the mean diameter of the leading follicle reached ≥18 mm or >3 follicles reached a mean diameter of ≥16 mm followed by TVU-guided oocyte retrieval 36 hours later and fertilization was carried out by intracytoplasmic sperm injection (ICSI).

Embryo grading

The day-3 embryos are graded as good (G grade) if it contains 6-9 symmetric cells with no fragmentation, as fair (F grade) if cells are symmetric but there is only minor fragmentation and as poor (P grade) if cells are asymmetric with no or moderate fragmentation (19). Women had embryos of grade G1 were excluded from the study and those had no fertilization or embryos of P, F or G2 grade received the 12-wk VDST and were re-evaluated for AFC and the previously estimated lab parameters levels and undergo another session of OS and ICSI.

Protocol for VDST

All patients were provided with 12-wk vitamin D3 ST as a once-daily oral dose of 5000 IU soft gels (Sunvite Mega Potency Vitamin D3 5000 IU, Puritan's Pride, Inc., Oakdale, NY, USA), which was proved to be safe for correction of VDD [20]. VD soft gels must be taken with a meal to aid in the absorption of this fat-soluble vitamin [21].

Study outcomes

1. The primary outcome is the effect of VDST on the quality of the embryo on the 2nd session of ICSI for women with POR.
2. The secondary outcomes included:
   - The relation between the extent of change of serum 25-OHVD and embryo quality grade
   - The predictability of Pre-VDST serum levels of studied lab parameters and the Pre-VDST embryo grade and between the extent of change in serum levels of studied parameters and the Post-VDST embryo grade.

Statistical analysis

Obtained data were presented as mean, standard deviation, numbers, and percentages, median and interquartile range. Results were analyzed using paired t-test for analysis of differences between Pre- and Post-VDST variables and Chi-square test (X2 test) and Mann-Whitney test for analysis of non-numeric data. Pearson's correlation analysis was applied to evaluate correlations between variables and the grade of embryos. A receiver characteristic curve was used to determine the predictors of embryo grades among the correlated variables. Statistical analysis was conducted using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) for Windows statistical package. P-value <0.05 was considered statistically significant.

Results

During the study duration from June 2019 till April 2021, 416 infertile women attended the ART Unit and were evaluated for inclusion criteria, 311 women were excluded mostly for having infertility due to other causes than POD or out of the enrolment age range, 17 women refused to sign the written consent to attend two session of ICSI if the 1st had failed and 6 women were excluded for having sufficient VD serum level (>75 nmol/ml), while 82 women with mean age of 36±1.8 (range:33-39 years) and BMI of 31.6±1.7 (range: 26.6-34.6 kg/m2) were enrolled in the study and completed the study protocol (Fig. 1).

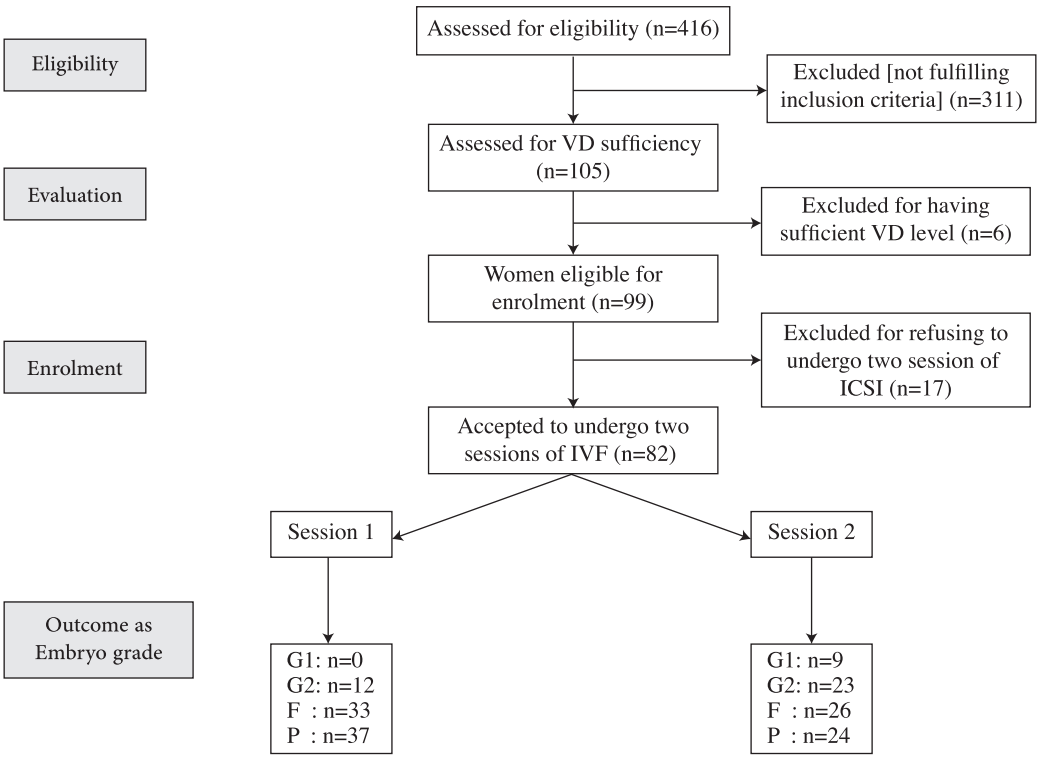

Figure 1: Consort Flow sheet

At the time of enrolment, 77 women (93.9%) had VDD, while only 5 women (6.1%) had insufficient VD levels. After the end of 12-wk VDST, no woman had severe VDD, 65 women (79.2%) had VDD, 14 women (17.1%) had insufficient VD level and 3 women (3.7%) had sufficient VD level with significantly (P=0.0002) lower frequency of women had VDD after VDST in comparison to Pre-VDST. Moreover, the mean value of 25-OHVD estimated Post-VDST was significantly (P=0.0007) higher than its Pre-VDST level with a mean percentage of increase of 31.7 (±10.8%). There were non-significant (P=0.055) differences between studied women as regards the Pre- and Post-VDST frequency of women who had serum TNF-α levels higher than the median of TNF-α level estimated in control samples. However, the mean value of serum TNF-α level estimated Post-VDST was significantly (P=0.0006) lower in comparison to the mean value of Pre-VDST estimated levels with the percentage of decrease of 12.8 (±7.4). On contrary, women's frequency according to serum IL-4 levels estimated Post-VDST showed a significant (P=0.00001) difference in comparison to Pre-VDST frequency in relation to the median value of IL-4 estimated in control samples. Also, Post-VDST levels of IL-4 were significantly (P<0.0001) higher in comparison to Pre-VDST levels with the percentage of increase of 18.66 (±6.28) as shown in table 1 & figure 2.

Table (1): Pre- and Post-VDST laboratory data of studied women

Data			Control (n=10)	Pre-VDST (n=82)	Post-VDST (n=82)	P-value
Serum 25-OHVD (nmol/ml)	Frequency according to level of VD sufficiency	Sufficient (>75)		0	3 (3.7%)	0.0002
		Insufficient		5 (6.1%)	14 (17.1%)	
		Mild VDD		45 (54.8%)	40 (48.8%)	
		Moderate VDD		18 (22%)	25 (30.4%)	
		Severe VDD		14 (17.1%)	0	
	Mean (±SD) level		78.4±2.8	26.7±13.1	34.7±16.6	0.0007
	Percentage of increased level			31.7±10.8		
Serum TNF-α (ng/ml)	Relation to median of control level (2.1)	<2.1		5 (6.1%)	14 (17.1%)	0.055
		2.1-3.15		69 (84.1%)	64 (78%)	
		(≥4.2)		8 (9.8%)	4 (4.9%)	0.0006
	Mean (±SD) level		1.9±0.47	3.07±0.77	2.67±0.7	
	Percentage of decreased level			12.8±7.4		
Serum IL-4 (ng/ml)	Relation to median of control level (1.4)	<1.4		16 (19.5%)	1 (1.2%)	0.00001
		1.4-2.1		66 (80.5%)	72 (87.8%)	
		≥2.8		0	9 (11%)	
	Mean (±SD) level		1.44±0.34	1.85±0.4	2.27±0.42	<0.0001
	Percentage of increased level			18.66±6.28		

Data are presented as mean; standard deviation (SD); numbers and percentages; Pre-VDST: before VDST; Post-VDST: after VDST; VDST: Vitamin D supplemental therapy; VDD: Vitamin D deficiency; 25-OHVD: 25-Hydroxy VD; TNF-α: Tumor necrosis factor-α; IL-4: Interleukin 4; P-value indicates the significance of difference between Pre- and Post-VDST levels; P<0.05 indicates the significant difference; P>0.05 indicates the non-significant difference

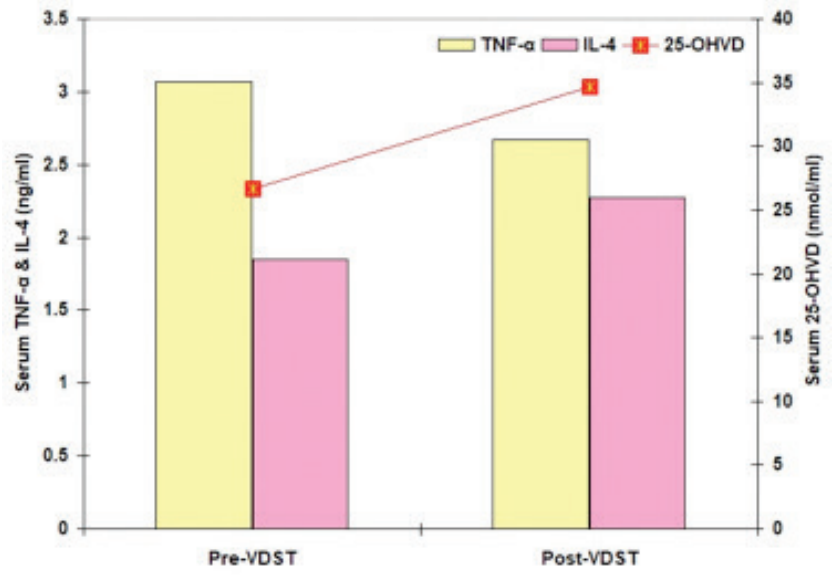

Fig. (2): Mean serum TNF-α, IL-4 and 25-OHVD levels estimated Post-VDST compared to its Pre-VDST levels

Interestingly, serum AMH estimated Post-VDST showed a non-significant (P=0.155) difference in comparison to Pre-VDST levels with the percentage of increase of 6.18 (±3.23). However, the median number of AFC increased significantly (P<0.0001) after VDST in comparison to AFC before VDST with a mean percentage of increase of 85.3 (±69%) and 16 women (19.5%) had AFC >7 (Table 2). Unfortunately, embryo grading before VDST defined 37 embryos of poor quality, 33 of fair quality, and only 12 embryos of G2 quality; on contrary, embryo grading after VDST defined 24 embryo of poor grade, 26 embryos of fair grade, 23 embryos of G2 grade and 9 of G1 grade with significantly (P=0.0011) lower frequency of embryos of poor and fair grades after VDST in comparison to before VDST.

Table (2): Serum AMH and AFC and grading of the obtained embryos of studied women Pre- and Post-VDST

			Pre-VDST (n=82)	Pre-VDST (n=82)	P-value
AMH (ng/ml)	Mean (±SD) level		0.65±0.17	0.69±0.18	0.155
	Percentage of increased level		6.18±3.23		
AFC	Frequency according to AFC	<5	79 (96.3%)	42 (56.1%)	<0.0001
		5-7	3 (3.7%)	24 (29.3%)	
		>7	0	16 (14.6%)	
	Median [IQR] level		3 [2-3]	4 [3-7]	<0.0001
	Percentage of increased level		85.3±64.1		
Embryo grading	Good	G1	0	9 (11%)	
		G2	12 (14.6%)	23 (28%)	
	Fair		33 (40.2%)	26 (31.7%)	0.0011
	Poor		37 (45.2%)	24 (29.3%)	

Data are presented as mean; standard deviation (SD); numbers and percentages; Pre-VDST: before VDST; Post-VDST: after VDST; VDST: Vitamin D supplemental therapy; VDD: Vitamin D deficiency; 25-OHVD: 25-Hydroxy VD; TNF-α: Tumor necrosis factor-α; IL-4: Interleukin 4; AMH: Anti-Müllerian hormone; P-value indicates the significance of the difference between Pre- and Post-VDST levels; P<0.05 indicates the significant difference; P>0.05 indicates the non-significant difference

Pre-VDST embryo grading showed a negative significant correlation with woman's age, and serum levels of TNF-α, while showed negative non-significant correlation with women's BMI and showed positive significant correlation with both AFC and serum levels of 25-OHVD and IL-4. Moreover, AFC before VDST showed a positive significant correlation with serum levels of 25-OHVD and IL-4, while showed negative significant correlation with serum levels of TNF-α. Also, serum levels of 25-OHVD showed a negative significant correlation with BMI, and serum levels of TNF-α, which showed a positive significant correlation with serum levels of IL-4. ROC curve analysis for these variables as predictors for the grading of the oncoming embryo excluded age and AMH and defined high serum levels of 25-OHVD and IL-4, and high AFC as significant positive predictors for high quality embryo and high serum levels of TNF-α and high BMI as significant negative predictor for the high-quality embryo (Fig. 3). Verification of these predictors using Regression analysis defined high AFC and serum levels of 25-OHVD and IL-4 levels as the significant positive predictors for high quality embryo (Table 3). To identify the pre-VDST important laboratory marker for prediction of quality of the oncoming embryo, The Automatic Linear Modeling analysis defined vitamin

D status as manifested by the 25-OHVD serum level as the most important predictor by 65% and improved anti-inflammatory status as defined by serum IL-4 as an important predictor by 35%, but excluded serum TNF-α level as a predictor (Fig. 4).

Table (3): Statistical analyses of clinical and laboratory findings at the time of enrolment and embryo grading of the Pre-VDST ICSI session

Variables	Embryo grade		VD		TNF-α		IL-4	
	Spearman's correlation analysis							
	r	P	r	p	r	p	r	P
Age	-0.338	0.002	-0.129	0.249	0.099	0.374	0.175	0.116
Body mass index	-0.208	0.061	-0.225	0.042	0.362	0.001	0.02	0.861
Antral Follicular Count	0.693	<0.001	0.474	<0.001	-0.247	0.026	0.312	0.004
Anti-Müllerian hormone	0.175	0.115	0.024	0.828	-0.027	0.807	0.155	0.164
25-OHVD serum level	0.514	<0.001						
TNF-α serum level	-0.309	0.005	-0.474	<0.001				
IL-4 serum level	0.435	<0.001	0.307	0.005	0.218	0.049		
	ROC curve analysis				**Regression analysis**			
Variables	AUC	±SE	p	95% CI	β		P	
Age	0.345	0.090	0.078	0.168-0.522	Excluded			
Body mass index	0.268	0.072	0.008	0.126-0.410	Excluded			
Antral Follicular Count	0.843	0.066	<0.001	0.715-0.972	0.535		<0.001	
Anti-Müllerian hormone	0.596	0.075	0.267	0.451-0.744	Excluded			
25-OHVD serum level	0.857	0.057	<0.001	0.745-0.969	0.196		0.026	
TNF-α serum level	0.172	0.059	<0.001	0.055-0.288	Excluded			
IL-4 serum level	0.213	0.083	0.001	0.050-0.376	0.208		0.011	

VD: Vitamin D; VDST: Vitamin D supplemental therapy; TNF-α: Tumor necrosis factor-α; IL-4: Interleukin 4; 25-OHVD: 25-Hydroxy VD; "r": Pearson's correlation coefficient; AUC: Area under the curve; SE: Standard error; CI: Confidence interval; β: Standardized coefficient; P-value indicates the significance of the result; P<0.05 indicates the significant difference; P>0.05 indicates the non-significant difference

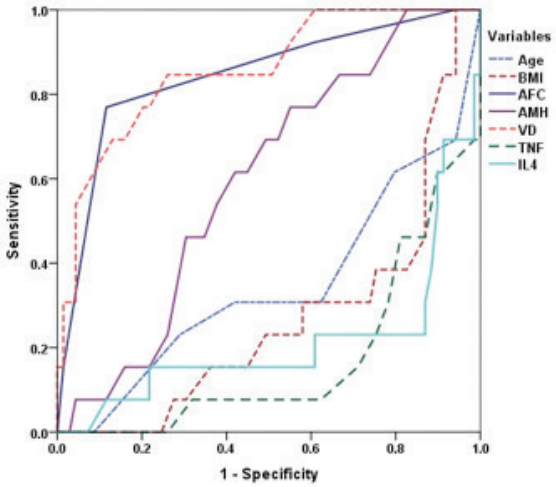

Fig. (3): ROC curve analysis of Pre-VDST variables as predictors of Pre-VDST embryo grade

Post-VDST embryo grading showed a positive significant correlation with Post-VDST AFC and percentage of increased serum level of 25-OHVD and IL-4 serum and ROC curve analysis defined increased number of AFC and a higher percentage of serum 25-OHVD level as the significant predictor for having good quality embryo (Table 4, Fig. 4).

Table (4): Statistical analyses of clinical and laboratory findings after VDST and embryo grading of the Pre-VDST ICSI session

Variables	Embryo grade		ROC curve analysis			
	r	P	AUC	±SE	p	95% CI
Post-VDST antral follicular count	0.777	<0.001	0.795	0.054	<0.001	0.689-0.901
% of increase of AMH level	0.160	0.152	0.467	0.068	0.643	0.333-0.601
% of increased 25-OHVD serum level	0.369	0.001	0.663	0.061	0.021	0.542-0.783
% of decreased TNF-α serum level	0.107	0.339	0.419	0.062	0.252	0.297-0.542
% of decreased IL-4 serum level	0.375	0.001	0.520	0.063	0.775	0.396-0.644

VDST: Vitamin D supplemental therapy; 25-OHVD: 25-Hydroxy VD; TNF-α: Tumor necrosis factor-α; IL-4: Interleukin 4; "r": Pearson's correlation coefficient; AUC: Area under the curve; SE: Standard error; CI: Confidence interval; P-value indicates the significance of the result; P<0.05 indicates the significant difference; P>0.05 indicates the non-significant difference

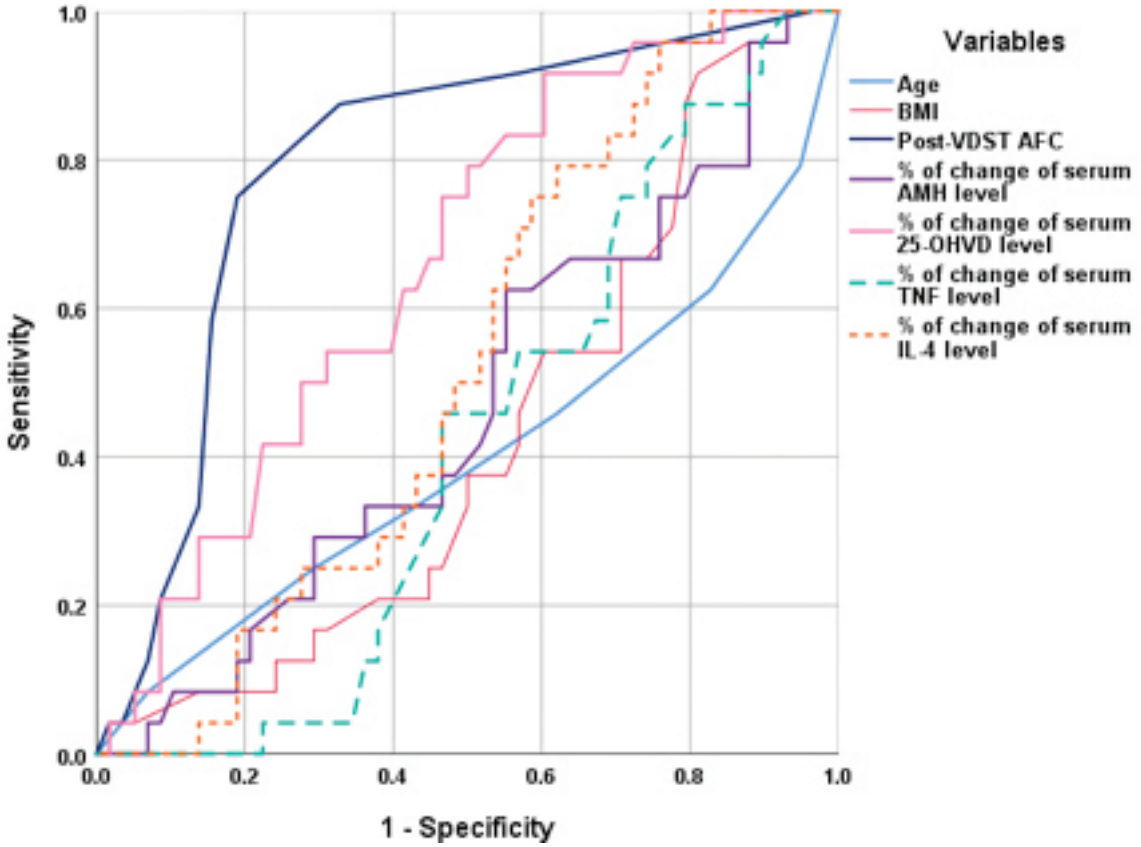

Fig. (4): ROC curve analysis of Post-VDST variables as predictors of Post-VDST embryo grade

Discussion

Throughout the period for case collection, 103 women younger than forty years had POD for a prevalence of 24.6%, a figure which is coincided with that previously reported [22] and 82 were had vitamin D deficiency-to-insufficiency. These findings spotlight on important coincident events including POD, vitamin D deficiency (VDD), and age; this triad could be attributed to the delayed age of marriage, weak exposure to ultraviolet rays to help to synthesize VD and sedentary life that induced obesity. In support of these assumptions, statistical correlation analysis detected a negative significant correlation between obesity and antral follicular count (ANF), serum level of 25-OHVD, and embryo grading after the initial ICSI trial.

