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Acknowledgments

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

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2- Books:

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

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Contents :

Letter from the Editor:

Dear colleagues,

Happy New Year 2021. Very interesting subjects are included in this issue. Adding azithromycin to the standard cephalosporin administered to women in selective CS reduces maternal infections and maternal episodes of fever. Preconceptional vitamin D supplementation is an easy method for decreasing incidence of early pregnancy loss. Paracervical block is a safe and effective analgesia option for Transvaginal Ultrasound Guided Oocyte Retrieval.

Best regards.

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Addition of azithromycin to the routine pre-cesarean prophylaxisagainst infection, effective or not?

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Abstract

Purpose: There is debatable data regarding the addition of azithromycin to the routine antibiotic prophylaxis of post-cesarean section (CS) infection. In this work, we tried to evaluate its potential benefit to protect against both post-cesarean maternal and fetalinfections.

Methods: The study included 230 women who were intended to have selective CS at Mansoura university hospital. They were randomly subdivided into115 women that received azithromycin plus standard cephalosporin and another115 women that received standard cephalosporin alone. The main outcome is evaluation of post-cesarean section infection as endometritis, wound sepsis, etc… across the puerperal period.

Results: In comparison between the test group and the control group, there was significant reduction of re-admission during puerperium(0% vs 6.5%, p=0.01). Endometritis manifestations were significantly reduced, including: puerperal Fever >38OC (4.3% versus 15.2%), uterine tenderness (3.2% versus 11.9%), abdominal pain (1.1% versus 14.1%), offensive vaginal discharge (2.1% versus 13%) and purulent drainage from uterus (2.1% versus 9.8%). Wound infection manifestations were significantly reduced, including; erythema around incision (2.1%versus 8.7), induration around incision (3.2 versus 12%) and purulent discharge from incision site (1.1% versus 9.8%). Also, the need for further antibiotic during puerperium was significantly reduced (3.2% versus 11.9%). There was no significant difference between the 2 groups regarding neonatal outcomes.

Conclusion: Azithromycin plus the standard cephalosporin administered to women in selective CS reduces maternal infections and maternal episodes of fever, with no clear benefit on the neonatal outcome.

Keywords: Azithromycin, Post-cesarean section infection

INTRODUCTION

Cesarean delivery is considered as one of the major procedures for saving both maternal and fetal lives. The incidence of cesarean deliveries, both repeat and primary, has risen lately in Egypt to be doubled between 2005 and 2014, and reach according to Egypt demographic and health survey to 52% of all deliveries. Egypt now is in the third rank worldwide between countries with the highest rates of caesarean section after Dominican Republic and Brazil(1).

Post-partum infection globally represent one of the leading direct causes ofmaternal morbidity and mortality which in turn elongate

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the length of hospital stay and add burden to the healthcare costs.Caesarean delivery is counted as one of the major risk factors for postpartum infection (2). Compared to planned vaginal delivery, women who underwent elective caesarean section had double to triple risk to be re-hospitalised for wound complications and/or infections (3).

Surgical site infection (SSI) and endometritis are the main two sub-groups of the post-caesarean section infections. SSI refers to skin and subcutaneous infection at the site of the incision. Endometritisrefers to infection of the endometriallining of the uterus with/without its wall (4).

The American College of Obstetricians andGynecologists (ACOG) recommendations 2011 endorsed theuseof an antimicrobial prophylaxis within 60 minutes from the onset of the caesarean incision and to be as soon as possible in cases of unscheduled caesarean deliveries.The appropriateantimicrobial coverage can be effectively provided bythe first generationcephalosporin or aminoglycosides combined with clindamycin in cases of hypersensitivity tocephalosporins(5). According to Cochrane review 2014,penicillinsare equivalent tocephalosporinsas regard to their prophylactic activity (6).

A single dose of one gram ofcefazolin provides the adequate and the broad spectrum antimicrobial coverage for normal and overweight women.However, the doseshould be adjustedfor more obese women(5).

Adding azithromycin to the routine pre-cesarean cephalosporin prophylaxis against infection may add great benefits to ladies delivering by CS. Having a longer half-life (68 hours) with its higher tissue concentrations and lesstransplacental passage compared with other antibiotics plus its antimicrobial activity against both aerobes and anaerobes, as well as Ureaplasmas, all making azithromycin a valuable addition to the prophylaxis against the post caesarean section infections. It significantly decreases the risk ofendometritisand SSI when added to the routine pre-cesarean cephalosporin prophylaxis (7&8).