In line with these data, Moreno-Santos et al. [23] found the active form of VD can modify adipose tissue physiology via its receptor, decreasing the expression of pro-inflammatory cytokines in adipose tissue and regulation of activation of insulin growth binding proteins in visceral adipose tissue and so VDD was associated with obesity and diabetes. Thereafter, Xu et al. [24] documented that the cumulative live birth rates for women with the poor ovarian response (POR) declined with increasing age and very low rate was associated with women aged >43 years old, so natural cycle IVF is of no benefit for these patients.

Recently, Bennouar et al. [25] found severe VDD was positively related to obesity with a higher risk of metabolic syndrome was in women than men. Moreover, Shea et al. [26] found women who had premature ovarian insufficiency were more likely to be obese and have decreased physical activity and de Sales Souza et al. [27] found VDD showed a high prevalence in adolescents with overweight living and is associated with increased cardiometabolic risk factors.

In a trial to explore the underlying mechanisms for this triad, Zanol et al. [28] experimentally detected strong negative correlations between adipocyte hypertrophy in animals maintained on a high carbohydrate diet and ovarian reserve, AMH levels and corpora lutea number with an increased number of atretic follicles. Also, Kang et al. [29] experimentally suggested that diet-induced obesity may lead to impaired function of T cells with inhibition of its autophagy, thus inducing the deregulation of T cell homeostasis, which underlie aggravation of inflammation that is commonly observed with obesity.

Vitamin D supplemental therapy (VDST) induced a significant improvement of AFC, but a non-significant increase in AMH serum levels in comparison to that reported before receiving VDST. Moreover, embryo quality grading was significantly improved in these women on receiving a second ICSI trial after receiving VDST and these grades were correlated with the new AFC and serum levels of 25-OHVD, but not with AMH levels. In line with these findings, Zhang et al. (22) documented that the AFC is better than AMH for predicting POR, the AFC had to be the preferred indicator for predicting ovarian response to subsequently develop an optimal individualized controlled ovarian hyperstimulation protocol.

The obtained results indicated a necessity for having within normal VD serum levels for maintenance and/or improvement of ovarian function and outcomes of ART and go in hand with experimental studies that supported a potential role for VD in follicular development; where VD receptor was detected in goat granulosa cells and VDST was found to seriously affect its proliferation by regulating cellular oxidative stress and cell cycle-related genes, and steroidogenesis [30] and a recent study documented that antral follicle is a target tissue for direct VD action that could regulate follicular development and function through increasing hormonal secretion by small and medium-sized follicles [31].

As another support for the efficacy of VDST to improve ovarian function, Refaat & El-

Boshy [32] experimentally treated VD sufficient female rats with supra-physiological VD3 supplements and detected improved ovarian functions by regulating the hypothalamic-pituitary-ovarian hormones with subsequent increased hormonal levels, increased counts of large follicles and corpora lutea and levels of mRNAs and proteins of targeted molecules.

Interestingly, the current study detected a reciprocal relation between the ratio of a pro to anti-inflammatory serum cytokines' levels; TNF-α/IL-4 ratio, and follicle development and improvement of embryogenesis, and such relation most probable was through vitamin D as evidenced by the negative significant correlation between AFC, serum 25-OHVD and embryo grade and serum levels of TNF-α, while the relations were positive with pre-VDST serum IL-4 levels and statistical analyses defined post-VDST percentage of increased IL-4 serum levels as a significant predictor for embryo grading and correlated with that of serum 25-OHVD.

These findings point to the possibility that improved pro/anti-inflammatory milieu after VDST may be behind the improvement of ovarian function and support that documented by Wu et al. [33] who found women with POR and VDD had significantly higher peripheral blood natural killer cell levels and cytotoxicity, CD19 + B and CD19+/5+ B-1 cell levels and significantly higher Th1/ Th2 cell ratio due to increased serum TNF-α levels and decreased serum IL-10 levels on comparison to women with POR and normal VD and women with normal OR and VD levels. Also, Chu et al. [34] detected inverse correlations between both total and free 25-OHVD levels with high-sensitivity C-reactive protein and leukocyte count in healthy reproductive-age women. In support of this assumption, the current study could not detect improved levels of AMH despite the increased AFC with the non-significant correlation between the extent of increased AMH levels and other variables. In line with this assumption, Wong et al. [35] found serum 25-OHVD level is significantly associated with AMH level in women with PCOS but not in ovulatory women. Recently, Lawal [36] detected no relationship between serum VD and AMH levels in infertile and fertile women. Moreover,

In support of the relation between the sufficiency of VD status and ovarian tissue and function through its impact on inflammatory milieu, multiple recent studies detected an inverse relationship between development and severity of ovarian cancer and vitamin D status and epidemiologic evidence indicates that VDST is associated with decreased cancer mortality [36, 37].

Conclusion

The diminished ovarian reserve may lie behind unexplained infertility in women younger than 40 years old. VDD, obesity, and deregulated immune milieu in direction of inflammation may predispose or aggravate poor ovarian response to ovarian stimulation for ICSI procedures. VDST improved ovarian function and quality of oocytes with subsequent improvement of embryo quality most probably through equalization of immune milieu to the direction of anti-inflammatory.

Limitations

Lack of exercise as an adjuvant to VDST to help to reduce obesity with its associated inflammatory mediators is a limitation of this study. Also, repeated courses of VDST were to be evaluated as management policy.

Recommendations

Estimation of serum 25-OHVD for infertile women especially those who had DOR and/ or obesity before attempts of ART and for women with VDD or insufficiency VDST is mandatory to improve the chance to get embryos of good quality and to save resources.

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Oxytocin versus carbetocin for prevention of postpartum hemorrhage in high risk cases: Randomized controlled trial

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Abstract

Objective: To assess the effectiveness of oxytocin compared to carbetocin in patients with high risk factors for atonic postpartum hemorrhage who are undergoing elective caesarean section for prophylaxis against postpartum hemorrhage.

Subjects and methods: This randomized controlled study was conducted on 100 pregnant women undergoing elective caesarean section and had high risk factors for atonic PPH. Group (1): were given oxytocin 10 (IU) and Group (2): were given 1 ml of carbetocin. The study was approved by the Ethics Committee, and all patients gave their informed consent before inclusion in the study.

Results: We found that there was a statistically significant differences between the two groups as regard the need for additional uterotonic agents, need for uterine massage, estimated mean operative blood loss, hemoglobin 24 hours after operation and incidence of major obstetric hemorrhage (P-value < 0.05).

Conclusion: The current study has demonstrated that carbetocin can be alternative to traditional oxytocin in the prevention of postpartum hemorrhage in high-risk women undergoing elective caesarean section.

Key words: Oxytocin, carbetocin, high risk, postpartum hemorrhage, elective caesarean section, randomized controlled study.

INTRODUCTION

Postpartum haemorrhage (PPH) following CS is an important cause of maternal mortality and morbidity associated with the procedure. (1)

uterine atony is the main causative factor of postpartum haemorrhage which accounts for 80% of cases, especially in caesarean sections. (2)

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The aim of using uterotonics in the third stage of labour as an active management to reduce the amount of bleeding, the need for blood transfusion and maternal deaths. (3)

Oxytocin has a short biological half-life (<10 Min.), requiring an intravenous infusion when prolonged uterotonic effect is needed, mainly with women at high risk of PPH. (4)

Oxytocin has a high therapeutic index and in addition to treatment of PPH its use as a prophylactic uterotonic agent is justified as well. (5)

It is considered as the initial option for prevention of uterine atony in patients undergoing caesarean sections. NICE recommend intravenous administration of 5 International Units (IU) of oxytocin during caesarean section. (6)

Carbetocin is a synthetic oxytocin analogue that has a biological activity 10 times that of the parent drug. It has 4–10 times longer half-life in comparison to oxytocin. Therefore, it is given as a single dose of 100 mcg, through IV or IM route; thus eliminating the need for infusion. (7)

It was hypothesized to use single dose of carbetocin to act as 16 hours intravenous oxytocin infusion, regarding the increased uterine tone and reduced risk of postpartum haemorrhage in elective caesarean section. (8)

Several data of literature had suggested that prophylactic carbetocin administered intraoperative may be a good alternative to oxytocin to prevent postpartum haemorrhage, but which uterotonic agent is ideal for prophylactic use is being debated. So, primary prevention of postpartum haemorrhage starts with the evaluation of identifiable risk factors. (9)

Patients and methods

This study was conducted on 100 pregnant women undergoing elective caesarean section during the period from January 2022 to Juli 2022 in our Obstetrics and Gynaecology Department; the institutional ethical review board approved the study.

Inclusion and exclusion criteria:

Pregnant women aged 20–40 years with gestational age more than 37 completed weeks and at high-risk condition for atonic postpartum hemorrhage such as multiple pregnancy, polyhydramnios, Placenta previa, Fetal macrosomia, Previous uterine atony or Anemic patients were included in this study. Whereas, women with low risk factors for atonic PPH or with medical disorders, hypersensitivity to oxytocin and carbetocin and coagulopathy were excluded from the study.

Sample size:

Fifty participants were included in each group depending on the following equation

$$SS = \frac{Z^2 \times (P) \times (1-P)}{C^2}$$

Z = Z-value (e.g., 1.96 for a 95 percent confidence level).

P = Percentage of population affected.

C = Confidence interval (sampling error), expressed as decimal (e.g.,.04 = +/- 4 percentage points).

- Group (1): (n=50) were given oxytocin 10 (IU).
- Group (2): (n=50) were given 1 ml of carbetocin.

Ethical consideration:

Written informed consent was taken from the participants after they were informed about the purposes and objectives of the study. Confidentiality and privacy were maintained throughout the study. Collected data will not be used for any other purpose.

Full history was taken and abdominal US for each case, also preoperative lab and cross matched blood was prepared, then divided randomly into 2 groups: Group (1): were given oxytocin 10 (IU) and Group (2): were given 1 ml of carbetocin.

the drug was given by the anaesthetist immediately after cord clamping.

The effect was compared intra-operative by uterine tone, amount of blood loss (by number of soaked towels and vaginal packs weight), changes in pulse and blood pressure, need for message, need for blood transfusion and need for additional uterotonics, and post operative by uterine tone, vaginal blood loss, change in vital signs, and need for adding uterotonics. Hemoglobin and Haematocrit values were noted before CS and 24 hours postpartum.

Statistical analysis:

Collected data had been computerized and analyzed using Statistical Package for Social Science (SPSS) version 16. Descriptive statistics were used to describe variables; percent, proportion for qualitative variables. Mean, SD, range for quantitative variables. Comparison between groups was done using chi-Square test for qualitative variables, independent t- test for quantitative variables. P values with significance of less than 0.05 were considered statistically significant.

Results

The demographic characteristics showed no statistically significant difference (P value > 0.05) among the 2 groups as regard maternal age, gestational age and BMI (table 1).

No significant difference (P-value > 0.05) between the 2 groups regarding various risk factors of uterine atony (table 2).

However, there were statistically significant differences (P-value < 0.05) between the studied groups regarding need for additional uterotonic agents and uterine massage for the control of intraoperative bleeding and maintaining uterine tone. But there was no statistically significant difference (P-value > 0.05) as regard need for transfusion of blood or blood products postpartum (table 3).

There was a statistically significant difference (P-value < 0.05) among the studied groups regarding HB levels 24 hours post operative but there was no significant difference regarding HCT value 24 hours post operative (table 4).

There was a statistically significant difference (P-value < 0.05) among the 2 groups as regard mean operative blood loss as mean blood loss after carbetocin administration was about 176 ml less than after oxytocin administration (table 5).

There was a statistically significant difference (P-value < 0.05) among the 2 groups as regard SBP and DBP after CS as there is significant decrease in it 30 min. after operation in the carbetocin group than the oxytocin group (table 6).

Regarding the incidence of major obstetric hemorrhage among the two groups as blood loss more than 1000 cc was much less in the carbetocin group than oxytocin (table 7).

Also, there was a statistically significant difference (P-value <0.05) between the 2 groups regarding time elapsed for the second dose of uterotonic agents (table 8).

Discussion

Defective function of the uterine musculature or atony is considered the most common cause of PPH. This belief is based on the fact that in many cases of moderate haemorrhage there was no evidence of retained placental tissue or tears, and the bleeding has persisted until uterine contraction was achieved. (10)

This study included 100 pregnant women at high risk to develop postpartum haemorrhage; 50 patients received 100 Microgram of IV carbetocin as a uterotonic agent. Another 50 patients received 10 IU of IV bolus oxytocin.

In our study, demographic features were statistically insignificant among the two groups.

In this study, one of our primary outcomes

was to compare the occurrence of major obstetric haemorrhage (> 1000 ml), only 5 patients in the carbetocin group developed such haemorrhage compared to 15 patients in the oxytocin group.

Dansereau et al, (1999) found that the carbetocin group had a decreased incidence of PPH than the oxytocin group, and that matches with our study. (11)

According to the study of Dansereau et al, (1999) the incidence of the need for therapeutic oxytocics in the carbetocin group was decreased than oxytocin group (4.7% vs. 10.1%; $P < 0.05$), and that was similar to our study results.

Attilakos et al, (2010) found that 33.5% of women in the carbetocin group needed additional oxytocics vs 45.5% of women in the oxytocin group. Therefore, significantly more women required additional oxytocics in the oxytocin group, and that matches with our present study.

In the present study, number of patients in the carbetocin group who required at least one uterine massage were less (12 patients) than oxytocin group (23 patients).

In the present study, mean operative blood loss; estimated by the operative team was 176 ml less in the carbetocin group compared to the oxytocin group.

A randomized study by Borruto et al., (2009) declared that a single 100 microgram IV injection of carbetocin was as effective as a continuous 2hrs infusion of oxytocin in controlling intraoperative blood loss after placental delivery. (12)

In the present study, haemoglobin drop among patients received carbetocin was much less than those received carbetocin. Seow et al. (2017) found that estimated blood loss was more in the oxytocin group. There was a high drop in HB in this group. This matches with the present study. (13)

In the present study, Carbetocin produced more hypotension in the patients than oxytocin. For the carbetocin being more potent and having longer duration of action compared to oxytocin which already causes hypotension as a side effect, this may explain the more hypotension that occurs with the former.

In the present study, only 3 patients in the carbetocin group received transfusion of blood or blood products, unlike the oxytocin group (6 patients). That was statistically insignificant.

At Attilakos et al. (2010) study, there were no statistically significant difference in the number of women requiring blood transfusion between the carbetocin and oxytocin groups. (9)

However, the high-risk nature of the patients for developing uterine atony and postpartum haemorrhage with its implications and morbidities, and the higher effectiveness of carbetocin being requiring less interventions; these factors promote the use of carbetocin for such patients to decrease the incidence of possible complications and morbidities that may finally lead to surgical interventions with more costs

Conclusion

We concluded that carbetocin can be an alternative to traditional oxytocin in the prevention of postpartum hemorrhage in high-risk women undergoing elective caesarean section. Single dose of IV carbetocin100 mcg is more effective as compared to IV oxytocin 10 IU.

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Legends to tables:

Table (1): Comparison between the studied groups regarding demographic data.

Table (2): Comparison between the studied groups regarding different high-risk factors.

Table (3): Comparison between the two groups regarding need for additional uterotonics, need for uterine massage and need for BL. Transfusion.

Table (4): Comparison between the studied groups regarding hemoglobin and hematocrit.

Table (5): Comparison between the studied groups regarding mean operative blood loss.

Table (6): Comparison between the studied groups regardingSBP and DBP.

Table (7): Comparison between the two groups regarding major obstetric haemorrhage.

Table (8): comparison between the two groups regarding time for second dose of uterotonics.

Legends to figures:

Figure (1): Need for additional uterotonic ttt among the studied groups.

Figure (2): Need for uterine massage among the studied groups.

Figure (3): HB level before and 24 hrs postpartum among the studied groups.

Figure (4): Total blood loss (ml) intra operative among the studied groups.

Figure (5): SBP before and 30 min. after Operation among the studied groups.

Figure (6): DBP before and 30 min. after Operation among the studied groups.

Figure (7): Incidence of major obstetric Hge among the studied groups.

Figure (8): Time (min) for second dose of uterotonic agents among the studied groups

List of abbreviation:

BMI: Body mass index.

CS: Caesarean section.

HB: Haemoglobin.

HCT: Haematocrit.

PPH: Postpartum haemorrhage.

SBP: Systolic blood pressure.

DBP: Diastolic blood pressure.

SD: Standard deviation.

SPSS: Statistical Package of Social Science.

US: Ultrasound.

Table (1)

	Drug used								P value
	Group (2)				Group (1)				
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	
Age (years)	29.56	5.45	22.00	37.00	28.70	4.32	19.00	36.00	0.368
Age (years)	26.00	1.38	24.00	28.00	26.10	1.34	24.00	28.00	0.826
GA (weeks)	38.16	.37	38.00	39.00	38.24	.59	38.00	41.00	1

Table (2)

		Drug used				P value
		Group (2)		Group (1)		
		Count	%	Count	%	
Risk	1. History of pp Hge	13	26.0%	9	18.0%	0.786
	2. Macrosomic baby	22	44.0%	26	52.0%	
	3. Polyhydramnios	7	14.0%	8	16.0%	
	4. Twin pregnancy	6	12.0%	4	8.0%	
	5. uterine wall fibroid	2	4.0%	3	6.0%	

Table (3)

		Drug used				P value
		Group (2)		Group (1)		
		Count	%	Count	%	
1. Need for additional uterotonic ttt	**Yes**	9	18.0%	18	36.0%	0.043*
	No	41	82.0%	32	64.0%	
2. Need for uterine massage	**Yes**	12	24.0%	23	46.0%	0.021*
	No	38	76.0%	27	54.0%	
3. Need for BL transfusion	**Yes**	3	6.0%	6	12.0%	0.295
	No	47	94.0%	44	88.0%	

Table (4)

	Drug used								P value
	Group (2)				Group (1)				
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	
HB before (mg/dl)	10.87	0.69	10.00	12.00	11.16	0.81	10.00	12.80	0.06
HB after (mg/dl)	10.12	0.92	7.90	11.50	9.47	1.01	7.90	11.50	0.001*
HCT % before	32.57	3.38	27.00	37.00	33.38	5.71	25.60	43.00	0.386
HCT % after	29.24	2.76	24.00	33.00	28.64	3.32	24.00	33.00	0.329

Table (5)

	Drug used								P value
	Group (2)				Group (1)				
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	
Total blood loss (ml)	706.64	177.11	387.00	1030.00	882.74	168.72	443.00	1100.00	< 0.001*

Table (6)

	Drug used								P value
	Group (2)				Group (1)				
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	
SBP before op.	112.76	10.35	100.00	130.00	113.56	9.63	90.00	130.00	0.690
SBP 30min. after op.	91.44	7.19	80.00	115.00	97.48	8.99	80.00	115.00	<0.001
DBP before op.	67.70	9.99	50.00	80.00	69.38	6.12	60.00	80.00	0.313
DBP 30min. after op.	56.84	7.70	50.00	70.00	62.68	6.75	50.00	70.00	< 0.001

Table (7)

		Drug used				P value
		Group (2)		Group (1)		
		Count	%	Count	%	
Major obstetric Hge	**Yes**	5	10.0%	15	30.0%	0.012*
	No	45	90.0%	35	70.0%	

Table (8)

	Drug used								P value
	Group (2)				Group (1)				
	Mean	SD	Min.	Max.	Mean	SD	Min.	Max.	
Time elapsed for second dose (min)	55.16	2.95	50.00	59.00	94.68	3.09	90.00	100.00	<0.001

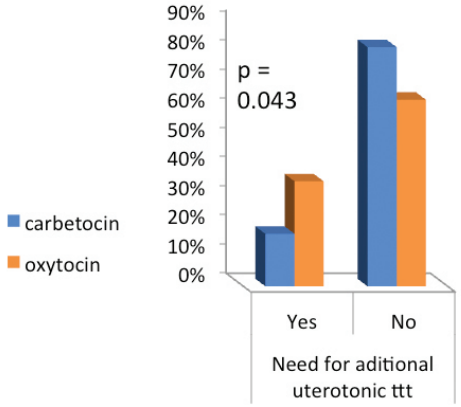
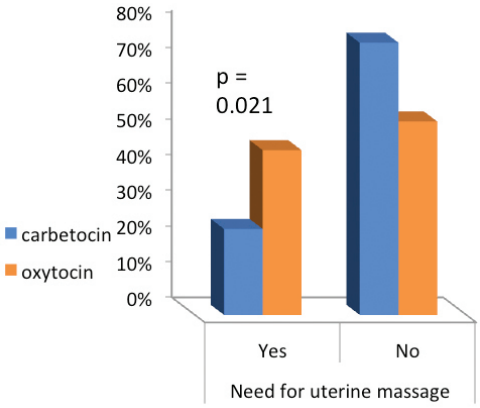


Fig. (1)



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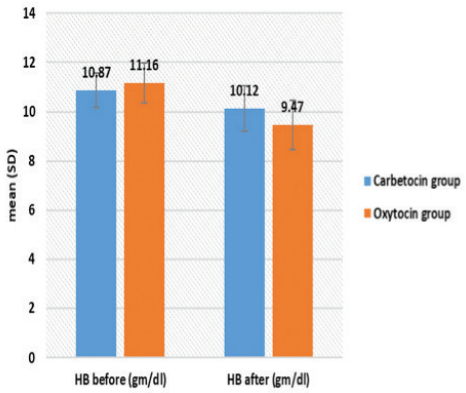

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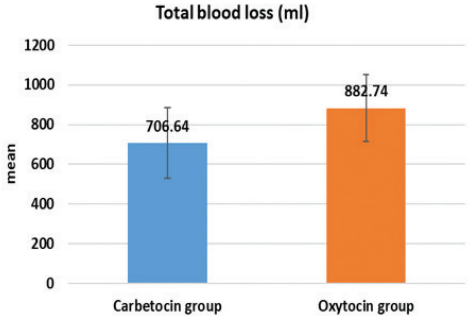

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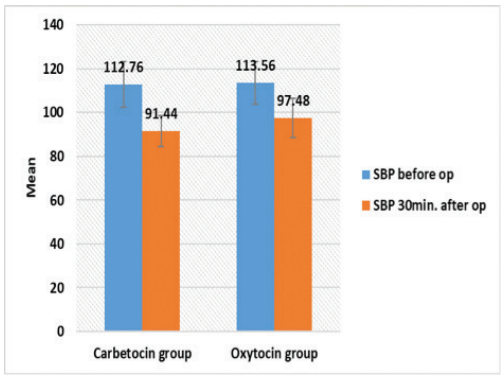

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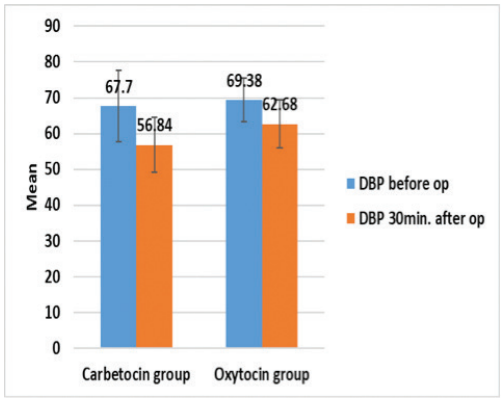

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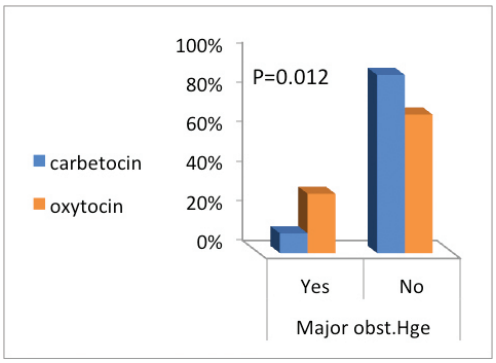

Fig. (7)

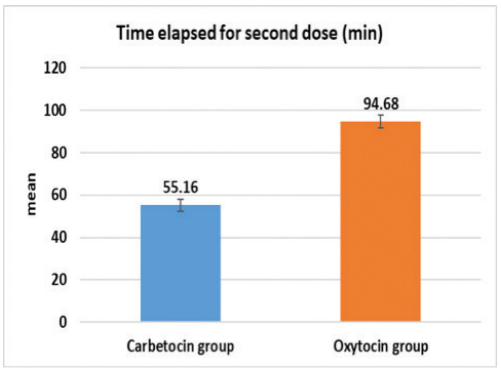

Fig. (8)

The value of sliding sign in evaluation of intra-abdominal adhesions in pregnant women undergoing elective cesarean section

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Abstract

Background: Incident of adhesions is a common and serious post-operative complication. Pre-operative prediction of adhesions is essential to help the surgical team in better counseling and preparation. Yet, the pre-operative assessment of adhesions is lacking. The sonographic sliding movement gives a reflection of the free mobility of the underlying viscera, hence; can predict the presence or absence of adhesions.

Objective: To study the accuracy of U/S sliding sign in pre-operative prediction of adhesions, in pregnant patients prior to CS procedure in general, and to re-adjust this data among different BMI classes in particular.

Methodology: A prospective, double-blinded study that included 235 pregnant patients at term, who were candidates for elective CS at Kasr Al-Ainy Hospital, Cairo University. We documented the type of previous surgery done. A single sonographer recorded the sliding sign pre-operatively. The surgeons reported the degree of adhesions quantitatively, according to a scoring system. Moreover, we documented the operative delivery time (time from skin incision to time of fetal delivery), and the incidence of visceral injury. Data was further re-analyzed in reference to each BMI class individually.

Results: : A total number of 235 pregnant women were recruited, with a mean age; 30 years, and mean BMI; 29kg/m2. The prevalence of adhesions was 48.51% (19.15% mild, and 29.36% marked adhesions). 0.85% of the cases had visceral injury. The prevalence of adhesions increased with the increase in number of previous CS; 22.8%, 34.2%, and 43% in cases with previous one, two, three or more CS respectively. 68.51% (n=161) of the recruited patients had positive sliding sign, while 31.49 % (n=74) had negative sliding sign. Positive sliding sign correctly identified 152 out of 166 patients who had no or mild adhesions, while negative sliding sign correctly identified 60 out of 66 patients who had marked adhesions. Accordingly, the sensitivity, specificity, PPV, NPV and accuracy of sliding sign in predicting intra-operative

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adhesions were 86.96%, 94.41%, 81.08%, 94.41% and 90.21% respectively. We further re-analyzed the data in relation to different BMI classes. The sensitivity, specificity, PPV, NPV, and accuracy specific to each BMI group were; 94.12%, 96.08%, 88.89%, 98%, 95.59% respectively in the normal weight group (n=68); 90%, 93.62%, 85.71%, 95.65%, 92.54% respectively in overweight cases (n=67); and finally, 81.25%, 86.76%, 74.29%, 90.77%, 85% respectively in the obese group (n=100). The median operative delivery time was significantly longer in patients with negative sliding sign compared to those with positive sliding sign (18.9 minutes versus 11 minutes).

Conclusion: The pre-operative assessment of the sliding sign is useful in the prediction of intra-abdominal adhesions, prior to CS procedure, especially in normal weight and overweight cases. The negative sliding sign correlates with longer operative delivery time.

Keywords: adhesions, sliding sign, cesarean section, body mass index.

Introduction

In nowadays practice, cesarean section (CS) is the most commonly surgical procedure done by obstetricians (1), and its rate is dramatically increasing worldwide (2).

On the other hand, the incident of intra-abdominal adhesions is a well-known complication that may occur following CS, with a documented incidence ranging from 24% to 58.5% in literature (3,4), and rising in a linear fashion with the increased number of previous CS (5).

These adhesions subject the patients to the risks of; difficult and lengthy repeat procedure, more blood loss, infection, visceral injury, and even hysterectomy (5). Secondary to prolonged operative time, the fetus as well is set at risk of perinatal complications (6). Long term sequale has been noted, in form of; infertility, ectopic pregnancies, and chronic pelvic pain (1,7,8). Thus, it is very helpful indeed, to detect the cases susceptible of adhesions preoperatively. This will guide the practitioners in proper patient's counseling regarding the possible risks, and proper preoperative preparation in terms of the availability of cross-matched blood, the operative setting, the anaesthetic staff awareness, the need for call of senior consultants and/or multidisciplinary team (9-13). All these precautions will eventually eliminate the maternal and neonatal morbidity in high risk cases.

Currently, surgeons lack the presence of a reliable assessment tool for the preoperative diagnosis of adhesions in cases with prior CS (14). The ultrasound (U/S) sliding sign has been suggested by some researchers to have a high predictive value in the detection of adhesions in cases with endometriosis, and chronic pelvic inflammation (9,15). More recently, the U/S sliding sign was proven to be both accurate and reproducible in such cases (16).

So, our aim was to investigate the proposed hypothesis regarding the accuracy of the U/S sliding sign in the preoperative prediction and assessment of adhesions in cases with prior pelvic surgeries, and to re-adjust the proposed accuracy in set of different BMI categories.

Materials and Methods

This is a prospective observational study, held at Obstetrics and Gynecology department, Kasr El-Aini Hospital, Cairo University, during the time interval from March 2021 till July 2021, after receiving the approval from our local ethical committee.

A total number of 235 cases were enrolled in the study, having term pregnancy (as evident by reliable dating from the first date of last menstrual period, or first trimesteric U/S), candidate for elective CS, and with a history of a previous open pelvi-abdominal surgery (CS, myomectomy, oopherectomy, salpingectomy, appendectomy, ovarian

cystectomy). Cases in need of immediate termination of pregnancy or emergency CS (as for cases with antepartum hemorrhage, fetal distress, prolapsed pulsating cord, obstructed labor), and cases diagnosed as having abnormally invasive placenta were initially excluded from the study.

All enrolled cases were subject to full history taking, and clinical examination to ensure fulfillment of inclusion criteria, and to receive informed consent.

BMI (weight in kg/ height in m2) was recorded for each patient, and accordingly the cases were further subdivided into three groups: group A (normal, BMI; 18- 24.9), group B (overweight, BMI; 25- 29.9), and group C (obese, BMI; ≥30), in reference to the WHO classification of BMI (17).

Pre-operatively, transabdominal ultrasound (TAS) - Medison U/S machine with frequency 2-6 MHz curvilinear abdominal probe - was done by a single sonographer, to ensure fetal viability, gestational age, and placental location. Using the real time TAS, pelvic sliding sign (relative motion between the maternal abdominal and uterine wall) was documented, as formerly described by Drukker et al (13).

To ensure uniform technique, all cases were set in supine position, asked not to empty the bladder, and instructed to breathe deeply, accentuating their respiratory movements while the sonographer was recording a video clip in sagittal plane, so as to determine freely glided movement of a certain structure in relation to adjacent structures.

Noting structures that glide easily, one against the other was considered as positive sliding sign ,whereas, no motion of the structure was considered a negative sliding sign. For being more precise, stable echogenic visceral point (the uterus) was marked as point A, the patient was asked to take a deep inspiration and exhalation while the sonographer recorded a clip, the movement of point A was observed, and the new area was marked as point B. The visceral slide was destined to be the longitudinal distance between point A and point B. A positive sliding sign was recorded if the anterior uterine wall was seen sliding across the abdominal wall more than one cm and a negative sliding sign with no evidence of such relative motion, as formerly described by Baron et al (11).

CS was done within 24 hours from the TAS examination, a detailed description of adhesions was provided through direct observation by the surgeons. Intra-operative adhesions encountered were graded according to the severity using a standardized scoring system; proposed by Bolnick et al (18) in literature. (0: no adhesions, 1: minimal or filmy adhesions, 2: moderate or thick adhesions, 3: absence of free space between the uterus and the anterior abdominal wall).

Other intra-operative recorded data included; the duration of the intervention (from skin incision to delivery of the baby) expressed in minutes, and the occurrence of bladder and bowel injury.

To avoid inter-observer bias, and to interpret the data objectively; the sonographer was blinded to the type of previous surgery the patient had. For the same reason, the operating surgeons were blinded as well to the TAS sliding sign result.

Our primary concern was to assess the accuracy of the U/S sliding sign in diagnosing the presence and the degree of severity of intra-abdominal adhesions. Other secondary outcomes included; re-adjustment of the accuracy of sliding sign in prediction of intra-abdominal adhesions in relation to the patient's BMI category, the operative time (skin incision time to delivery time), and visceral (bladder or bowel) injury.

Statistical Analysis

Sample size calculation was based on the sensitivity of sliding sign detected by ultrasound scan done to full term pregnant

women in predicting the presence of intra-abdominal adhesion after prior CS. Prior data indicated that the sensitivity of the sliding sign in predicting intra-abdominal adhesion after CS ranged from 56% to 76.2%, with an average of 66.1%. If the prevalence of intra- abdominal adhesions after CS is 38% (19), According to the sensitivity estimation formula with 95% confidence 80% power setting type I error probability to 0.05. We needed to study at least 225 third trimester pregnant women to be able to reject the null hypothesis. Calculations were done using Flahault et al equation (20).

Data were coded and entered using the Microsoft excel version 2013. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between groups were done using unpaired t test in normally distributed quantitative variables while non-parametric Mann-Whitney test was used for non-normally distributed quantitative variables. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. P-values less than 0.05 were considered as statistically significant.

Results

We examined a total number of 235 pregnant women, at term (completed 37weeks gestation), who were candidates for elective CS, from March 2021 to July 2021.

The age, BMI, and gestational age in terms of mean ± standard deviation (SD) were; 30 ± 5.53 years, 29 ± 5 kg/m2, and 38 ± 0.87 weeks respectively. All cases were multiparas, in whom 114 cases (48.51%) were noted to have intra-abdominal adhesions; as evident by the operative findings.

In review of the past medical history; 32 cases (13.62%) had medical disorders, in form of hypertension, diabetes mellitus, cardiac disease, anemia, and epilepsy. As for the history of previous operations; 217 cases had previous CS, 15 cases had appendectomy, and 3 cases had open myomectomy. (Figure 1)

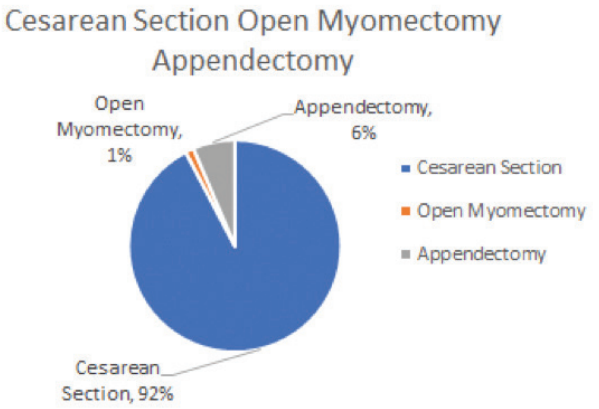

Figure (1): The number of previous pelvi-abdominal surgeries among the study group

In those with history of previous CS, we reported the incidence of adhesions in relevance to the number of previous CS. The intra-abdominal adhesions were encountered in; 22.81% (n= 26), 34.21% (n= 39), and 42.98% (n= 49) in cases with previous 1 CS, previous 2 CS, and previous ≥ 3 CS respectively.

The results of TAS sliding sign were documented separately from those noted intra-operatively (the presence & degree of adhesions, operative time, and visceral injury).

161 cases elicited a positive sliding sign, in whom, 117 cases (72.67%) had no adhesions, while; 35 cases (21.73%), and 9 cases (5.59%) & had mild & marked adhesions respectively. On the other hand, 74 cases had a negative sliding sign, in whom, 60 cases (81.08%) had marked adhesions, while; 10 cases (13.51%) and 4 cases (5.4%) had mild and no adhesions respectively (Figure 2). A statistically significant difference (P value <0.001) was noted between both groups; cases with positive and negative sliding sign in prediction of adhesions. The positive

sliding sign predicted absence of adhesions, whilst, the negative sliding sign was in favor of presence of marked adhesions.

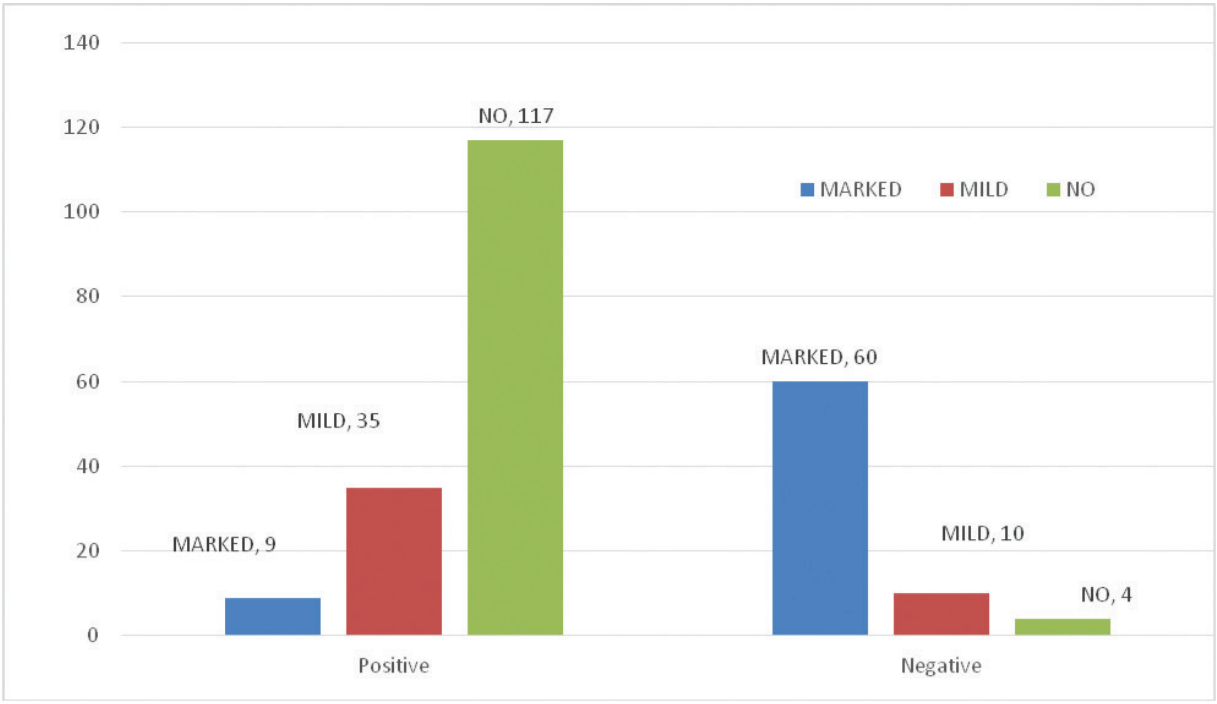

Figure (2) : Association between sliding sign and adhesions.

We considered cases with mild adhesions & those with no adhesions to be set in the same categorical class. Thereafter, the association between the U/S sliding sign and adhesions noted intra-operatively are summarized in Table (1). Accordingly, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the diagnostic performance of the sliding sign in predicting intra-operative abdominal adhesions, were; 86.96%, 91.57%, 81.08%, 94.41%, and 90.21% respectively.

Table (1): Comparison between positive & negative sliding signs and the operative adhesions

Sliding sign	no or mild adhesions	marked adhesions	Total	p-value*
Positive	**152**	**9**	**161**	**<0.001**
Negative	**14**	**60**	**74**	
Total	**166**	**69**	**235**	

*chi square test

Further classification of the cases according to their BMI, highlighted the different predictive value of the sliding sign in each group individually (Table 2). In the normal weight group (n= 68); 50 cases had positive sliding sign (1,13,36 cases with marked, mild, and no adhesions respectively), while 18 cases had negative sliding sign (16,2,0 cases with marked, mild, and no adhesions respectively). In the overweight group (n= 67); 46 cases had positive sliding sign (2,4,40 cases with marked, mild, and no adhesions respectively), while 21 cases had negative sliding sign (18,2,1 case with marked, mild, and no adhesions respectively). In the obese group (n= 100); 65 cases had positive sliding sign (6,18,41 cases with marked, mild, and no adhesions), while 35 cases had a negative sliding sign (26,6,3,cases with marked, mild, and no adhesions).

Table (2): Comparison between positive & negative sliding signs and adhesions at different BMI categories.

	Normal weight group		
Sliding	MARKED	MILD	NO
Sign	Adhesions	Adhesions	Adhesions
Positive	**1 (2%)**	**13 (26%)**	**36 (72%)**
Negative	**16 (88.89%)**	**2 (11.11%)**	**0 (0%)**
Total	**17**	**15**	**36**
P value	**<0.001**		
	Overweight group		
Sliding	MARKED	MILD	NO
Sign	Adhesions	Adhesions	Adhesions
Positive	**2 (4.35%)**	**4 (8.7%)**	**40 (86.96%)**
Negative	**85.71 (2%)**	**2 (9.52%)**	**1 (4.76%)**
Total	**20**	**6**	**41**
P value	**<0.001**		
	Obese group		
Sliding	MARKED	MILD	NO
Sign	Adhesions	Adhesions	Adhesions
Positive	**6 (9.23%)**	**18 (27.69%)**	**41 (63.08%)**
Negative	**26 (74.29%)**	**6 (17.14%)**	**3 (8.57%)**
Total	**32**	**24**	**44**
P value	**<0.001**		

Similarly, we considered cases with mild adhesions & those with no adhesions to be set in the same categorical class, and re-analyzed the data in reference to their BMI category. Accordingly, the sensitivity, specificity, PPV, NPV and accuracy of the diagnostic performance of the sliding sign in predicting intra-operative abdominal adhesions, were; 94.12%, 96.08%, 88.89%, 98.00% and 95.59% respectively in the normal BMI group, and 90.00%, 93.62%, 85.71%, 95.65% and 92.54% respectively in the overweight group, and 81.25%, 86.76%, 74.29%, 90.77% and 85.00% respectively in the obese group.

As a secondary outcome, the operative delivery time, defined as the time elapsed from skin incision to the delivery of the baby, was assessed (in minutes). It was significantly longer (p value < 0.001) in cases with negative sliding sign (median, range; 18.3, 16-21) compared to those with positive sliding sign (median, range; 11.6, 9-14).

In relation to the presence and degree of adhesions, the operative delivery time was significantly longer in cases with marked adhesions (19.8± 2.9), compared to those with mild adhesions (11.7± 2.6), or no adhesions (11± 3.2).

To sum up the outcome of the study participants, 161(68.51%) cases elicited a positive sliding sign, while 74 (31.49%) cases had a negative sliding sign. No adhesions were encountered in 121 cases (51.49%), whilst 45 cases (19.15%) had mild adhesions, and 69 cases (29.36%) had marked adhesions. The incidence of visceral (bladder/bowel) injury was 0.85% (n= 2).

Discussion

Occurrence of adhesions is a common post-operative complication (2). In face of increasing rates of CS worldwide, the prevalence of adhesions in turn is relatively high (3,4), and rising in a linear fashion with the increased number of previous CS (5,21). The prevalence of adhesions in our study group was 48.51% (19.15% mild and 29.36% marked adhesions). The incidence of adhesions increased with higher number of previous CS; 22.8% , 34.2% and 43% in cases with previous one, two, three or more CS respectively.

Several strategies have been proposed to predict the presence of adhesions, as; previous surgical history, characters of striae gravidarum, and skin scar evaluation (14,22,23). However, these attempts lack reproducibility, and unreliable history taking is usually the case in many situations.

Sigel et al (24), proposed the U/S sliding test to detect intra-abdominal adhesions as an attempt to avoid bowel injury. It has been reported that; the sliding movement of the viscera underneath the abdominal wall, elicited by deep inhalation, can be observed by U/S. The absence of this movement-negative sliding sign- can be used as a useful marker for the presence of intra-abdominal adhesions.