Material and Method

Study design

This study was carried out as a prospective randomized non-blinded controlled trial.

Study subject

The estimated sample size is 230 patientswho were intended to have selective CS (this number including 10% increase to the estimated minimal sample size).The estimated sample size was calculated with a level of confidence of 95%. Those patients were randomly subdivided into two groups: testgroup includes 115 women who received Azithromycin plus standard cephalosporin, and control group includes 115 women who received standard cephalosporin alone.

Setting

Obstetrics and gynecology department, Mansoura university hospitals (tertiary hospital), Mansoura University, Egypt.(Figure 1).

Inclusion criteria

Women > 28weeks viable gestation, women undergo unscheduled / selective cesareans with either labor (spontaneous or induced) or ruptured membranes.

Exclusion criteria

Vaginal delivery, elective or scheduled cesarean prior to labor or membrane rupture, known Azithromycin (or other macrolide) allergy, clinical chorioamnionitis or any other active bacterial infection (e.g. pyelonephritis, pneumonia, abscess), fetal demise or major congenital anomaly, significant liver disease, significant renal disease or on dialysis, active congestive heart failure or pulmonary edema, active diarrhea at time of delivery, and immunosuppressed patients.

Drug administration

All patients were received the standard routine cephalosporin (1gm ceftriaxone) before the surgical incision.Azithromycin (500 mg) was given concurrently with the standard cephalosporin bolus in the test group.

Outcome measures

Endometritis and\or wound infection and\or post-cesarean infections (occurring within 4-6 weeks of delivery) defined as follows:Endometritis is the presence of two or more of the following signs with no other recognized cause: fever > 38 C, abdominal pain, uterine tenderness, or purulent drainage from uterus.Wound infection is the presence of either superficial or deep incisional surgical site infection characterized by cellulitis/erythema and induration around the incision or purulent discharge from the incision site with or without fever.Other infections include pelvic septic thrombosis, abdominal or pelvic abscess, pyelonephritis, pneumonia or maternal sepsis.

Results

In this work we tested the effect of addition of azithromycin to the standard pre-cesarean section cephalosporin prophylaxis (the test group), in comparison to the standard pre-cesarean section cephalosporin prophylaxis alone (the control group). There was no significant difference regarding the socio-demographic, obstetric data and conditions among studied groups(Table 1 and 2).

Endometritis, wound infection needed antibiotics and re-admission during puerperium were significantly reduced in the test group in comparison to the control group (P= 0.006, 0.02 and 0.01 respectively) (Table 3).

On comparison between the 2 studied groups we found that puerperal Fever >38Oc, uterine tenderness, abdominal pain, offensive vaginal discharge and purulent drainage from uterus were significantly increased in the control group. Also, we found that the need for further antibiotic during puerperium was significantly increased the same group. (Figure 2, and Table 4).

On comparison between the 2 studied groups(Figure 3, and Table 5) we found that erythema around incision, induration around the incision, purulent discharge from the incision site, need for further antibiotic during puerperium and white blood cells count(WBCs) at 7th day postpartum were significantly increased in the control group receiving cephalosporin alone .

There was no significant difference regarding neonatal outcomes between both the test and the control groups.(Table 6).

Discussion

Cesarean section (CS) has dramatically increased over the last decade in Egypt to reach up to institution based rate of 67.3% (9). Although it is considered as an important lifesaving operation for both mother and child in many situations, however, this does not omit the fact that it is one of the most important risk factor for postpartum maternal infection which can increase maternal morbidity and mortality (10). Current recommendations for antibiotic prophylaxis in cesarean delivery include the standard administration of a broad-spectrum antibiotic before the skin incision (11). Unscheduled CS represent about 60% to 70% of all caesarean deliveries and up to 12% of women undergoing selective cesarean sectiondelivery had puerperal infection when the standard pre-incision prophylaxis was used(12). These two facts point the importance of availability of more effective antibiotic regimens for protection against post CS infections.

The current study aimed to evaluate the efficacy of addition of Azithromycin with its strong antibacterial and bacteriostatic effects against atypical pathogens (13)to the standard cephalosporin (ceftiaxone) which has strong antibacterial effect against E. coli and Enterobacteriaceaein reducing the risk of post-cesarean infection compared to cephalosporin alone among women undergoing unscheduled cesarean delivery.