So, the aim of our study was to investigate the value of U/S sliding sign in the prediction of intra-abdominal adhesions in cases with prior pelvic surgeries.

Formerly, some sonographic signs were viewed as features suggestive of adhesions, as; the adherence of the CS scar to the anterior abdominal wall, lack of CS scar mobility when the uterus was pushed with the examining probe, elongated pulled up cervix, non-visualization of the full bladder between the uterine fundus and anterior abdominal wall, retroverted uterus forming an angle with the distended urinary bladder (10,25).

More recently, dynamic U/S assessment has been used to evaluate the possibility of adhesions before laparoscopic procedures (26). A systematic review conducted by Limperg et al (27), reviewing 25 articles that included 1609 patients, concluded that the U/S visceral slide has a high NPV for the absence of adhesions. This review was concerned about the risk of bowel adhesions, and safe laparoscopic entry, thus the area around the umbilicus was the only site of interest in their research.

All the previously mentioned studies examined the prediction of adhesions on small uteri, in gynecological practice.

In obstetric practice, Baron et al (11), and Drukker et al (13) were pioneer investigators to assess the integrity of this diagnostic tool in patients with previous CS. For pre-operative prediction of intra-abdominal adhesions, the sliding sign had 76.2% sensitivity, 92.1% specificity, 84.2% PPV, 87.5% NPV, 9.64 positive likelihood ratio (LR), and 0.26 negative LR, as stated by Baron et al (11); whilst, Drukker et al(13) declared a 56% sensitivity, 95% specificity, 12.1 positive LR and 0.46 negative LR. The study conducted by Baron et al (11) was limited by the small number of recruited cases. Moreover, one third of their cases had a high BMI, which may have affected the results interrogation. On the other hand, Drukker et al (13), overcame these pitfalls in their study by recruitment of large number of cases (n=370), and excluding those with high BMI (≥40). Still, their scope of work focused on the detection of severe adhesions only, and evaluated the sliding sign at one location only, though dense adhesions were probably better detected at different sites.

In our data analysis; the positive sliding sign correctly identified 152 out of 166 patients who had no or mild adhesions, whereas, the negative sliding sign correctly identified 60 out of 66 patients who had marked adhesions. So, we reported 86.96% sensitivity, 91.57% specificity, 81.08% PPV, 94.41% NPV,

and 90.2% accuracy of the sliding sign in prediction of intra-abdominal adhesions.

Comparably, Bukar et al (28), claimed a sensitivity & a specificity 100% in each in determining the presence or absence of intra-abdominal adhesions, and further analyzed the data according to the degree of adhesions, with a sensitivity and a specificity; 65%, 82.98% respectively in cases with moderate adhesions, and 25%, 98.41% respectively in cases with severe adhesions. Still, their results should be interpreted with caution, as their sample size was relatively small (n=67), and they did not take into consideration the factors that may affect U/S accuracy (as high BMI), together with the lack of intraobserver variable analysis.

To the best of our knowledge, we are the first to assess this diagnostic modality in relation to different BMI categories. The sensitivity, specificity, PPV, NPV, and accuracy specific to each BMI group were; 94.12%, 96.08%, 88.89%, 98%, 95.59% respectively in the normal weight group (n=68); 90%, 93.62%, 85.71%, 95.65%, 92.54% respectively in overweight cases (n=67); and finally, 81.25%, 86.76%, 74.29%, 90.77%, 85% respectively in the obese group (n=100). The diagnostic performance of U/S sliding sign in prediction of intra-abdominal adhesions is comparable in the normal weight and overweight classes, but relatively decreases in obese cases. This comes in agreement with the fact that U/S accuracy is highly correlated with the abdominal wall thickness (13,29).

On the contrary, Shu W(12) in examining the sliding sign in 112 Asian women claimed that it has modest ability in the detection of dense uterine-abdominal adhesions, with 53.3% sensitivity. Still, this study described the adhesions qualitatively, though the quantification of post CS adhesions is feasible, and was recommended by Shu W herself to be adopted by clinicians and investigators (30,31).

In assessment of secondary outcome parameters, Bukar et al, documented the duration of establishing the sliding sign by sonographers (mean ± SD; 7.56 ± 2.86 seconds), to highlight its feasibility in the preoperative preparation, being not time consuming, but they did not report the operative time (28).

We documented the operative delivery time in all cases, and correlated it with the sliding sign results as an independent factor. The operative time was significantly longer in patients with negative sliding sign compared to those with positive sliding sign (18.9min versus 11min). This may highlight the added value of the sliding sign in prediction of the state of difficulty of the surgical procedure irrelevant to the presence or absence of adhesions. Similarly, it has been stated in literature that cases susceptible of adhesions will exhibit a longer operative time (5,31,32). These data may help the surgical team when known prior to the procedure done.

There are several aspects of strength in our study; its prospective nature and double-blinded study design, only one expertise in U/S was assigned to the role of U/S sliding sign documentation, the quantitative assessment of intra-abdominal adhesions in terms of scoring, the relatively large number of our study population in comparison to other researches, and finally, the further analysis of the sliding sign accuracy in relation to different BMI classes.

On the contrary, we have to admit that there have been some limitations. The lack of reporting the blood loss and the fetal outcome may have provided us with more added values for the sliding sign evaluation. We preferred to focus on the accuracy in relation to adhesions, and secondarily assessed the operative delivery time and re-analyzed the data in reference to BMI classes. The inter- and intra- observer variability among surgeons in reporting the degree of adhesions is another limitation. But from our point of view, this will be inevitably met in

such research, being conducted by different operators. The U/S probe as it targets the midsection of the lower uterine segment wall, adhesions involving the omentum and pelvic side walls cannot be predicted. Thus, we advise to set many locations for eliciting the sliding sign to achieve a better predictive value.

In conclusion, the U/S sliding sign can be a useful tool in the preoperative prediction of intra-abdominal adhesions, especially in normal weight and overweight patients. This in turn will enhance a more comprehensive counseling, better preoperative preparation and multidisciplinary management.

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Prognostic value of the proper timing of bladder dissection in surgical management of placenta accreta spectrum. A randomized controlled trial

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Running title: timing of bladder dissection in PAS

Abstract

Background and aim: the management of PAS during CD is already a challenge owing to the presence of maternal comorbidities and suspected mortality. This study aimed to determine proper timing of bladder dissection during CD thus improving the outcome via decreasing blood loss and urinary tract injuries.

Methods: a randomized controlled trial included 100 patients planned for elective CD at Mansoura University Hospitals, Egypt from July 2020 to July 2021. They were assigned into two equal groups. Group 1, bladder was dissected earlier before uterine incision meanwhile in group 2, bladder was dissected late after uterine incision and fetal extraction. Outcome measures were intra and postoperative blood loss, pre and postoperative hemoglobin levels, associated urinary tract injuries and emergent hysterectomy.

Results: baseline patients' characteristics did not show any statistically significant change in both groups [p>0.05]. The mean estimated blood loss during and after cesarean delivery was significantly lower in group 2 compared to group 1 (2654.12 ± 1412.48 ml vs. 3356.2 ± 1906.63, p=0.039). Similarly, the need for additional packed RBCs and plasma were significantly higher in group 1 than group 2 (p values are 0.001 and 0.046 respectively). Also, there was more urinary bladder injuries and emergent hysterectomy in group 1 compared to group 2 (10 vs 4 cases, p =0.04 and 12 vs 5 cases, p =0.02 respectively). Therefore, the mean operative time (±SD) is longer in group 1 (p = 0.02). On the other hand, there were irrelevant differences regarding the need for platelet or Voluven transfusion, postoperative Hb level, maternal or neonatal ICU admission, PPH, fever or hospital stay time (p values >0.05).

Conclusion: surgical management of PAS by CD proved

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that bladder dissection after delivery of the baby is much better in terms of decreasing blood loss, urinary tract injuries and emergent hysterectomy than if done earlier.

Keywords: Placenta accreta, bladder dissection time.

Synopsis: the proper time of bladder dissection during CD in patients with PAS was confirmed to be after uterine incision and fetal extraction as this proved less intra and postoperative complications.

Introduction

Placenta previa is broadly defined as a placenta inserted totally or partially into the lower uterine segment. In these situations, there are various degrees of placental tissue invasion ranging from just attachment to the decidua up to deep invasion involving the surrounding structures including the cervix, the bladder, or even the bowel [1]. This group of placental abnormalities, with varying degrees of invasion, has been recently redefined into the placenta accreta spectrum (PAS). When the invasion is limited to the deep endometrium it would be named "adherent placenta accreta", while the myometrial invasion is categorized as "placenta increta", and invasion of the full thickness of the myometrium up to the uterine serosa or adjacent organs is called "placenta percreta" [2-4]. Surely; there is a rapid increase in the incidence of PAS over the past few decades from approximately 1/2500 to 1/500 [5]. This increase was attributed mainly to an increase in cesarean delivery (CD) rates; however, other causes may be implicated as interruption with the lining of the uterus namely manual placental delivery, uterine curettage, hysteroscopic endometrial resection, or even previous minor hysteroscopic surgery [6]. Unexpectedly, there were some reported cases with no previous history of uterine surgery as those proved with some cases of submucous myoma, uterine adenomyosis, and bicornuate uterus [6]. Ultrasonographic evaluation is recommended as the first-line modality for diagnosing PAS with some suggestive features such as loss of the normal retroplacental clear zone, reduced retroplacental myometrial thickness, presence of intra-placental lacunar spaces, a decrease of the uterine-bladder interface, and connecting or anastomosing vessels between the placenta and urinary bladder [7]. Indeed, PAS is responsible for one of the main causes of obstetric hemorrhages with subsequent significantly increased maternal morbidity and or mortality [8]. More than half of the direct maternal deaths are attributed now to PAS and its dilemma of management as there is increased liability for urinary tract injuries and rarely bowel injuries. This is estimated to be 4% in developed and up to 14% in developing countries [8].

This study was held to evaluate the role of proper timing of bladder dissection during the management of morbidly adherent placenta in CD or cesarean hysterectomy thus modifying the operative techniques aiming to reduce the likelihood hemorrhage and urinary tract injuries.

Materials and Methods

This prospective interventional randomized controlled trial was conducted at the department of obstetrics and gynecology, Mansoura University Hospitals, Mansoura, Egypt from July 2020 through July 2021. The local research ethical committee at Mansoura faculty of medicine (institutional research board "IRB") approved the study with IRB number [20.9.2151]. Therefore, the study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Patients allocated for the study verbally consented and then each participant gave written consent before being included in the trial. The study involved 100 patients from those admitted for a planned CD due to placenta previa with variable degrees of accretion. To include this number in the study,

the sample size was prepared before starting as the authors conducted a pilot study using uterine exteriorization with mean ± SD of the estimated intraoperative blood loss was 550 ± 250 ml. Assuming alpha = 0.05 and beta = 0.2 (power = 80%) and using the 2-tailed Student t-test, 45 subjects were required in each group to detect a difference of 150 ml between groups which are considered to be the least clinically significant effect. To allow for subject dropouts, 50 subjects were assigned to each group. Allocated patients were divided randomly using a computer-generated table into two equal groups. Group 1 [G1], where the bladder was dissected early before uterine incision, and group 2 [G2], in whom the bladder was dissected late after uterine incision and baby extraction. All patients included were those having previous one or more CDs, gestational age of at least completed 37 weeks with the viable fetus and normal fetal heart rate tracing, and PAS with anteriorly located placenta previa. All patients were judged clinically for exclusion of evidence of active labor. Moreover, an ultrasound was performed for fetal well-beginning the day before or on the morning of surgery. On the other hand, patients who were hemodynamically unstable before the skin incision (admitted with severe bleeding, operated as an emergency, coagulation defects, hemoglobin level <9gm/dl), placenta separated spontaneously, the previous history of bladder injury, posteriorly located placenta, and those with no evidence of gross placental invasion at the time of surgery were already excluded. Patients who had any type of medical disorders or evidence of active labor were also excluded. Patients' demographic data were gathered from both groups including mainly, full history, thorough clinical examination, routine laboratory investigations, data of 2D ultrasound, and color doppler for features suggestive of PAS. Caesarean deliveries were performed under general anesthesia by the same senior obstetrician after consulting a senior anesthetist. General anesthesia was decided in all cases as viewed by the consultant anesthetist as it would be better for patients' control of general parameters where a long time is expected with more manipulation of the bowel or expected urinary tract complications. In the first group, the bladder was dissected before uterine incision from lateral to medial to improve the line of cleavage followed by a uterine incision higher than the previous scar. In the second group, the bladder was dissected from lateral to medial after the uterine incision was done above the level of the placenta and after the delivery of the baby. In each group we looked for blood loss and if there were any urinary tract injuries. The uterus in both groups was repaired with a continuous unlocked suture in 2 layers using Vicryl 1 suture (Johnson & Johnson, USA). The peritoneum was left un-sutured while the muscle layer was opposed and approximated with Vicryl 0. The rectus sheath was closed by Vicreyl 1, and finally, the skin was closed with sub-cutical suture by Proline double zero in both groups of patients. Estimation of blood loss started after skin incision by two trained nurses, one for each group. They were responsible for blood and amniotic fluid collection in two separate suction sets and weighing the surgical towels before and after the operation. Those with documented intraoperative urinary complications were urgently managed by a senior consultant urologist on duty and their postoperative care was followed up according to his surgical advice. Some cases with severe lacerations of the lower uterine segment and difficult repair or complicated by severe intractable intraoperative bleeding were urgently secured by salvage hysterectomy. Post-partum blood loss during the first 24 hours after the operation was estimated by weighing soaked napkins and blood accumulated in the intraperitoneal drains when applied. Preoperative hemoglobin was measured 2 hours before surgery and again 24 hours after the operation. Urinary tract injuries and the need for a hysterectomy were counted in both groups. Neonatal outcomes

including APGAR score, admission to the neonatal intensive care unit (NICU), and neonatal deaths were also evaluated in the two groups. Data were collected, tabulated, and statistically analyzed by IBM computer using SPSS v25 (IBM©, Chicago, IL, USA).

Statistical Analysis

Statistical analysis was done by SPSS v25 (IBM©, Chicago, IL, USA). Shapiro-Wilks test and histograms were used to evaluate the normality of the distribution of data. Quantitative parametric data were presented as mean + standard deviation (+SD) and were analyzed by unpaired student t-test. Quantitative non-parametric data were presented as the median and interquartile range (IQR) and were analyzed by the Mann-Whitney test. Qualitative data were presented as numbers and percentages and were compared by chi-square (X2) or Fisher's Exact test when appropriate. A two-tailed P value <0.05 was considered statistically significant.

Results

A total of 238 patients with elective CD were enrolled in the study, of them 138 patients were excluded due to failure to fulfill the inclusion criteria (figure 1). Baseline patients' characteristics are shown in Table (1); there were no significant differences between both groups regarding maternal age, gravidity, parity, body mass index, fetal gestational age, number of previous CD(s), and preoperative hemoglobin levels, history of early obstetric complications including mainly abortion or ectopic pregnancy as well as intrauterine manipulation specifically myomectomy, curettage or hysterotomy [p>0.05]. Also, the type of placenta previa did not show any statistically significant change in both groups [p>0.05] as presented in table (1).

The mean estimated blood loss during and after cesarean delivery was significantly lower in group 2 compared to group 1 (2654.12 ± 1412.48 ml vs. 3356.2 ± 1906.63, p=0.039). Similarly, the need for additional packed RBCs and plasma were significantly higher in group 1 compared to group 2 (3.98 ± 1.80 vs 2.84 ± 1.65, p =0.001 and 1.86 ± 1.77vs 1.22 ± 1.36, p =0.046 respectively), as shown in table (2). Also, there was more liability for bladder injuries and emergent hysterectomy in group 1 compared to group 2 (10 vs 4 cases, p =0.04 and 12 vs 5 cases, p =0.02 respectively). Therefore, the mean operative time/minutes ± SD is found significantly longer in group 1 compared to group 2 (86.5 + 10.2 vs 55.4 + 8.2, p = 0.02) as described in table (2). On the other hand, there were irrelevant differences regarding the need for platelet or Voluven transfusion between both groups together with postoperative Hb level as well as maternal or neonatal ICU admission and postpartum maternal complications including PPH, fever or prolonged hospital stay time (p values >0.05) as evidenced in table (2).

Discussion

The main findings of the study confirmed that, the bladder dissection after uterine incision and fetal delivery is much less liable to be accompanied with bladder injuries, intraoperative bleeding and emergent hysterectomy than it would be done earlier. Furthermore, the operative time and the need for blood and blood products transfusion is much decreased.

Recently, PAS is deemed as a well-known serious obstetric situation with significant maternal morbidity and sometimes mortality. The principal hazards emerging with any type of PAS comes mainly from heavy obstetric bleeding that is mostly managed by unplanned surgeries. Therefore these patients are commonly put in danger, in addition to surgical risks, for hazards of blood and blood elements transfusion up to disseminated intravascular coagulopathy, adult respiratory distress syndrome and even renal failure in some occasions [10, 24]. This makes

some authors to publish at some time that caesarean hysterectomy is considered as the gold standard management for PAS [12,13]. But, according our opinion, it is really a catastrophe in female who do not complete her family and the time may come soon to change this notion. The results of the current study demonstrated blood loss in large amounts in both groups but still significantly lower in group 2 than group 1 (p=0.039) with less usage of intra and postoperative transfusion of packed RBCs and plasma (p =0.001 and 0.046 respectively). This could be explained by the fact that dissection of the urinary bladder before uterine incision takes more time due to stretch of the lower uterine segment with adherent bladder. Another explanation is that when the bladder is dissected before uterine incision and delivery of the fetus, it led to increase blood loss from varicosities and engorged vessels caused by compression and stretch of the lower uterine segment by the presenting part and implanted lower down placenta. Moreover, many of these patients (12 cases) had emergent salvage hysterectomies which prolonged more and more the operative time and increased patients' susceptibility for intraoperative bleeding. This comes in accordance with facts proved by other authors [11, 14 and 15].

Also, the data of this study evidenced that bladder dissection and mobilization were observed easier when done after uterine incision, baby extraction and more and easier when the placenta is completely removed with less liability for bladder injury or the need for postoperative urological care and follow up. Logically, this is again is clarified by easy manipulation of the tissues after delivery of the fetus and retraction of lower uterine segment and regaining of the bladder tissues to its normal size with better control of the bleeding beds. This comes in agreement with some authors [15, 16] who demonstrated a challenging surgery to do bladder dissection prior to uterine incision and newborn delivery with more association of severe bleeding. The problem is raised more and more when the placental invasion reaches the percreta type, so they advised some modifications of the surgical maneuvers to preserve the uterus, maintain integrity of the bladder and before deciding to hysterectomy. So, if bladder invasion was expected in such situations, the bladder was dissected away from the uterus with clamping of any blood vessels, devascularization of the area and the placental bed and preserving the bladder wall as much as possible. Similar maneuvers were presented by some local and international authors for the same purpose [16-19] but the dome of the bladder was opened by some to secure the vasculature form inside [16]. According to our experience from this work, when such situations met as suspected by preoperative Doppler ultrasound study or MRI, or assured intraoperatively, the bladder was opened only in presence of senior urologist to remove the invading placental tissues, then close the uterus or did hysterectomy according to our decision and left the bladder to be repaired by the urologist. An advice which we should highlight for all obstetricians, when suspecting placenta percreta and bladder invasion, the obstetrician should consult a urologist to attend the operation. Our advice was raised earlier by some surgeons to decrease urinary complication during the management of PAS [20, 21]. On the other hand, data from some previous published results cannot come in accordance with our findings because they verified that bladder dissection before uterine incision and baby delivery gained good results in terms of decreased blood loss, intraoperative blood transfusion and urinary tract injuries [22, 23].

As mentioned, caesarean hysterectomy was considered by some as the only solution for PAS [12,13] but the decision was very difficult for the obstetricians attending the cases of this study and resorted to only after failure of all other conservative surgical and medical

measures on an attempts to preserve the uterus in our patients and after discussion with other members of the surgical team in the theatre. Our results appeared satisfactory regarding this point, as in group 2 we achieved to preserve the uterus in 90% of cases, salvage hysterectomy in 5 cases only, meanwhile, in the group 1 hysterectomy was the solution in 24% of cases. Here the authors can state that most case from both groups [4 from group 2 and 9 from group 1] had percreta type of placental invasion. Surprising to us was the postoperative data, as the authors did not find any significant difference in both groups regarding Hb level, hospital stay time, mean (+SD) of blood loss, need for blood or circulation correcting fluids as well as maternal and neonatal outcome. This might be explained by the fact that the management of patients was in a tertiary care hospital with well-equipped staff members at operating theatre and all available facilities for intra and postoperative care. These findings were contrary to data observed earlier by some other authors [17, 18, 24].

Unquestionably, this study had some limitations including a relatively small sample size compared to thousands admitted yearly in tertiary place of the study, being unicentric and lack of long follow up for the patients to estimate remote urinary morbidities and the fertile function of the preserved uteri.

Table [1]: Patients' epidemiological data in both groups.

Variable	Group 1 (n = 50)	Group 2 (n = 50)	P value
Age/years (± SD)	31.8 ± 6.14	30.74 ± 4.49	0.327
Gravidity	4	5	0.744
Parity	3	3	0.153
BMI	26.7 ± 2.02	27.5 ± 1.82	0.67
Previous CD(s)	50 (100%)	50 (100%)	1
Hx of Abortion	18 (36%)	19 (38%)	0.836
Ectopic	2 (4%)	2 (4%)	1
Gestational age in weeks	37.68 ± 1.62	37.76 ± 0.72	0.750
Hx of myomectomy Hx of D&C Hx of Hysterotomy	1 (2%) 10 (20%) 2 (4%)	1 (2%) 11 (22%) 1 (2 %)	1 1 0.495
Preoperative Hb (+ SD)	11.43 + 1.2	11.84 + 1.3	0.46
Types of the placenta Grade1 Grade2 Grade3A Grade3B	 19 (38%) 21 (42%) 8 (16%) 2 (4%)	 14 (28%) 18 (36%) 17 (34%) 1 (2%)	 0.207

Data were presented as mean (± SD) or frequency (%). CD: cesarean delivery, Hx; history, D&C; dilatation and curettage. P value was set as significant when <0.05.

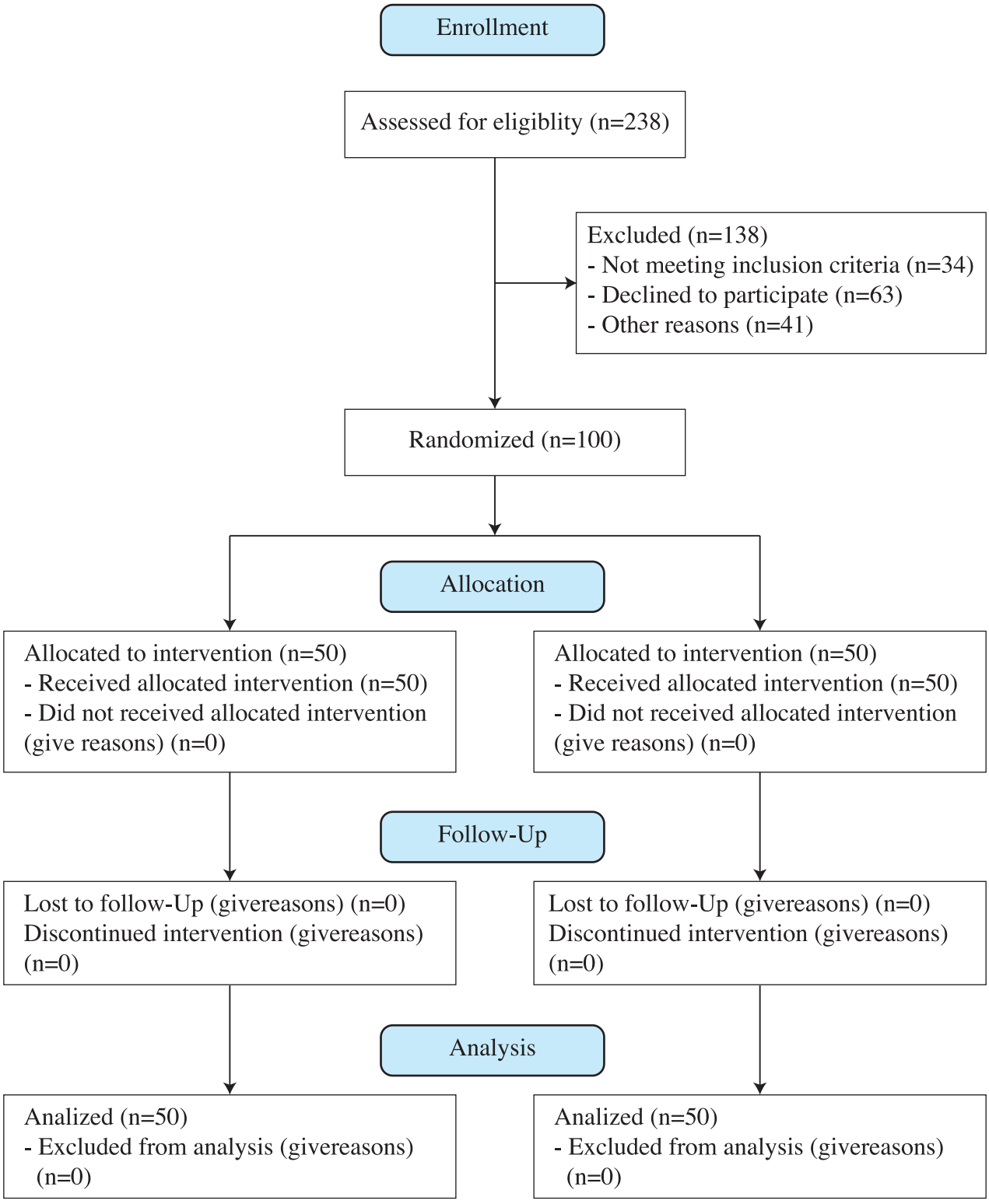
Table [2]: Operative and postoperative data in the two studied groups.

Variables	Group 1 (n = 50)	Group 2 (n = 50)	P value
Blood loss (Mean ± SD)	3356.2 ± 1906.63	2654.12 ± 1412.48	0.039*
Bladder injury			
NO	40 (92%)	46 (80%)	0.04*
yes	10 (20%)	4 (8%)	
Cesarean hysterectomy	12 (24%)	5 (10%)	0.02*
Operative time/minutes (Mean ± SD)	86.5 + 10.2	55.4 + 8.2	0.02*
Parameters (Mean ± SD)			
RBCS	3.98 ± 1.80	2.84 ± 1.65	0.001*
Plasma	1.86 ± 1.77	1.22 ± 1.36	0.046*
Platelet	0.20 ± 0.78	0.08 ± 0.27	0.309
Voluven	0.14 ± 0.40	0.08 ± 0.27	0.387
Postoperative Hb	11.04 ± 1.15	10.85 ± 1.22	0.397
Hospital-stay time	6.24 ± 4.54	7.5 ± 4.25	0.278
Maternal ICU admission	0 (0%)	0 (0%)	-----
Postpartum hemorrhage	1 (2%)	1 (2%)	1
Postpartum fever	1 (2%)	0 (0%)	1
Neonatal outcome			
APGAR score at 5 minutes	9.1 + 0.8	8.9 + 0.9	0.368
ICU admission	0 (0%)	0 (0%)	-----

Data were presented as mean ± SD or frequency (%). ICU; intensive care unit. P value is statistically significant when <0.05.

Conclusions: bladder dissection after uterine incision and fetal extraction is better than if done before uterine incision and fetal delivery, as the latter is commonly accompanied with more bleeding, blood transfusion as well as bladder injuries with more liability for emergent hysterectomy and affection of the future fertility.

Patients' Flow Diagram



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Tables' legend:

Table [1]: Patients' epidemiological data in both groups.

Table [2]: Operative and postoperative data in the two studied groups.

Figure [1]: Patient's flow chart.

The value of Middle cerebral and umbilical arteries Doppler indices in pregestational diabetic versus normal pregnancies in prediction of adverse neonatal outcome

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Synopsis

Maternal DM is not associated with significant Doppler abnormalities. UA and MCA had low sensitivity in the prediction of adverse neonatal outcome

Impact statement

What is already known on this subject?
Doppler velocimetry was introduced as an important fetal well-being test.

the results of Doppler studies in pregnancies complicated by diabetes are conflicting

- What do the results of this study add?

The sensitivity and specificity of umbilical artery Doppler in the prediction of adverse neonatal outcomes among diabetic patients were 25% and 88.89%, respectively, while, the sensitivity and specificity of middle cerebral artery Doppler were 20.83% and 91.67%, respectively.

- What are the implications of these findings for clinical practice and/or further research?

Maternal DM is not associated with significant abnormalities in Doppler indices of placental or fetal circulation.

Both UA and MCA had low sensitivity in the prediction of adverse neonatal outcome. These cannot be used as a single evaluation test for fetal well being.

Abstract

Objectives: To study the impact of pregestational DM on fetal middle cerebral (MCA) and umbilical arteries (UA) Doppler indices and hence, evaluating their diagnostic performance as predictors for adverse neonatal outcome.

Methods: The study included 2 equal groups of 60 patients each, thus making up a total of 120 patients; control group of healthy pregnant women and study group included pregnant patients known to have pregestational diabetes. The study group was furtherly subdivided into two equal subgroups of 30 patients each. This sub classification was made according to to HbA1C levels namely; controlled diabetics (defined as those having HbA1C < 6.5 %) and uncontrolled diabetics (defined as those having values of HbA1C ≥ 6.5 %). UA, MCA Doppler indices (resistance index and pulsatility index) and Cerebroplacental Doppler ratio were measured for each patient. Neonatal outcome was assessed and recorded following delivery. The following parameters were assessed: neonatal blood sugar, 1min and 5min Apgar score and admission to neonatal intensive care unit.

Results: The sensitivity and specificity of umbilical artery Doppler in the prediction of adverse neonatal outcomes among diabetic patients were 25% and 88.89%, respectively, while, the sensitivity and specificity of middle cerebral artery Doppler were 20.83% and 91.67%, respectively. The resistance index and pulsatility index of MCA and UA were not of significant corelation with any of the neonatal outcomes (Pearson's r ranged -0.07 to 0.13, $p > 0.05$).

Conclusion: maternal DM is not associated with significant abnormalities in Doppler indices of placental or fetal circulation. In addition, both UA and MCA had low sensitivity in the prediction of adverse neonatal outcome.

Keywords: Diabetes mellitus, Umbilical artery, Middle cerebral artery Doppler ultrasound and adverse neonatal outcome.

Introduction

The prevalence of Diabetes mellitus (DM) in the pregnant population in the United States is stated to be 6-7%. It is classified into pre-gestational DM (PGDM) and gestational DM (GDM) (1).

Pregestational DM poses an increased risk for the mother, the fetus and the neonate (2). Congenital malformation (e.g., cardiac or Musculo-skeletal) occurs more frequently in pregestational diabetic women and approximately 50% of such women deliver macrosomia babies with consequent risk of birth related trauma, and the development of type 2 diabetes mellitus, metabolic syndrome, vascular and cardiac diseases later in life (3 & 4). On the other hand, long standing preexisting DM before current pregnancy poses a higher risk of vasculopathy involving the uterine arteries thus resulting in abnormal development of the uteroplacental circulation and consequently placental insufficiency & restricted fetal growth (5). Those with suboptimal glycemic control have higher risk of developing such complications (6).

The pregnancy outcome in this pregnant population could be improved by approaching different targets. Health education should be delivered to all patients ensuring following adequate balanced diet, adhering to drug treatment, and the role of optimum glycemic control on pregnancy outcome (7).

Fetal surveillance in high-risk pregnancies has been a matter of concern, with the aim of achieving optimum pregnancy outcomes in such group of However, till the present date, no method of fetal surveillance was proved to be superior (8). Doppler velocimetry was introduced as an important fetal well-being test. It measures the blood flow through arteries and veins, such as umbilical and middle cerebral arteries. However, the results

obtained regarding the use of Doppler studies in pregnancies complicated by diabetes were conflicting. In fact, despite their widespread application, few studies have investigated their role as effective tools in ameliorating perinatal outcomes among such population of pregnant mothers. (1).

Shabani Zanjani and his colleagues (9) studied the Doppler indices of fetal brain hemodynamics among pregnant women with GDM compared to healthy ones and reported that the pulsatility index (PI) of the middle cerebral artery (MCA) was Increased among diabetic group compared to the healthy group. On the other hand, Niromanesh and his colleagues (10) compared the umbilical artery (UA) and MCA Doppler indices as fetal well-being tests in diabetic pregnant women (whether gestational or pregestational) and they claimed that UA Doppler indices were better than MCA indices in the prediction of adverse neonatal outcomes but both had low sensitivity.

Hence, we performed this study to evaluate of effect pregestational diabetes on fetal middle cerebral and umbilical arteries Doppler indices and to evaluate their validity as predictors of poor neonatal outcome in pregnancies complicated by diabetes.

Methodology

The current study is a Cross-sectional one conducted in the Obstetrics & Gynecology department (Kasr El-Aini Hospital – Faculty of Medicine - Cairo University) in the time period from July 2019 to October 2020. One hundred and twenty pregnant women (aged from eighteen to forty years old) with living health singleton fetus between 34-37 weeks gestation (confirmed by either the 1st day of the LMP or 1st trimesteric ultrasound scan) were recruited and they were divided into two equal groups of 60 each; Control group that included healthy pregnant women and Study group that included pregestational diabetic pregnant patients. The latter group was furtherly subdivided into two subgroups according to HbA1C levels namely; controlled diabetics (included 30 diabetic pregnant women with controlled DM defined as HbA1C less than 6.5%) and uncontrolled diabetics (included 30 diabetic pregnant women with uncontrolled DM defined as HbA1C equal to, or more than 6.5%). The study was approved by the Hospital Ethical Committee & was registered at ClinicalTrial.gov registry (The registry number: NCT03915990).

Diabetic women with either complicated diabetes or any other concomitant chronic disorder (e.g., hypertension or renal disease) were excluded. Patients with growth-restricted (EFW less than the 10th percentile for the corresponding gestational age) or malformed fetuses were excluded. Patients with any superimposed medical disorders, oligohydramnios (AFI below the fifth percentile) or rupture of membranes in the current pregnancy were also omitted.

Informed consent was obtained from all participants (after explaining the aim of the study and discussing the potential hazards) then all candidates who met the eligibility criteria were subjected to the following: full history taking, thorough physical examination (including maternal body weight and the 1st day of the LMP) followed by obstetric ultrasound to confirm the eligibility of the current pregnancy to participate in the study (by confirming gestational age & excluding fetal anomalies or oligohydramnios) and to assess the fetal weight and amniotic fluid index (to detect presence of macrosomia and polyhydramnios (defined as EFW above 90th percentile for gestational age and AFI more than 95th percentile, respectively). Laboratory investigations (complete blood picture, fasting and post prandial blood sugar, liver & kidney functions and HbA1C estimation) were also done.

Doppler ultrasonography assessment was done using Samsung SonoAce R3 abdominal probe convex linear transducer 3.5 MHZ

equipped with color and pulsed Doppler capabilities (SonoAce R3, SAMSUNG MEDISON CO., Gangnam-gu, Seoul, Korea). As regard umbilical artery (UA) Doppler, participants were examined in a semi-recumbent position with a left lateral tilt. The uterine content was scanned and an area of amniotic cavity with many free loops of cord was selected. The characteristic sound and shape of the umbilical artery were identified using a pulsed wave Doppler applied on a free loop of cord. The image was frozen when at least 3 consecutive waves of similar height appeared on the screen and umbilical artery Resistance index (RI) and pulsatility index (PI) were calculated. The final values were obtained after a minimum of 3 separate readings were averaged. Umbilical artery Doppler evaluations were performed during fetal apnea (to nullify effect of fetal breathing movements on waveform variability) and avoided during fetal activity (11). Abnormal UA Doppler velocimetry was considered when UA indices exceeded the 95th centile for the corresponding gestation or when the diastolic flow was either reversed or absent (figure 1&2).

As for the evaluation of the middle cerebral artery (MCA) Doppler indices, the fetal brain was scanned at the level of the biparietal diameter and a transverse view was obtained then the probe was advanced towards the base of the skull till the level of the lesser wing of the sphenoid bone. The middle cerebral artery was identified using color flow imaging being the major lateral branch of the circle of Willis that runs anterolaterally at the margin between the anterior and the middle cerebral fossae. The flow velocity waveforms were obtained by placing the pulsed Doppler sample gate on the middle portion of the artery. The image was frozen when at least 3 consecutive waves of similar height appeared on the screen and MCA RI &PI were calculated. The final values were obtained after a minimum of 3 separate readings were averaged. As fetal head compression may alter intracranial arterial waveforms, subsequently, no or minimal pressure should be applied to maternal abdomen during the scan (12). Abnormal MCA Doppler velocimetry was considered when MCA indices were below the 5th centile for the corresponding gestational age (figure 3&4). All ultrasounds and Doppler evaluations were done by the same sonographer (Rasha El-komy).

The following data were recorded; gestational age at Doppler study and termination, presence of macrosomia or polyhydramnios, Doppler indices for UA & MCA, mode of delivery and neonatal outcomes (i.e., birth weight, 1- & 5-minutes Apgar score, blood sugar at birth, admission to neonatal intensive care unit). Abnormal perinatal outcomes were considered in the presence of any of the following four events: 1-& 5- minutes Apgar scores below 7, neonatal blood sugar less than 50 mg/dl (neonatal hypoglycemia) and neonatal intensive care unit (NICU) admission. Patients with at least one adverse neonatal event were categorized in the abnormal neonatal outcome group.

Primary outcome measured the difference in Doppler indices values (RI&PI) for umbilical and middle cerebral arteries between the control (non-diabetic) and the study group (diabetics whether controlled or uncontrolled). The sensitivity and specificity of umbilical artery and middle cerebral artery Doppler indices (RI&PI) as predictors for adverse neonatal outcomes among diabetic women were assessed as secondary outcomes.

The sample size was calculated according to the figures obtained from Shabani Zanjani and his colleagues (9) using the Pulsatility Index (PI) of the left MCA Doppler. The mean PI for MCA Doppler in the first group (gestational diabetic cases) was 2.07 and the second group (non-diabetic cases) was 1.85. The standard deviation (SD) used for calculation was 0.40. The ratio of enrollment for the study to control was 1:1. The power

was set at 0.8 and Alpha error at 0.05. This gave us the sample of 52 patients in each group, we raised the sample size by 15% to avoid dropouts thus giving us 60 cases in each study arm. Sample size was calculated using Sample Size Calculator ClinCalc.com last accessed on 2/4/2017.

Data were coded and entered using the statistical package SPSS version 25. Data was summarized using mean, standard deviation, median, minimum and maximum for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test in normally distributed quantitative variables, while non-parametric Mann-Whitney test was used for non-normally distributed quantitative variables (13). For comparing categorical data, Chi square (|2) test was performed. Exact test was used instead when the expected frequency was less than 5 (14). Correlations between quantitative variables were done using Spearman correlation coefficient (15). Logistic regression was done to detect independent predictors of cases (16). P-values less than 0.05 were considered as statistically significant.

Results

This prospective study included one hundred and twenty patients who met the inclusion criteria. Flow of patients was demonstrated in figure 5.

Study versus control group analysis

Patients' characteristics and laboratory parameters together with pregnancy characteristics and neonatal outcomes were summarized in table 1. Regarding adverse neonatal outcome, there was a statistically significant difference between the study and control group in 3 out 4 parameters defined in methodology namely; the frequency of neonatal hypoglycemia, 1-minute Apgar score at 1minute (below 7) and Apgar score at 5 minutes (below 7) (table 2).

Our results showed no statistically significant difference between the mean RI & PI of **umbilical artery** in the study group (RI= 0.63& PI=0.93) and the control group (RI = 0.61 & PI = 0.95; P value =0.057 &0.659, respectively). Similarly, there was no statistically significant difference between the mean RI &PI of the **middle cerebral artery** between the study group (RI = 0.8 & PI=1.73) and the control group (RI=0.83 & PI=1.81; P value =0.07 &0.322, respectively). Consequently, the difference between the mean **Cerebroplacental Doppler ratio** (MCA/UA PI) in both groups was not statistically significant (1.94 in the study group versus 1.92 in the control group; P=0.754) (table 3).

Controlled diabetics versus uncontrolled diabetics

Patients' characteristics and laboratory parameters together with pregnancy characteristics and neonatal outcomes were summarized in table 4. Regarding adverse neonatal outcome, there was no statistically significant difference between the controlled and uncontrolled group in all parameters defined in methodology (table 5).

There was no statistically significant difference between the mean RI &PI of **umbilical artery** in the controlled group (RI = 0.62 & PI = 0.92) and the uncontrolled group (RI = 0.64 & PI= 0.94; P value =0.5 & 0.764, respectively). Similarly, there was no statistically significant difference between the mean RI & PI of the **middle cerebral artery** between the controlled group (RI= 0.81 & PI = 1.77) and the uncontrolled group (RI = 0.79& PI = 1.69; P value =0.52& 0.488 respectively). Hence, the difference between the mean **Cerebroplacental Doppler ratio** (MCA/UA PI) in both groups was not statistically significant (1.98 in the controlled diabetic group versus 1.9 in the uncontrolled diabetic group; P value =0.46) (table 6).

Predictive value of umbilical artery & middle cerebral artery Doppler indices in the diabetic group

Adverse neonatal outcomes were detected in 24 neonates out of 60 among those included in the study group. Umbilical artery Doppler indices (abnormal RI & PI) correctly identified only six of them, while, middle cerebral artery Doppler indices correctly identified only five of them. Based on data, the sensitivity and specificity of umbilical artery and middle cerebral artery Doppler tests in the prediction of adverse neonatal outcomes were calculated and the results are summarized in table (7).

Logistic regression to detect independent predictors of cases

We performed a multivariate logistic regression to identify factors associated with cases compared to control group. We have found that only PPBS was more likely to be associated with the study group (odds ratio (OR): 1.32, 95% Confidence Interval: 1.05-1.66, p= 0.018) (table 8).

Correlation analysis of umbilical artery & middle cerebral artery Doppler indices with neonatal outcome

The RI and PI of umbilical artery in the study group were not significantly correlated with the Apgar score at 1 minute (Pearson's r = -0.07, p = 0.58), Apgar score at 5 minutes (r = -0.14, p = 0.28) or neonatal hypoglycemia (r = -0.12, p= 0.35). Similarly, the RI and PI of middle cerebral artery were not significantly correlated with Apgar score at 1 minute (r = -0.03, p = 0.80), Apgar score at 5 minutes (r = 0.07, p = 0.58) or neonatal hypoglycemia (r= 0.13, p = 0.34) (table 9).

Discussion

Diabetes is a multisystem chronic disease that requires vigilant medical care and implementation of different strategies for risk reduction beside optimum glycemic control. Patient support and health education form the cornerstone of management of those cases in order to prevent acute complications and reduce the risk of long-term complications. Several strategies have been developed in order to improve the outcome of diabetes. (17).

The care of women with pregestational diabetes should be delivered ideally by multidisciplinary team in a multidisciplinary setting that consists of an endocrinologist, maternal-fetal medicine specialist, dietitian and diabetes educator, when available (17).

Fetal surveillance is an important tool in the care of such pregnancies, complicated with PGDM or GDM. Doppler velocimetry is one of the most important methods of antenatal surveillance. In the present study, we examined whether UA and MCA Doppler measurements could help to sort out fetuses at risk of jeopardized outcomes in case of maternal DM.