186 ladies undergoing unscheduled CS were included in the current study. They were subdivided into two groups, study group: included 94 subjects that received Azithromycin (500 mg) plus standard cephalosporin (ceftiaxone 1 gm), and control group: included 92 subjects that received standard cephalosporin (ceftiaxone 1 gm) alone.

There was no significant difference regarding the socio-demographic, obstetric data and conditions among studied groups (Table 1 and 2).

In our study there was significant reduction of re-admission during puerperium among the test group in comparison to the control group(0% vs 6.5%, p=0.01). Also, all signs and symptoms of endometritis (including; puerperal Fever >38OC,

uterine tenderness, abdominal pain, offensive vaginal discharge and purulent drainage from uterus) were significantly reduced among the test group in comparison to the control group. Also, all signs and symptoms of wound infection (including; erythema around incision, induration around incision, purulent discharge from incision site, need for further antibiotic during puerperium and WBCs at 7th day postpartum) were significantly reduced among the test group in comparison to the control group.

In accordance with our findings, Tita et al. found the adjunctive azithromycin prophylaxis for caesarean delivery in 1019 cases (study group) showed a significant protective effect against maternal endometritis, wound infection (2.4% vs 6.6%, $p<0.001$) and serious maternal adverse events (1.5% vs 2.9%, $p=0.03$) in comparison of addition of placebo in 994 control cases.This effective prophylaxis may be explained by its ability to cover againstureaplasma species, which are reported to be frequently associated with post CS infections (14).

In the same context, Ward and Duff 2016 evaluated the effect of addition of azithromycin to a first generation cephalosporin drug (cefazoline) before skin incision on prevention of postpartum endometritis in comparison to cefazoline alone after cord clamping. Although the difference in the timing of administration and the cephalosporine generation used, but their findings were consistent with ours as regard the significant protection against postpartum endometritis in azithromycin group (8).

Harper et al., 2017 and Skeith et al., 2017 tested the cost effectiveness of addition of azithromycin to the routine cephalosporin given as a prophylaxis against post-partum infections in both scheduled and non-scheduledCS. The research group found addition of azithromycin is both effective in reduction of post-partum infectious complication plus being of less cost compared with the cost of treatment of such complications (15and16).

In contrast, Johnson et al., 2019 in a retrospective study found addition of azithromycin to cephalosporines add no value in prevention of post-partum infections. Being a retrospective study and included only 100 participant making this study need further research to stand in front the growing evidence of the efficacy of addition of azithromycin to the routine prophylaxis in cases of selective CS(17).

In the present study, there was no significant difference between the 2 groups regarding neonatal outcomes in accordance withTita and his colleagues (14) and Ward and Duff(8).In contrast, Oluwalana and his colleagues found azithromycin administered to women in labor reduces maternal and neonatal infections as otitis and conjunctivitis probably due to the effect on colonization of Staphylococcusaureus and Streptococcus species in the newborns, because these bacteria are major causes of both conditions. This contrast in the results can be explained by the fact that Oluwalana compared administration of azithromycin in test group with giving only placebo in the control group, both were delivering vaginally(18). We recommend performance of larger trials designed to assess the effect of prophylactic use of azithromycin on severe morbidity with studies that allow doing cultures for ureaplasma and other bacteria.

Ethical approval

The study was approved by the Mansoura Faculty of Medicine Institutional Research Board (MFM-IRB).

Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and material are confirmed.