Our results showed that fetal and neonatal risks were higher with pregnancies complicated by pregestational diabetes in comparison to their healthy counterparts. However, this difference was not shown to be present in the measurements of umbilical artery (UA), middle cerebral artery (MCA) and cerebroplacental ratio Doppler. Furthermore, neonatal outcome was jeopardized in uncontrolled diabetic group compared to controlled diabetic group, but this was not statistically significant. In addition, our results failed to demonstrate any difference of statistical significance in umbilical artery, middle cerebral artery Doppler indices and cerebroplacental ratio between the two subgroups of pregnant patients with pregestational diabetes. Both UA and MCA assessments had low sensitivity in the prediction of adverse neonatal outcome (25% and 20.83%, respectively) whereas their specificity were 89% and 92%, respectively.

Our results showed that maternal DM does not adversely affect Doppler indices. This finding was in harmony with the results reported in a study by Salvesen and his co-workers (18). They investigated the effect of maternal diabetes on both placental and

fetal circulation in relation to any changes in fetal blood pH, PO2, and hematocrit; but the study included only well-controlled diabetic pregnancies (65 cases). They concluded that there was no association between maternal DM abnormal Doppler indices of placental or fetal circulation.

Likewise, Ben-Ami and his colleagues (19) evaluated the role systolic to diastolic ratio (S/D ratio) of the umbilical artery in the prediction of perinatal outcome in diabetic pregnancies. Their study included 92 diabetic pregnant women of gestational age from 28 to 40. the sensitivity and specificity of the Doppler studies as a predictor of poor perinatal outcome had a sensitivity and specificity of 39% and 92%, respectively, thus failing to prove any association of maternal diabetes to S/D ratio abnormalities in fetuses who had an inferior outcome to their counterparts. They concluded that the S/D ratio of the umbilical artery was not superior to other surveillance tests in the management of diabetic pregnancies.

The current results showed that sensitivity of both UA and MCA Doppler tests was low for neonatal outcomes. These findings are not different than the results reported by Niromanesh and his colleagues (10). They compared MCA and UA Doppler indices for the evaluation of fetal well-being in 103 pregnant mothers with pre-gestational or gestational diabetes mellitus. Those who had abnormal UA or MCA Doppler test results were candidates for pregnancy termination either by labor induction or cesarean section, which was decided upon set criteria. The outcomes included one- and five-minute Apgar score, admission to NICU, metabolic disturbances as acidosis, hypoglycemia, hypocalcemia, in addition to gestational age at delivery, and neonatal death. 48 women had poor perinatal outcomes; 17.5% and 9.7% of women had abnormal UA and MCA Doppler test results, respectively. They concluded that though abnormal UA and MCA Doppler tests were associated with adverse neonatal outcomes; yet both tests had low sensitivity in the prediction of adverse neonatal outcomes (ranged between 20% and 60%).

In another study, Niromanesh and his colleagues (20) compared between the efficacy of the non-stress test (NST) to that of the umbilical artery (UA) Doppler assessments in the prediction of adverse perinatal outcomes in 50 pregnant women with GDM. Totally, 22% and 12% of women had an abnormal UA Doppler and a non-reactive NST respectively; 13 women had poor outcomes. Women with non-reactive NST ($p<0.001$) and abnormal UA Doppler ($p=0.033$) had a higher prevalence of poor neonatal outcome. The sensitivity and specificity of the NST in predicting different poor outcomes were 76.9% and 97.3% respectively, whereas that UA Doppler in predicting different poor outcomes were 30.8% and 94.6% respectively. Accordingly, they concluded that NST was far more superior to UA in the prediction of adverse perinatal outcomes in patients with GDM.

Moreover, Yalti and his colleagues (21) stated that Umbilical velocimetry, is an assessment tool for placental function only and is not a direct reflection of the fetal status. In their study, sensitivity, positive predictive values of umbilical artery Doppler indices alone were 30 and 50 per cent respectively.

To the contrary, Shabani Zanjani and his colleagues (9) studied the effects of GDM on Doppler parameters (fetal MCA and UA) in comparison to normal pregnancies. The study was performed on 66 pregnant women, including 33 women with GDM and 33 healthy pregnant patients. Peak systolic and diastolic velocities, PI, RI and systolic diastolic ratio (SD) were recorded in UA as well as both right and left fetal MCAs for every recruited pregnant woman by means of Doppler ultrasonography. The mean gestational age at the time of examination was 34.45 weeks in GDM group. Although, the study group had higher Doppler indices values compared to their healthy counterparts; yet this was not

statistically significant. However, only the left fetal MCA-PI, was significantly higher in GDM group; for which they concluded that gestational diabetes may contribute to an elevated PI in the fetal MCA. However, the small sample size with the consequent low statistical power and the lack of access to follow up data, were two major limiting factors to this study.

To the best of our knowledge, the current study is the first one that compared fetal middle cerebral and umbilical arteries Doppler indices in pregestational diabetic versus normal pregnancies. Most of the former studies focused more on the blood flow of umbilical artery and fetal vessels in patients with gestational DM (GDM). Furthermore, we evaluated any difference that could be impacted upon the Doppler indices by glycemic control. We also excluded patients with other concomitant medical disorders to avoid the effect of other confounding variables on Doppler indices.

The first shortcoming of this study was the small sample size that led to low statistical power in between the groups of comparison. Although the rate of poor neonatal outcomes in our sample was about 40%, the outcomes were not associated with abnormal Doppler test results of fetal circulation. This may be due to the outcomes were not severe enough to affect fetal circulation. Further studies and systematic reviews are warranted to reach a precise answer about the best surveillance test for fetal evaluation among diabetic mothers.

In conclusion, maternal DM is not associated with abnormal changes in Doppler indices of placental or fetal circulation (irrespective of the glycemic control). In addition, both UA and MCA assessments had low sensitivity in the prediction of adverse neonatal outcome.

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Declarations

Ethics approval and consent to participate Kasr Alainy ethical committee approval.

Consent for publication all participants agave their consent for publication

Informed consent: Informed consent was obtained from all individual participants included in the study.

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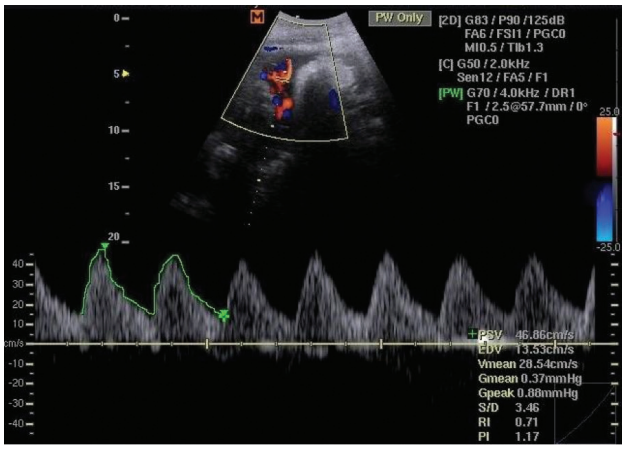

Figure (1): Umbilical artery Doppler for uncontrolled diabetic with adverse neonatal outcome (1-min APGAR score was less than 7).

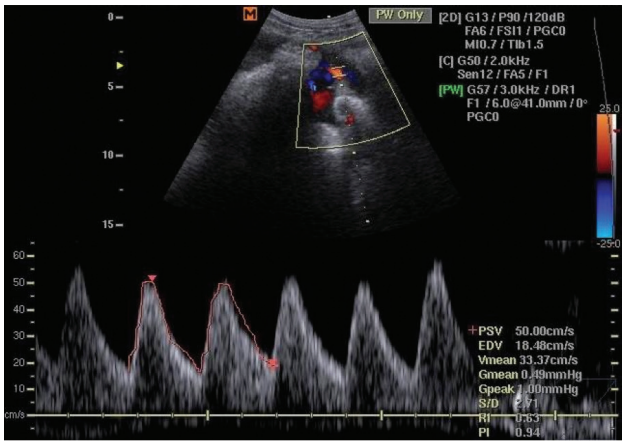

Figure (2): Umbilical artery Doppler for non-diabetic pregnant woman.

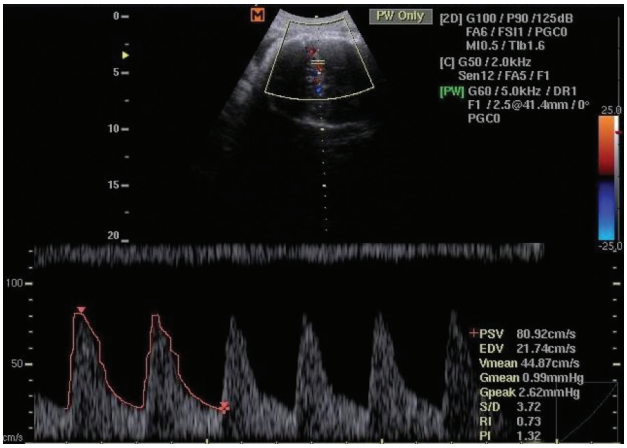

Figure (3): Middle cerebral artery Doppler for controlled diabetic women with adverse neonatal outcome (neonatal blood sugar 40mg/dl& 1-min APGAR score less than 7).

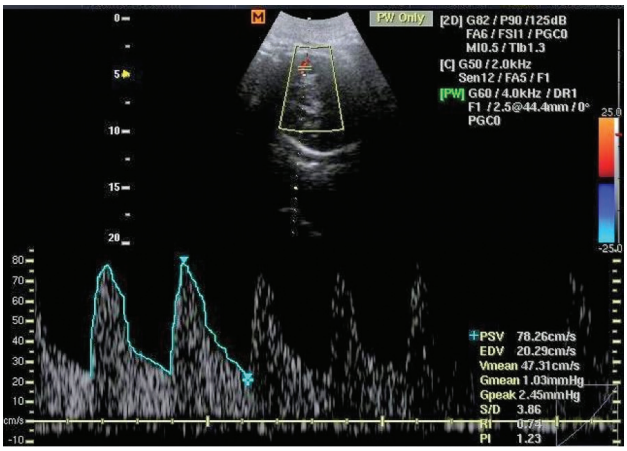

Figure (4): Middle cerebral artery Doppler for healthy pregnant women.

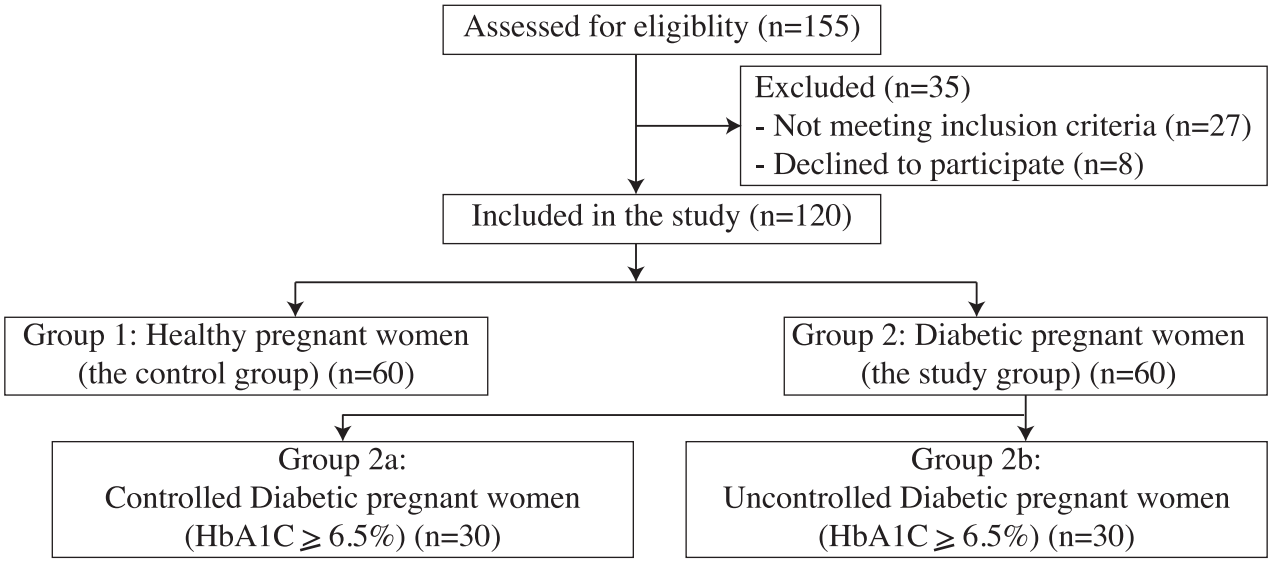

Figure (5): flow chart of the participants.

Table (1): Patients characteristics and laboratory parameters, pregnancy characteristics and neonatal outcome in the study and control groups.

Variable	Study group	Control Group	P value
Maternal Age (Y, Mean ± SD)	30.45 ± 5.80	27.77 ± 5.76	0.012
BMI (kg/m², Mean ± SD)	30.37 ± 4.468	30.8 ± 4.337	0.591
Primigravidas (No., %)	12 (20%)	18 (30%)	0.606
FBS (mg/dl, mean ± SD)	107.00 ±17.62	78.00 ± 6.63	< 0.001
PPBS (mg/dl, mean ± SD)	166.63 ±21.99	114.83 ± 6.69	< 0.001
HBA1C (%,mean ± SD)	6.69 ±1.16	5.75 ± 0.46	< 0.001
Cesarean delivery (No., %)	40 (66.7%)	30 (50%)	0.064
GA at termination (wks, mean ± SD)	36.45 ± 0.746	36.20 ± 0.879	0.096
Neonatal birth weight (g,mean ± SD)	3315.17±455.41	3116.83±214.99	0.003
Neonatal blood sugar (mg/dl,mean ± SD)	68.27±22.07	79.92±18.83	0.002
1 min Apgar score (mean ± SD)	6.73 ± 1.039	7.47 ± 0.965	< 0.001
5 min Apgar score (mean ± SD)	8.08 ± 1.43	8.77 ± 1.17	0.005

Table (2): Frequency of adverse neonatal outcomes in study and control groups

Variable	Study group	Control Group	P value
Hypoglycemia (<50mg/dl - No., %)	14 (23.3 %)	2 (3.3%)	0.001
1 min Apgar score <7 (No., %)	18 (30%)	6 (10 %)	0.006
5 min Apgar score <7 (No., %)	12 (20%)	4 (6.7%)	0.032
NICU admission (No., %)	7 (11.7%)	3 (5 %)	0.186

Table (3): Gestational age at Doppler studies, Ultrasonographic findings & Doppler indices in study & control groups.

Variable	Study group	Control Group	P value
GA at Doppler study (wks, mean ± SD)	36.28±0.88	36.12 ±1.01	0.338
Macrosomia (No., %)	15 (25%)	2 (3.3%)	0.001
Polyhydramnios (No., %)	14 (23.3%)	4 (6.7%)	0.011
UA RI (mean ± SD)	0.63 ± 0.07	0.61 ± 0.06	0.057
UA PI (mean ± SD)	0.93 ± 0.25	0.95 ± 0.13	0.659
MCA RI (mean ± SD)	0.8 ± 0.1	0.83 ± 0.06	0.07
MCA PI (mean ± SD)	1.73 ± 0.45	1.81 ± 0.37	0.322
MCA/UA PI (mean ± SD)	1.94 ± 0.42	1.92 ± 0.29	0.754

Table (4): Patients characteristics and laboratory parameters, pregnancy characteristics and neonatal outcome in controlled and Uncontrolled diabetics groups.

Variable	Controlled DM	Uncontrolled DM	P value
Maternal Age (Y, mean ± SD)	30.53 ± 6.91	30.37 ± 4.55	0.913
BMI (kg/m^2, mean ± SD)	30.8 ± 4.506	29.93 ± 4.464	0.457
Primigravidas (No., %)	10 (33.3 %)	2 (6.7 %)	0.01
FBS (mg/dl, mean ± SD)	97.17 ± 13.24	116.83 ±16	< 0.001
PPBS (mg/dl, mean ± SD)	158.33 ± 23.50	174.93 ±17.01	0.003
HBA1C (%, mean ± SD)	5.95 ± 0.49	7.43 ±1.16	< 0.001
GA at termination (wks, mean ± SD)	36.37 ± 0.928	36.53 ± 0.507	0.393
Cesarean delivery (No., %)	16 (53.3%)	24 (80%)	0.028
Neonatal birth weight (g,mean ± SD)	3230.83± 322.86	3400.33± 550.18	0.150
Neonatal blood sugar (mg/dl, mean ± SD)	68± 22.03	68.53± 22.47	0.926
1 min Apgar score (mean ± SD)	6.77 ± 1.104	6.7 ± 0.988	0.806
5 min Apgar score (mean ± SD)	8.2 ± 1.448	7.97 ± 1.426	0.532

Table (5): Frequency of adverse neonatal outcomes in controlled and Uncontrolled diabetics groups.

Variable	Study group	Control group	P value
Hypoglycemia (<50mg/dl - No., %)	6 (20%)	8 (26.7 %)	0.542
1 min Apgar score <7 (No., %)	8 (26.7%)	10 (33.3%)	0.573
5 min Apgar score<7 (No., %)	5 (16.7%)	7 (23.3%)	0.519
NICU admission (No., %)	3(10%)	4 (13.3%)	1

Table (6): Gestational age at Doppler studies, Ultrasonographic findings & Doppler indices in controlled and Uncontrolled diabetics groups.

Variable	Controlled DM	Uncontrolled DM	P value
GA at Doppler study (wks, mean ± SD)	36.2 ±1.06	36.37±0.67	0.471
Macrosomia (No., %)	7 (23.3%)	8 (26.7%)	0.766
Polyhydramnios (No., %)	6 (20%)	8 (26.7%)	0.542
UA RI (mean ± SD)	0.62 ± 0.06	0.64 ± 0.08	0.5
UA PI (mean ± SD)	0.92 ± 0.23	0.94 ± 0.26	0.764
MCA RI (mean ± SD)	0.81 ± 0.09	0.79 ± 0.1	0.52
MCA PI (mean ± SD)	1.77 ± 0.45	1.69 ± 0.45	0.488
MCA/UA PI(mean ± SD)	1.98 ± 0.37	1.9 ± 0.47	0.46

Table (7): The sensitivity, Specificity, positive and negative predictive values and accuracy of umbilical artery & middle cerebral artery Doppler in the prediction of adverse neonatal outcomes among diabetic patients

	Umbilical artery Doppler		Middle cerebral artery Doppler	
Statistic	**Value**	**95% CI**	**Value**	**95% CI**
Sensitivity	25%	9.77% to 46.71%	20.83%	7.13% to 42.15%
Specificity	88.89 %	73.94% to 96.89%	91.67 %	77.53% to 98.25%
PPV	60 %	32.09% to 82.64%	62.50%	30.49% to 86.36%
NPV	64 %	57.86% to 69.71%	63.46 %	58.04% to 68.56%
Accuracy	63.33%	49.90% to 75.41%	63.33%	49.90% to 75.41%

PPV = Positive Predictive Value – NPV=Negative Predictive Value

Table (8): Logistic regression to detect independent predictors of cases

		P value	OR	95% C.I.	
				Lower	Upper
Cases	**Age (yrs)**	0.759	0.969	0.794	1.184
	FBS	0.147	1.183	0.943	1.484
	PPBS	0.018	1.319	1.049	1.659
	HBA1C	0.594	0.504	0.040	6.270
	Neonatal birth weight	0.377	0.998	0.993	1.003
	Neonatal blood sugar	0.300	0.960	0.890	1.037
	Apgar score at 1 min	0.486	0.496	0.069	3.557
	Apgar score at 5 min	0.539	0.610	0.126	2.952

Table (9): Correlation analysis of umbilical artery & middle cerebral artery Doppler indices with neonatal outcome

		RI (umbilical artery doppler)	PI (umbilical artery doppler)	RI (middlle cerebral artery)	PI (middlle cerebral artery)
Apgar score at 1 min	Correlation Coefficient	-0.073-	-0.073-	-0.033-	-0.035-
	P value	0.577	0.580	0.802	0.791
	N	60	60	60	60
Apgar score at 5 min	Correlation Coefficient	-0.141-	-0.141-	0.071	0.067
	P value	0.284	0.281	0.589	0.612
	N	60	60	60	60
Neonatal blood sugar	Correlation Coefficient	-0.123-	-0.120-	0.126	0.126
	P value	0.348	0.359	0.337	0.336
	N	60	60	60	60

Effects of adding oestradiol supplementation in luteal phase in patients undergoing ICSI long agonist fresh embryo transfer cycles

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Abstract

Background: Luteal phase support (LPS) is a crucial step in ICSI/IVF cycles for embryo implantation. It has been agreed that progesterone supplementation is an integral part of luteal phase support and implantation. Different additional supplementations have been proposed in addition to progesterone; of which estradiol was one. This study was done to compare the additive role of ostradiol supplementation to progesterone for luteal phase support compared to progesterone alone in ICSI cycles.

Objectives: to assess the effect of oestradiol supplementation in addition to progesterone during luteal phase on the implantation rate in patients undergoing long agonist ICSI/IVF cycles.

Methods: A prospective randomized controlled double blinded study, two-hundred and thirty six patients undergoing their first ICSI cycle using the long agonist protocol, were enrolled in this study. Participants were then randomized into two equal groups of 118 patients each; **Group A:** received a dose of 400 mg progesterone twice daily in the form of vaginal or rectal suppositories, in addition to (2x2) placebo oral tablets (similar to estrogen tablets). **Group B:** received 400 mg progesterone twice daily in the form of vaginal or rectal suppositories, in addition to oestradiol valerate oral tablets in a dose of 4mg/day (2x2). In both groups, medications were started from the day of ovum pickup and for 14 days after embryo transfer. Participants were further divided in to two groups, according to their oestradiol levels. Implantation rate was set as the primary outcome, secondary outcome included chemical, clinical pregnancy and miscarriage rate per cycle.