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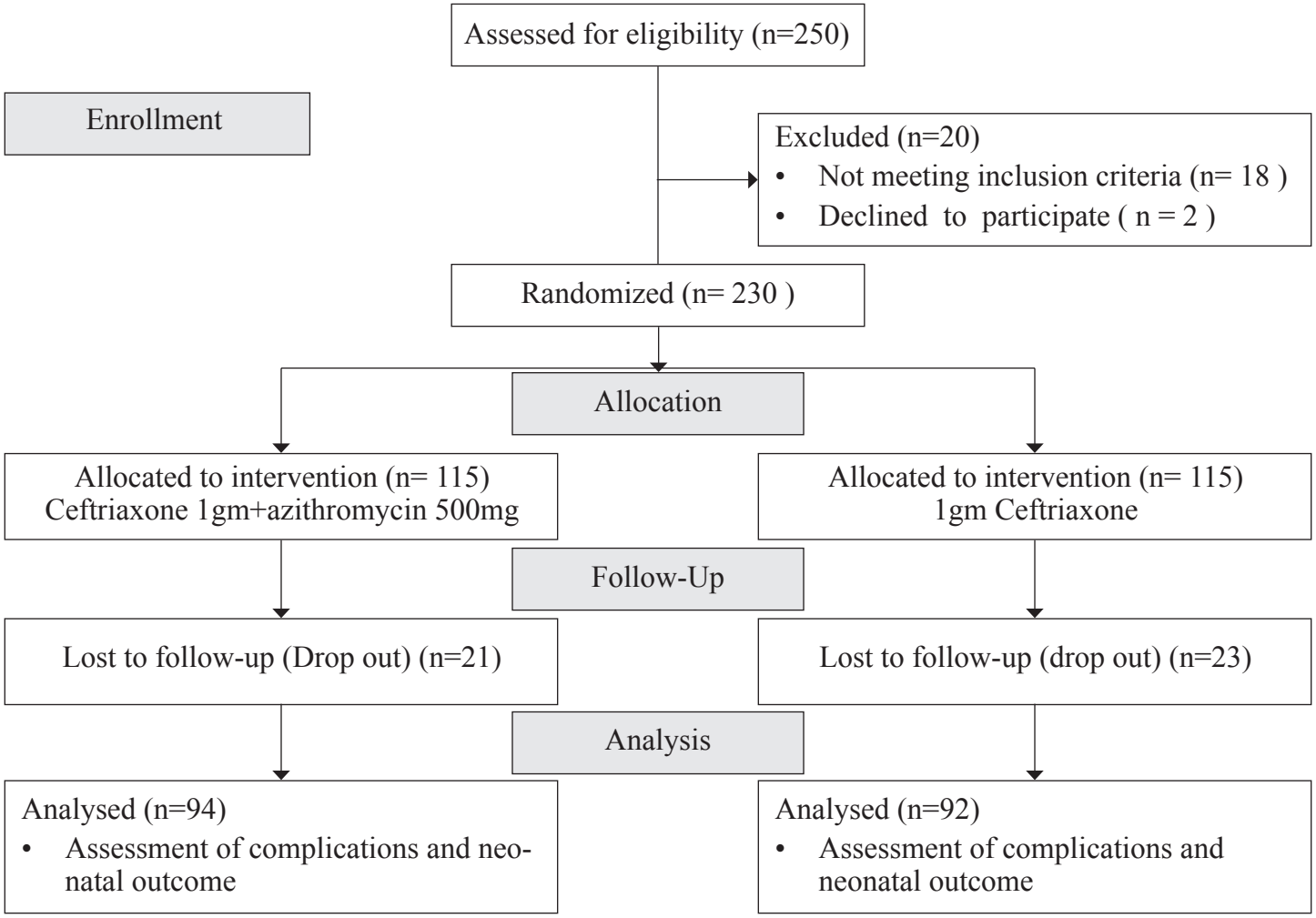
Figure 1: Consort flow of the study.