Results: The implantation rate was significantly higher in the progesterone only group (Group A) compared to oestradiol and progesterone group (GroupB) (12.88% vs 7.98% respectively)

Conclusion: Supplementation of oestradiol to progesterone in luteal phase support confers no additional benefit to progesterone alone.

Further studies are required to elucidate the role of oestradiol in the luteal phase support in ICSI cycles.

Key words: Oestradiol, Progesterone, ICSI, luteal phase support, implantation rate .

Introduction

Adequate luteal phase function is a crucial part of embryo implantation and both pregnancy development and maintenance. In normal ovulatory cycles, progesterone secreted by the corpus luteum helps in pregnancy maintenance until the placenta takes over at 7 weeks. Therefore, a defective progesterone secretion, and hence a defect in the luteal phase would hamper the development and maintenance of the pregnancy process (1,2).

In controlled ovarian stimulation cycles, the multifollicular development and supra physiological levels of oestradiol and progesterone induce negative feedback on luteinizing hormone (3,4); thus resulting in luteal phase dysfunction, which in turn affects ICSI/IVF outcome negatively (5). This dysfunction has been observed in both GnRH agonist and antagonist protocols, hence luteal phase support became an integral step in ICSI/IVF cycles (6).

Progesterone plays an important role in the implantation process through different mechanisms at both cellular and humoral levels. At cellular level, progesterone reduces intracellular calcium concentration, and at humoral level it plays as an immunomodulatory. Progesterone prepares the endometrium and improves its receptivity for embryo implantation (7). Successful implantation requires the synchronization of the endometrium receptivity with the embryo, which is effectively achieved by progesterone supplementation (8).

In natural ovarian cycles, estrogen is secreted in addition to progesterone by the corpus luteum, thus suggesting that estrogen supplementation may play a role in luteal phase support in IVF/ICSI cycles (7). A met analysis in 2015, concluded that there was no role to oestradiol supplementation in addition to progesterone in IVF/ICSI implantation and pregnancy rates (9). Two further studies also concluded that adding estradiol conferred no additional benefit (10,11).

The use of oestradiol has been a matter of controversy over the past decade (12), we have designed this study to assess the role of additional oestradiol supplementation to progesterone in long agonist ICSI cycles.

Sample size

According to Lukaszuk et al. (2005) (13), a group sample size of 112 in each group achieves 80% power and 0.05 significance level to detect a difference between the group proportions of 0.1405. The proportion in the treatment group is assumed to be 0.0980 under the null hypothesis and 0.2385 under the alternative hypothesis. The proportion in the control group is 0.0980. The statistical test used is the two-sided Z test with pooled variance. The sample size increased by 5 % to be 118 in each group for dropout.

Patients and Methods

We have conducted a prospective randomized controlled double blinded study, two hundred and thirty six patients undergoing GnRH long agonist protocol, with fresh embryo transfer were recruited at the Assisted Reproduction Treatment unit, Obstetrics and Gynecology department, Cairo University hospitals. The participants were recruited in the period between May 2019 and May 2022.

The study was conducted after the approval of

the the ethical committee of the Gynecology and Obstetrics; and was registered at the Clinical trial.gov (registration no.NCT03832894).

The included women had signed written informed consent before participating in this study after being informed of the purpose, interventions, outcome, and possible complications.

Inclusion criteria were female patients between 20- and 38-years old, undergoing GnRH long agonist protocol, with fresh embryo transfer on day 3, or day 5. Grade 1 and Grade 3, quality embryos were transferred under ultrasound guidance. Women were excluded if they had karyotypic abnormalities in either partner, uterine abnormalities, grade 3 / grade 4 (G3-G4) quality embryos, estradiol level 10,000 or more at time of trigger, egg /sperm donation/embryo donors, polycystic ovary syndrome (PCOS) patients, poor responders and patients with severe male factor.

All women in both groups were subjected to detailed history and clinical examination to ensure adherence to inclusion criteria. Trans-vaginal ultrasound (TVS) [vaginal probe 6.5MHz, Mindray, China] was done on day 2 to 5 to assess antral follicular count, uterus and adnexa then long agonist protocol was started.

Participants were then randomized into two main groups: Group A: Received 400 mg progesterone twice daily in the form of suppositories (Prontogest 400mg) either through the vagina or rectum, in addition to 2x2 placebo oral tablets (similar to estrogen tablets) for luteal phase support,. This was started on the day of oocyte retrieval and for 14 days after embryo transfer. Group B: Received a dose of 400 mg progesterone twice daily in the form of suppositories either vaginally or rectally, in addition to 2x2 oestradiol valerate oral tablets (Progynova 2mg, Bayer) in a dose of 4mg/day (2x2), for luteal phase support, from the day of ovum pickup and continued for 14 days following transfer of embryos.

In both groups, the participants were further subdivided into two subgroups, according to their estradiol levels: Subgroup A: patients with oestradiol levels less than 5000 pg/ml on the day of human Chorinic Gonadotropin (hCG) trigger. Subgroup B: patients with oestradiol level between 5000 -10,000 pg/ml on the day of hCG trigger. Randomization was done by withdrawing closed envelopes for each patient. Double blinding was applied (both patient and health administrator).

For both groups quantitative ß- HCG in serum was done after 14 days of embryo transfer and also TVS to detect clinical pregnancy at 6-7 weeks of gestation.

In the event of pregnancy in either group, same luteal phase support for group A and B was continued till 12 weeks gestation.

Statistical analysis

Pre-coded data was entered on the computer using Microsoft Office Excel Software Program 2018. Pre-coded data was then transferred and entered into the Statistical Package of Social Science Software program, version 25 (SPSS), to be statistically analyzed. Quantitative variables were described as mean ±SD, median, and range, while qualitative variables were described as frequency and percentage. For quantitative data, the Independent Sample t-test was used to compare normally distributed variables, and the Mann-Whitney U test was used to compare non-normally distributed variables. On the other hand, the Chi-square test/Fisher Exact test was used to compare qualitative variables. P-value was considered significant if less than 0.05.

Results

The results are illustrated in Tables 1, 2 and 3.

Table 1 displays different patient characteristics as age, BMI, type and duration of infertility and sociodemographic characteristics. As

shown both groups were properly matched regarding the aforementioned characteristics.

Table 2 shows the cycle characteristics as AFC, number of days of stimulation, dose of gonadotrophins, endometrial thickness on the day of embryo transfer and number of embryos transferred. Both groups were comparable except for the endometrial thickness which was significantly higher in the progesterone only group (groupA), compared to group the oestradiol and progesterone group (group B) [11 vs 10.5], with a p value 0.01.

Table 3 shows the implantation, chemical and clinical pregnancy rates and miscarriage rate among the study groups. The implantation rate was significantly higher in group A compared to group B (12.88% vs 7.98%), with a P value: 0.029.

The progesterone only subgroup with serum E2 levels higher 5000 pg.ml, showed significantly higher implantation rate than the oestradiol and progesterone subgroup with serum E2 levels greater than 5000 pg/ml; (13.38% vs 7.32%), p value 0.012.

The clinical and chemical pregnancy and miscarriage rates did not show any significant difference between both groups.

Discussion

There is no debate that progesterone supplementation is fundamental for luteal phase support. The question was there any beneficial role to adding oestradiol to progesterone on implantation and pregnancy rates in IVF/ICSI outcomes. This study was designed to compare the role of adding estradiol tablets in a dose of 4mg to vaginal progesterone in infertile patients with good prognostic factors (i.e good ovarian reserve indicated by their basal FSH and AMH levels ; undergoing their first ICSI/IVF cycle using the GnRh agonist long protocol.

In this randomized controlled trial, computer randomization together with double blinding of the two groups to estradiol and placebo, eliminated any element of bias that could be related to patient selection. Both groups were comparable regarding the age, basal hormone levels, BMI and smoking, thus making both groups comparable. Also both groups were comparable regarding the AFC, total dose of gonadotrophins, days of stimulation and number of embryos transferred, thus eliminating any bias in the implantation rate that could be due to number of embryos transferred.

The implantation rate was higher in group A (progesterone alone) 12.88%, whereas that for group B (progesterone and oestradiol valerate 4mg) was 7.98%, the p value 0.029% which makes it significantly different. The endometrial thickness was significantly greater in the progesterone only group 10.99 ± 1.24 Vs the oestradiol and progesterone group 10.31 ± 1.90, which was statistically significant p value 0.001%. The difference in endometrial thickness between both groups could explain the significantly higher implantation rate in the progesterone only group.

The chemical and clinical pregnancy rates were higher in the progesterone only group compared to the oestradiol and progesterone group (29.66% Vs 26.27%, p value 0.56), (27.12% Vs 22.03%, p value 0.364). However, this was not statistically significant. Pregnancy loss was higher in group B compared to group A 16.13% Vs 5.71%, again this was statistically insignificant.

Both groups had comparable serum E2 levels on the day of hcg trigger (2890.71 ± 1956.58 Vs 2843.89 ± 1834.91), with p value 0.850. This negates that the difference in implantation rates could be attributed to difference in serum E2 levels between the progesterone only group (group A) and the progesterone and oestradiol group (group B).However, serum E2 levels higher than 5000 pg/ml in progesterone only group was associated with significantly higher implantation rate (13.38%) compared to serum E2 levels of more than 5000 pg/ml

in the progesterone and oestradiol group (7.32%); p value 0.012. The significant difference between the major groups A and B in relation to serum E2 levels > 5000 pg/ml, could not be explained.

In a former systematic review of 4 articles from 2000 till 2016, Pinherio et al concluded that oestradiol addition to progesterone was not superior to progesterone alone in GnRH antagonist cycles. The patients included in different papers showed similar patient characteristics to our study and good prognostic factors, thus mitigating the bias that could be due to poor ovarian reserve. However, those studies used the GnRH antagonist protocol and not the long GnRH agonist protocol used in our study (10).

In a retrospective observational study that included 150 patients with 75 in each group, the pregnancy rate was 41% Vs 36 % in the oestradiol Vs control group. Again in this study, the GnRH antagonist protocol was the used protocol (11). It is believed that antagonist cycles show lower estradiol levels compared to agonist cycles. This marked decrease in serum pestradiol levels is secondary to increased serum progesterone levels seen in antagonist cycles (12). Despite that, addition of estrogen to progesterone in antagonist cycles conferred no superior results over progesterone alone in the aforementioned studies.

Another similar study to ours, but was open label; that included 160 patients divided equally in to two groups. The control group received progesterone suppositories 200 mg twice daily, and the intervention group, received oestradiol in the form of patch 100mcg/day in addition to progesterone. They concluded that supplementing oestradiol did not add any extra benefit, as the implantation rate did not differ between both groups (34.9% [51 of 146] vs. 28.9% [41 of 142], the ongoing pregnancy rate was the same in patients receiving oestradiol in addition to progesterone compared to progesterone alone (14).

Another meta analysis of different studies that was conducted in 2019 concluded that oestradiol supplementation in luteal phase in IVF/ICSI was beneficial compared to progesterone alone. However, they concluded that this benefit was only observed in GnRH agonist cycles only but its supplementation in antagonist cycles conferred no additional value on clinical pregnancy and implantation rates. This was contradictory to the fore mentioned studies which mainly evaluated its effect on antagonist cycles, and ours in which GnRh agonist protocol was the study protocol (15).

On comparing pregnancy rates in relation to serum E2 levels in the major groups A and B, , we could not establish any correlation between serum E2 levels and pregnancy rates. Previous studies showed such positive correlation (16, 17); other studies failed to establish any correlation of serum E2 levels with pregnancy rate (18). Further studies and met analysis are needed to evaluate the role of serum estradiol levels on IVF/ICSI outcomes.

The strength of our study is that it was double blinded which makes it unbiased. Furthermore, it involved one protocol and the patients were comparable regarding characteristics and IVF/ICSI prognostic factors, thus making the results more reliable. The limitation in our study was sample size, larger sample size might be more informative.

In conclusion, adding estradiol did not improve implantation rates, to the contrary, it compromised the outcome. More studies have to be done to evaluate if there is any role to adding oestradiol in luteal phase support in fresh embryo transfer cycles, and till then its use should not be recommended outside the scope of research.

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Results

Table 1: Characteristics of the study groups

	Group A (P only) (n=118)	Group B (E+P) (n=118)	P- value
Age (years)	29.66 ± 5.37 30 (19 - 39)	28.94 ± 5.21 28 (18 - 40)	0.297
BMI	28.51 ± 5.10 29 (19 - 42)	28.75 ± 4.62 29 (20 - 44)	0.698
Gravidity	0.69 ± 1.32 0 (0 - 8)	0.58 ± 1.02 0 (0 - 5)	0.826
Parity	0.25 ± 0.57 0 (0 - 4)	0.19 ± 0.45 0 (0 - 2)	0.411
Previous abortions	0.44 ± 1.14 0 (0 - 8)	0.39 ± 0.92 0 (0 - 5)	0.967
Occupation • Housewife • Employer	 101 (85.59%) 17 (14.41%)	 114 (96.61%) 4 (3.39%)	**0.005***
Residence • Urban • Rural	 73 (61.86%) 45 (38.14%)	 74 (62.71%) 44 (37.29%)	0.893
Type of infertility • Primary • Secondary	 76 (64.96%) 41 (35.04%)	 77 (65.25%) 41 (34.75%)	0.962
Infertility Duration (years)	4.62 ± 2.72 4 (1 - 15)	4.76 ± 2.97 4 (1 - 14)	0.961

Table 2: ICSI cycle characteristics of the study groups

	Group A (P only) (n=118)	Group B (E+P) (n=118)	P- value
AFC	12.60 ± 4.13 12.5 (3 - 23)	11.64 ± 3.43 12 (5 - 22)	0.052
Number of Days of Stimulation	12.63 ± 2.75 13 (4 - 21)	12.02 ± 2.61 12 (6 - 20)	0.082
Number of GN Ampoules	43.41 ± 14.54 39.5 (12 - 90)	42.99 ± 13.83 40 (18 - 90)	0.822
Endometrial thickness	10.99 ± 1.24 11 (8 - 14)	10.31 ± 1.90 10.5 (3 - 15)	**0.001***
Serum E2 Level	2890.71 ± 1956.58 2300 (305 - 10263)	2843.89 ± 1834.91 2468 (355 - 9550)	0.850
Day of ET	3.16 ± 0.82 3 (2 - 5)	3.06 ± 0.72 3 (2 - 5)	0.376
Number of Embryos Transferred	3.09 ± 0.83 3 (1 - 4)	3.19 ± 0.82 3 (1 - 4)	0.407
Number of gestational sacs	1.47 ± 0.76 1 (1 - 4)	1.15 ± 0.46 1 (1 - 3)	0.058

Table 3: Analysis of the study outcome

	Group A (P only) (n=118)	Group B (E+P) (n=118)	P- value
Implantation Rate	47/365 (12.88%)	30/376 (7.98%)	**0.029***
Implantation Rate • <5000 pg/ml • >5000 pg/ml	 5/51 (9.80%) 42/314 (13.38%)	 6/48 (12.50%) 24/328 (7.32%)	**0.670** **0.012***
Chemical Pregnancy	35/118 (29.66%)	31/118 (26.27%)	0.562
Chemical Pregnancy • <5000 pg/ml • >5000 pg/ml	 31/102 (30.39%) 4/16 (25.00%)	 24/103 (23.30%) 7/15 (46.67%)	 0.252 0.208
Clinical Pregnancy	32/118 (27.12%)	26/118 (22.03%)	0.364
Clinical Pregnancy • <5000 pg/ml • >5000 pg/ml	 28/102 (27.45%) 4 /16 (25.00%)	 20/103 (19.42%) 6/15 (40.00%)	 0.174 0.306
• Pregnancy loss	2/35 (5.71%)	5/31 (16.13%)	0.240

Evaluation of serum progesterone on the day of human chorionic gonadotropin administration as a predictor of pregnancy rate in ICSI

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Abstract

Introduction: Increase in serum P4 levels alongside high estradiol (E2) levels may lead to endometrial gland stroma asynchrony, which can lead consequently to implantation failure. Aim: Is to investigate the role of serum progesterone level on the day of hCG administration as a predictor of pregnancy in ICSI.

Methods: a prospective observational clinical study included fifty infertile women undergoing ICSI. The main outcome of the study was to evaluate the role of serum progesterone on the day of hCG administration as a predictor of pregnancy rate in ICSI.

Results: serum progesterone level at the day of hCG administration is not a useful predictor of pregnancy in cases undergoing ICSI. Conclusion: Serum progesterone at the day of hCG is not a useful predictor of pregnancy outcome. Further studies with larger numbers are needed to confirm or refute this finding.

Key words: Serum progesterone; prediction; pregnancy.

Introduction

In gonadotropin-releasing hormone (GnRH) analogues protocol, small increases in serum progesterone (P4) levels beyond a certain threshold value is seen at late follicular phase in IVF cycles. This varies between 5 and 35% with GnRH agonist protocol and between 9 and 38% in GnRH antagonists protocols [1-4].

Increase in serum P4 levels alongside high estradiol (E2) levels may lead to endometrial gland stroma asynchrony, which can lead consequently to implantation failure as a result of luteal phase defect, without influencing oocyte or embryo development [5-8].

The aim of the current study was to investigate the role of serum progesterone level on the day of hCG administration as a predictor of pregnancy rate in ICSI.

Patients & Methods

This is a prospective observational clinical study performed in the assisted reproduction unit of Air-Force Specialized hospital from jaunuary 2019 to December 2019 and was approved by the ethical committee of the hospital. The study included fifty infertile women undergoing ICSI with the following inclusion criteria age between 20 and 40 years, BMI < 35 kg/m2, basal FSH < 10 IU/ml and patients stimulated by the long protocol. While those who had previous history of low ovarian response to stimulation, previous failed ICSI, hydrosalpinx or any uterine pathology were excluded from the study. The main outcome of the study was to evaluate the role of serum progesterone on the day of hCG administration as a predictor of pregnancy rate in ICSI.

All cases were subjected to detailed history, general, abdominal and local examination, in addition to, investigations in the form of FSH, LH, PRL, E2 and thyroid functions in day 2 of the menstrual cycle before the induction protocol. Semen analysis and transvaginal ultrasound (TVS) were also done.

Induction protocol

Patients underwent controlled ovarian stimulation using the long GnRH agonist protocol for pituitary down-regulation. Ovarian stimulation was done by human menopausal gonadotropin (HMG)(Merional IBSA,Switzerland). The initial dose of HMG was individualized for each patient according to age, FSH level, antral follicle count (AFC) and BMI. Dose adjustments was performed according to ovarian response, which was monitored according to TVS and E2 levels. Serum progesterone was performed on the day of hCG administration (Chorioumon ,IBSA,Switzerland)which was given if 3 or more follicles reached 18 mm.

Ovum pick-up

TVS guided oocyte retrieval was performed 34-36 hours after hCG injection using 17-gauge needle under general anesthesia. The oocyte-corona complexes were denuded and ICSI performed 2 hours after incubation.

Embryo transfer

Two or three good quality embryo were transferred in day 3. B-hCG was performed 14 days after embryo transfer to define chemical pregnancy and TVS was performed at 5 weeks gestational age to define clinical pregnancy.

Progesterone measurement

Venous blood samples were collected in the day of hCG administration to measure serum progesterone. Samples were tested with a microparticle enzyme immunoassay Axsym System (Cobas e 411, Roche diagnostics. HITACHI), which has a sensitivity of 0.03 ng/ml.

Statistical analysis

Data were analysed using statistical program for social science (SPSS) version 18.0. Quantitative data were expressed as mean & standard deviation (SD). Qualitative data were expressed as frequency and percentage.

Independent-samples t-test of significance was used when comparing between two means. Spearman's rank correlation coefficient (rs) was used to assess the degree of association between two sets of variables if one or both of them were skewed, otherwise pearson correlation was used. P-value < 0.05 was considered significant, < 0.01 was considered highly significant and > 0.05 was considered insignificant.

Results

Nineteen cases out of fifty cases got pregnant. Comparison was done between non-pregnant and pregnant cases.

Table 1 shows comparison between pregnant & non-pregnant cases regards the basal characteristics

	Group A (n=31) mean & SD	Group B (n=19) mean & SD	P value
Age	28.37 +/- 4.38	29.03 +/- 3.09	0.569
BMI	27.68 +/- 5.83	28.52 +/- 2.79	0.560
FSH	6.35 +/- 1.86	5.91 +/- 1.74	0.409
AFC	10.60 +/- 4.46	11.11 +/- 4.36	0.694

Table 2 shows comparison between pregnant & non-pregnant cases regards No. of HMG ampoules, induction duration, E2 & P4 (day hCG), No. of retrieved oocytes. No. of MII, fertilized oocytes and No. of embryos transferred.

	Group A (n=31) mean & SD	Group B (n=19) mean & SD	P value
No. of HMG ampoules	3.42 +/- 1.05	3.42 +/- 0.86	0.961
Induction duration	11.85 +/- 1.53	11.39 +/- 1.35	0.2866
Oocyte	10.55 +/- 5.33	11.53 +/- 5.97	0.549
E2 (day hCG)	3898.77 +/- 1.948.1	4909.21 +/- 3567.7	0.426
P4 (day hCG)	1.67 +/- 1.54	1.39 +/- 0.92	0.477
No. of retrieved oocytes	12.58 +/- 8.65	14.55 +/- 7.62	0.418
No. of MII	8.68 +/- 7.00	9.89 +/- 4.71	0.509
Fertilized oocytes	6.67 +/- 5.15	7.29 +/- 3.24	0.641
No. of ETs	2.54+/- 0.50	2.63 +/- 0.49	0.572

Table 3 shows correlation between serum progesterone (day hCG) and E2 (day hCG), No. of retrieved oocytes, No of MII, fertilized ova, No. of good quality embryos, No. of E.Ts and expected oocytes in cases who got pregnant (n=19)

	P4 (day hCG)	
E2 (day hCG)	r	0.475
	P value	0.003
No. of retrieved oocytes	r	0.065
	P value	0.697
No. of MII	r	0.154
	P value	0.356
Fertilized ova	r	0.155
	P value	0.354
No. of good quality embryos	r	-0.033
	P value	0.855
No. of ETs	r	0.278
	P value	0.091
Expected oocytes	r	-0.204
	P value	0.219

Table 4 shows correlation between serum progesterone (day hCG) and E2 (day hCG), No. of retrieved oocytes, No of MII, fertilized ova, No. of good quality embryos, No. of E.Ts and expected oocytes in cases who didn't get pregnant (n=31)

	P4 (day hCG)	
E2 (day hCG)	r	0.280
	P value	0.031
No. of retrieved oocytes	r	0.163
	P value	0.212
No. of MII	r	0.217
	P value	0.096
Fertilized ova	r	0.144
	P value	0.271
No. of good quality embryos	r	-0.030
	P value	0.834
No. of ETs	r	0.012
	P value	0.930
Expected oocytes	r	-0.028
	P value	0.831

Discussion

The results of the effect of serum P4 level on day of hCG trigger as a predictor of pregnancy in IVF/ICSI cycles using either the GnRH agonist or antagonist protocol are variable. The Possible reasons for such discrepancies include the retrospective nature of most studies, differences in ovarian stimulation protocols, differences in cut-off values used for P4 aduring data analysis or inaccurate definitions in the references for elevated P4 levels in some cases. Other factors include variations in the statistical methods used for estimation of circulating P4 limit values and the precision of P4 measurements due to use of different immunoassays. Most investigators agreed that supraphysiological rise in P4 in late follicular phase has deleterious effect on pregnancy rates [9-16].

The results of the current study showed that serum progesterone level at the day of hCG administration is not a useful predictor of pregnancy in cases undergoing ICSI and a significant positive correlation between estradiol and serum progesterone levels measured at the day of hCG stimulation in both pregnant and non-pregnant cases, while there was no significant correlation between serum progesterone measured at the day of hCG administration and number of retrieved oocytes, number of MII oocytes, fertilized ova, number of good quality embryos, number of embryos transferred or expected oocytes.

The results of the current study partially agrees with the results of Sangisapu et al [17] who studied 306 cases of fresh IVF cycles and concluded that serum progesterone at the day of hCG trigger alone is not associated with IVF outcome; however, it differed from the results of the current study in that it found a significant association with the number of oocytes retrieved; however, Merviel et al [18] found that serum progesterone level on the night before and the day of hCG trigger predicted the likelihood of pregnancy.

Li et al [19], found that higher levels of serum estradiol and progesterone on the day of hCG trigger may affect endometrial receptivity and suggested the transfer of frozen embryos in a natural cycle to avoid this determintal effect of such high hormonal levels.

The current study has the advantage of being prospective, while the limitations of the current study include recruitment from a single center, small number of cases and lack of study of the effects of other hormones.

Conclusion

Serum progesterone at the day of hCG is not a useful predictor of pregnancy outcome.

Further studies with larger numbers are needed to confirm or refute this finding.

Conflict of interest & financial support:

None of authors has any conflict of interest to declare.

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Studying the Reproductive Performance among Women with Congenital Uterine Anomalies. An observational descriptive study at Mansoura University Hospitals

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Running title: The reproductive outcome of uterine anomalies

Abstract

Background and aim: To study the pattern of congenital uterine anomalies and their impact on reproductive outcomes.

Methods: A Prospective clinical study included 100 women of childbearing age with reproductive failure either infertility, repeated miscarriage, or preterm birth, and diagnosed to have congenital uterine anomalies either inside or outside Mansoura university hospitals. Hysterosalpingogram (HSG), hysteroscope, and or laparoscope were performed to confirm the undiagnosed cases. The main outcome measure was reproductive complications among studied patients.

Results: The mean age (+SD) of the studied cases was 27.07 (±4.04) years, with the frequency of complaints as failure to conceive being recorded as the commonest (45%), 27 cases as primary while 18 as secondary infertility with a duration shorter in primary than secondary infertility (3.01+ 1.1 vs 4.02+ 1.2 respectively) followed by recurrent pregnancy loss (39%), preterm labor (13%), and lastly acute abdominal pain (3%). On the other hand, anomalies demonstrated according to their frequencies were arcuate uterus (35%), bicornuate unicolis uterus (17%), incomplete uterine septum (15%), complete uterine septum (9%), didelphys uterus (7%), uni-cornuate uterus with a rudimentary horn (6%) and without a horn (6%), complete uterine agenesis (4%) and lastly T-shaped uterus (1%). Ninety-one patients got pregnant after intervention but 21 ended by abortion, and 27 had preterm labor meanwhile the rest (43) passed fetal maturity despite some complications already occurred in about 65% of them including low birth weight (17) premature placental separation (8), and rupture uterus (3). All cases planned for delivery were delivered by Lower segment cesarean section.

Conclusion: There is a strong association between congenital uterine anomalies and adverse reproductive outcomes. The arcuate uterus was the commonest congenital uterine anomaly found in the study and this occurred mostly among women presenting with secondary infertility or recurrent pregnancy loss.

Keywords: Uterine anomalies; hysteroscopy; reproductive outcome.

Synopsis: There is a large variety of uterine anomalies among females with poor reproductive performance. Despite many patients may be detected during fertility workups but a remarkable number could present with poor pregnancy outcomes.

Introduction

Globally, congenital uterine anomalies (CUAs) are present in 1-10% of women and constitute 2-8% of infertile women and 5-30% of women with a history of miscarriage. This discrepancy in the prevalence rates is mostly related to the use of different diagnostic tests and the use of non-standardized classification systems to diagnose these anomalies [1]. Common categories of anomalies are agenesis, hypoplasia, uni-cornuate uterus, uterus didelphys, bicornuate uterus, septate uterus, arcuate uterus, and diethylstilbestrol-exposure related anomalies namely T-shaped uterus [2, 3].

Clinically CUAs may present by a variety of symptoms such as pelvic pain, prolonged or otherwise abnormal bleeding starting from the time of menarche, inability to conceive, recurrent pregnancy loss, or preterm birth. Consequently, these anomalies could be suspected in girls and women who present with one or more of these disorders [4]. Also, it could be suspected with ectopic pregnancies in primigravida, malpresentation, and intra-uterine growth restriction of unexplained etiology [4,5]. Imaging modalities are largely relied up on diagnosing and classifying uterine anomalies. Of these, the commonly used utilities are two-dimensional ultrasonography (2DUS), three-dimensional ultrasonography (3DUS), hysterosalpingography, saline infusion ultrasonography, and magnetic resonance imaging (MRI). A 3DUS proved to be highly accurate in diagnosing uterine anomalies and may be equivalent to MRI [2]. Even after management, patients with CUAs appeared to have increased morbidity and mortality both for the mother and fetus owing to the presence of pregnancy or labor-related problems [5]. This study was conducted to study the prevalence and patterns of CUAS and their association with reproductive outcomes.

Patients and methods

This observational prospective study had been carried out on 100 women of childbearing age with reproductive failure after confirming the presence of uterine anomalies. The patients were selected from those attending outpatient clinics at the fertility care unit and Obstetrics and Gynecology Department at Mansoura University Hospitals, Egypt from April 2020 to April 2022. The study protocol was approved by the Medical Research Committee of the Faculty of Medicine, Mansoura University (IRB Code No. MS.20.03.1059). Confidentiality and personal privacy were respected at all levels of the study. All women had written consent to participate after a verbal discussion about the role of the study and any patient who refused to share was withdrawn immediately. The collected data was not used for any other purpose.

Inclusion criteria were women of childbearing age with an evident history of reproductive failure including infertility, recurrent miscarriages, or recurrent preterm birth, and diagnosed to have CUAs confirmed by hysterosalpingography (HSG), hysteroscope, laparoscope, or MRI either before inclusion or confirmed by one or more of these investigations as a part of reproductive

failure work up in the place of the study. Every patient was subjected to a full history taking, demographic details, complaint, duration and type of infertility, and previous obstetric history. Systematic physical examination including general, abdominal, and local pelvic examination was done for all followed by one or more of the mentioned radiological investigations done by an expert radiologist for the detection of anomalies in those who were not diagnosed before. Combined laparoscopy and hysteroscopy were done by the same senior gynecologist for all selected cases under general anesthesia as a routine management step for assuring the diagnosis and surgical intervention when needed. All patients were followed up for 6 months to evaluate the improvement in their reproductive state. Those who proved to be pregnant were then followed up till the end of the journey of their pregnancies and the pregnancy data and outcome were verified. All patients' demographic data and data gathered during the period of management and follow-up for pregnancy were collected and then subjected to statistical analysis

Statistical analysis and data interpretation

Data was fed to the computer and analyzed using IBM SPSS version 22.0. (Armonk, NY: IBM Corp). Quantitative data were described by using numbers (%) and mean (SD) and then were analyzed by unpaired student t-test whilst nonparametric qualitative data were presented as numbers and percentages and then compared by chi-square (X^2) or Fisher's Exact test when appropriate. A two-tailed P value <0.05 was set as statistically significant.

Results

A total of 100 patients fulfilled the inclusion criteria of this prospective study. The Demographic parameters were given in table (1). It showed the mean age (SD) of the cases involved was 27.07±4.04 while the anomalies demonstrated according to their frequencies were arcuate uterus (35%), bicornuate unicolis uterus (17%), incomplete uterine septum (15%), complete uterine septum (9%), didelphys uterus (7%), uni-cornuate uterus with a rudimentary horn (6%) and without a horn (6%), complete uterine agenesis (4%) and lastly T-shaped uterus (1%). On the other hand, the patients' complaints were arranged as failure to conceive (45%), recurrent pregnancy loss (39%), preterm labor (13%), and acute abdominal pain (3%). In those who were infertile, 27 cases presented as primary and 18 cases as secondary infertility with the mean duration (SD) being shorter in primary than secondary type (3.01+ 1.1 vs 4.02+ 1.2 respectively). Looking at the pattern of menses, menarche didn't occur in 4 cases (uterine agenesis) while the majority (87) had regular cycles, intermenstrual bleeding (irregular cycle) was the role in 7 cases and lastly, heavy frequent cycles [polymenorrhagia] was recorded in 2 cases only, table (1). Data from local examination and radiological as well as surgical investigations, namely hysteroscopy and laparoscopy, are represented also in the table (1). In 92 cases, local examination reported normal findings, however, double cervix was confirmed in 8 cases "all cases of uterus didelphys and 1 case of those who had complete uterine septum where the septum was extending to the external os". Investigations done for cases involved in the study whether inside our hospitals or brought by the patients from the start were gathered and revealed that 2D-US diagnosed the type of anomaly only in 24 cases, while 60 cases were confirmed by 3D-US, and MRI used to confirm the cases of uterine agenesis (4). Despite HSG was done in 96 cases being a basic investigation in such cases but cannot rule out all types of anomalies except after confirmed by hysteroscopy [65 cases] or combined hysteroscope and laparoscope [85 cases], table (1).

The relation between the patient's complaints and the type of anomaly diagnosed was represented in table (2). The data showed that the arcuate uterus, bicornuate and unicornuate uterus, and uterine agenesis are the anomalies commonly associated with abortion, infertility, and menstrual abnormalities including amenorrhea that was set as the role in those with agenesis [p- values, 0.045, 0.007, 0.001, 0.001 respectively], table (2).

The reproductive outcome after intervention and pregnancy follow-up data were shown in table (3). Preterm birth was demonstrated in 27 cases but more in those having incomplete uterine septum (p-value 0.002) compared to other types. First and second-trimester abortion was the role in 21 cases and 6 cases occurred in those suffering from the arcuate uterus (p-value, 0.001). On the other hand, low birth weight was demonstrated in 17 cases, 9 of which from those with an incomplete uterine septum (p-value, 0.001). Also, these data reported a higher incidence of fetal malpresentation (15) and rupture uterus (3) with the arcuate uterus and unicornuate uterus with rudimentary horn (p-value, 0.001 in both). Premature placental separation was recorded in 8 cases, 5 of these are from those with an incomplete uterine septum, and 3 from cases of unicornuate uterus with a rudimentary horn (p-value, 0.001 in both). Rupture uterus occurred in 3 cases all of which had unicornuate uterus with rudimentary horn, table (3).

Discussion

This study established that CUAs are present and could not be discovered except late after marriage while the patient is seeking fertility or management of pregnancy complications.

While some CUAs are proven to be asymptomatic and pass with normal reproductive outcomes but others proved to be associated with adverse effects [6]. The results of the current study proved that the mean age of the patients involved was 27.07±4.04 years and despite this appears slightly higher in developing countries like ours but comes in accordance with data proved by Nisha et al 2020. [7] and Cahen-Peretz et al 2019. [8] and contrary to the findings published earlier by Vyas et al. [9] who reported that the majority of CUAs related to gynecological cases were observed at 19.03 years. This discrepancy could be explained, according to the author's opinion, by the fact that some of the patients involved in our study might deny their pre-marriage complaints or knowledge about the presence of an anomaly and that they discovered this during the course of the management plane for infertility or obstetric complications. It is unlogic and unbelievable for example for those who had uterine agenesis (4) to be discovered only after marriage not complaining before from amenorrhea.

Forty-five cases of the studied patients in this research complained of infertility but 72.2% of them had secondary infertility compared to 27.8% who had primary infertility. This finding comes in disagreement with the results revealed by some authors who proved primary infertility higher than secondary infertility in patients with CUAs [7, 10, 11]. Again, the authors can give an explanation of this by the fact that all patients who had any type of uterine anomaly during the period of the study were involved, and a large proportion of them was found to have an arcuate uterus (35) which manifests commonly by obstetric complications rather than fertility problems.

As mentioned, the results of this study convinced that the arcuate uterus was the most frequent anomaly (35%) followed by bicornuate unicolis (17%), and the least was a T-shaped uterus (1%). These findings are similar to that proved by some national and international researchers [10, 18, 22] but contrary to some other authors who stated in their work that a septate uterus came out to be the most common anomaly [7, 19, 20] and to those who assured the unicornuate uterus to be the

highest followed by septate uterus [21]. For explaining this, according to our insight, some of the previous authors [19-21] investigated uterine anomalies in infertile women only, but we included infertile women and those coming with obstetric complications.

The most frequent complaint in patients explored by this study was a failure to conceive (45%) followed by recurrent pregnancy loss (39%), preterm labor (13%), and lastly acute abdominal pain (3%). However, this comes in contrast to the study published by Raj and Chavan 2019 [13] who showed dysmenorrhea as the commonest gynecological complaint followed by abnormal uterine bleeding, and then chronic pelvic pain in patients with CUAs. In the patients of this study, the mean (SD) duration of primary infertility appeared shorter in those with primary than secondary type (3.01+ 1.1 vs 4.02+ 1.2 respectively) and this agreed with the report of Ajayi et al 2015. [12].

The findings described from this study showed a statistically significant relationship between the patients' complaints and the type of anomaly present as those with uterus didylphus complained mainly of recurrent pregnancy loss, those with unicornuate uterus with rudimentary horn complained of acute abdominal pain and recurrent pregnancy loss while 80% of cases in whom incomplete septum was proved, failed conception was the main presentation. Also, in all cases of the complete uterine septum, the main presentation was a failure to conceive meanwhile those with arcuate uterus had a higher rate of recurrent pregnancy loss and preterm labor, and rarely presented with infertility or acute lower abdominal pain. These findings come in association with the data described before [13, 23] that studied the patterns of recurrent pregnancy losses and their correlations with CUAs. Interestingly and surprising to us, Chan et al 2011.[24] reported no difference in pregnancy rates when compared to women with CUAs and those with normal uteri.

After the intervention, obstetric-related problems were documented in nearly all cases (91) of the studied group after excluding 4 cases of uterine agenesis, no hope for fertility, and 5 cases who did not cope for follow-up. Of these pregnant and followed-up women, 27 cases had preterm labor, 21 cases had abortions, 17 cases had low birth weight, 15 cases had an abnormal fetal presentation, 8 cases had a placental abruption, and lastly 3 cases were urgently undergone laparotomy on rupture uterus. Similar findings were established by many other investigators [5, 14-17, 22, 24]. Here, the authors can state that these variable obstetric outcomes could be resorted to the associated abnormal uterine cavity, disturbed uterine vasculature, or endometrial receptivity that is commonly found in an animalized uterus.

In some previously published data, CUAs were reported to increase the risk for cesarean delivery by more than 13-fold [8, 17]. They explained that higher rates of cesarean section were mainly due to malpresentation and previous cesarean delivery, while other indications observed were non-progressive labor or non-reassuring fetal heart rate tracing were less common in the exposed group [8, 17]. A fact also proved in this work, as all delivered cases were by lower segment cesarean section except 3 cases that were subjected to urgent laparotomy for rupture uterus, and all had unicornuate uteri with rudimentary horns.

Certainly, this study had some shortcomings, namely the wide variables of CUAs involved as this brought an unequal number in each group and made specifying a certain obstetric complication to a specific anomaly difficult. Also, the lack of presenting data for the maternal and neonatal outcomes. Lastly, this study is a unicentric one that investigated some populations in one locality of the country, so the authors recombed for further multicenter study for obtaining more convenient results.

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Conclusion

there is strong evidence of poor reproductive outcomes and many obstetric complications in women with CUAs and canalization defects. Therefore, it is so beneficial for obstetricians and gynecologists to be notified of the potential problems that are actually increased depending on the type and severity of anomaly discovered.

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Reversible non-immune hydrops in twin pregnancy due to CMV infection which was treated by IV Gancyclovir "Case report"

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Key words: Cytomegalovirus CMV – Non-Immune Hydrops Fetalis NIHF – Gancyclovir – Case report

Abstract

A 28 years old primigravida 19 weeks pregnant carrying Diamniotic dichorionic twin pregnancy was suffering from jaundice due to CMV infection and fetal ascites which was successfully managed by IV Ganciclovir. Fetal infection by CMV leading to NIHF could be reversed completely by early detection and proper management using antiviral treatment. Despite the rarity of the cases and the difficulty of confirming the diagnosis, CMV should be considered in the differential diagnosis of NIHF, putting in consideration that early start of treatment will lead to better prognosis.

Introduction

Non-immune fetal hydrops is diagnosed when there is fluid accumulation in more than two extravascular spaces, in addition to soft tissue edema such as the skin or scalp, or fluid in body cavities such as ascites pleural effusion, pericardial effusion, or hydrocele (1, 2).

Many causes may result in such condition as (3): Intrauterine infections represent 7% of cases of non-immune hydrops, with a prevalence of congenital cytomegalovirus (CMV) infection of 0.64% (4).

IT is important to remember that NIHF represent almost 85% of all cases of hydrops fetalis. (3) The current case study is to present a case of non-immune hydrops due to CMV which was reversed by antiviral treatment.

Case Report

A 28 years old primigravida 19 weeks pregnant carrying Diamniotic dichorionic twin pregnancy was admitted to the hospital suffering from jaundice. She did investigations in the form of liver enzymes which were elevated, (magnetic resonance cholangio-pancreatography) which was normal. The virology studies revealed high levels of

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IgG & IgM for cytomegalovirus (CMV), but negative PCR results.

Obstetric US didn't reveal fetal anomalies, so she was discharged and instructed to follow-up in the outpatient clinic.

During follow-up four days later there was mild ascites in the first twin and severe ascites in the second twin as shown by ultrasound (figure 1). She started to have intravenous Ganciclovir twice daily for two weeks. Two weeks later there was marked improvement in the condition of both fetuses so oral acyclovir was continued for further 3 weeks with close follow-up by obstetric ultrasound, which showed disappearance of ascites in both fetuses (figure 2). Pregnancy carried on uneventful and delivery of two normal twins by cesarean section at 37 Weeks. Seven months follow up of both babies showed no CNS affection or sensory or hearing affection.

Discussion

Congenital CMV is the most frequent congenital infection complicating almost up to 2% of all pregnancies throughout the world. It is one of the leading causes of hearing loss and neurological deficits during infancy and childhood (6). Congenital CMV infection may also result intrauterine fetal death, cytomegalic inclusion disease, and pneumonia (6). Putting in mind that the congenital infection will mostly pass unnoticed as it is asymptomatic in 90% of the cases. (4) Also it is important to remember that the trans-fetal infection represent from 0.1% to 1% in cases of recurrent maternal infection with CMV. (7)

Sonographical signs of intrauterine CMV infection were reported as cerebral calcifications, cerebellar hemorrhage, hyperechogenic bowel, fetal hydrops, pericardial effusion, cardiomegaly, placentomegaly, and oligohydramnios (8).

To the best of our knowledge not many cases with congenital CMV infection had prolonged survival whether due to cerebral affection or due to multiple congenital anomalies. (7) In the current case not only did both fetuses survive, but also features of hydrops present in the ultrasound were reverted by Ganciclovir treatment suggesting that treatment may reverse the problem especially in early cases (the difference between the ultrasound done in the hospital and the follow-up ultrasound was about 4 days).

The diagnosis of CMV infection as a cause of fetal hydrops is challenging since the laboratory tests are not highly sensitive, also the rarity of the cases and the evidence of neonatal infection, putting in consideration the importance of early administration of treatment, so high index of suspicion is needed (7). Neither the less the infection is mostly recurrent infection as the CMV IgG titer was high which may explain also the mild form of fetal symptoms and the good response to the antiviral treatment. That is why the maternal immunity response during primary or recurrent viral infestation should be considered as one of the important items to explain the severity of the fetal infection.

F. D'Antonio et al after revising eight studies (618 women) concluded that prenatal valacyclovir administration in pregnancies with maternal CMV infection can reduce the risk of congenital CMV infection (9). Valacyclovir is an acceptable, tolerable and effective line of treatment in management of intrauterine vertical transmission of CMV (10).

Conclusion

CMV infection should be considered as one of the causes of fetal hydrops with a high index of suspicion, although the laboratory tests are not highly sensitive, YET early initiation of IV antiviral treatment would have a good response with good prognosis for the outcoming babies.

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