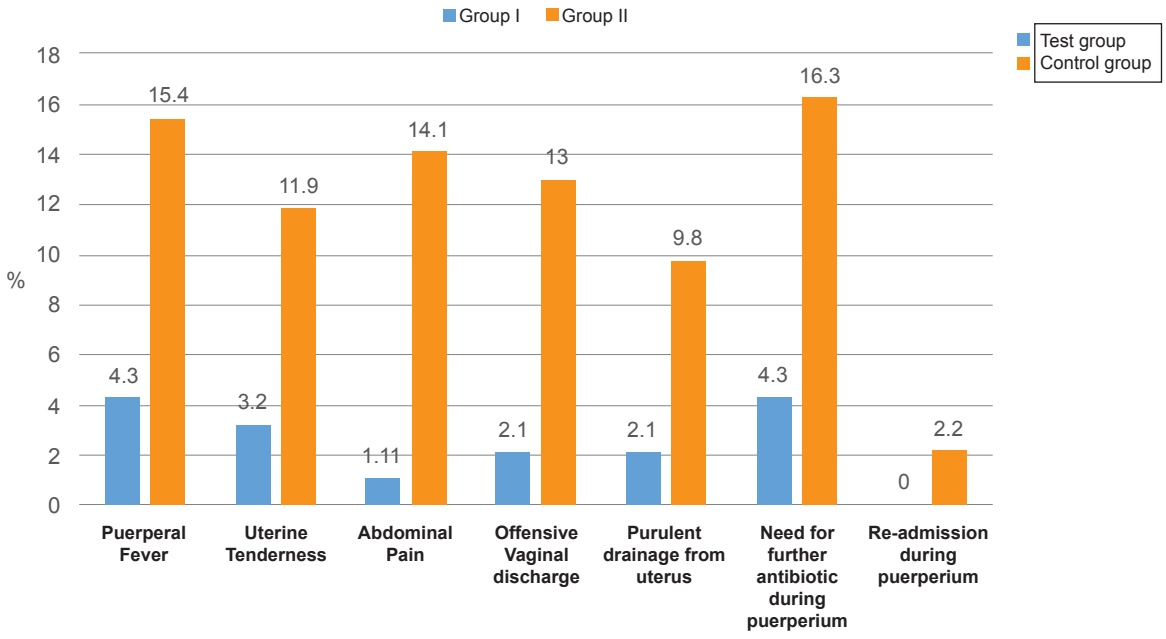


Figure 3: Wound infection distribution among studied cases

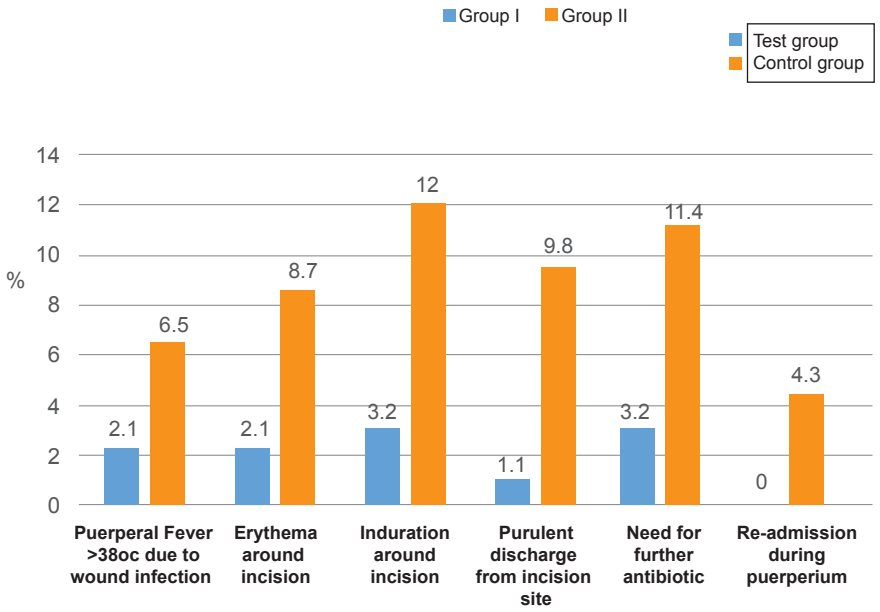


Table 1: Socio-demographic & obstetric data among studied groups.

	Test group N=94(%)	Control group n=92(%)	Test of significance
Age/years Mean±SD	27.44±5.06	27.59±4.97	t=0.22 P=0.83
Gravidity • **Primigravida** • **2nd** • **3rd** • **>3**	12(12.8) 14(14.9) 34(36.2) 34(36.2)	13(14.1) 16(17.4) 31(33.7) 32(34.8)	χ^2=0.35 p=0.95
Parity • **Nullipara** • **Primipara** • **2nd** • **≥3**	12(12.8) 18(19.1) 38(40.4) 26(27.7)	13(14.1) 19(20.7) 41(44.6) 19(20.7)	χ^2=1.25 p=0.74
Gestational age/weeks Mean±SD	39.15±0.98	39.13±0.99	t=0.13 p=0.89
Mode of previous deliveries : • **Vaginal** • **Cesarean**	8(9.8) 74(90.2)	3(3.7) 78(96.3)	χ^2=2.37 p=0.12

χ^2:Chi-Square test t: Student t test *statistically significant p: probability of error

Table 2: Maternal and fetal condition during labor among studied groups.

	Test group N=94(%)	Control group n=92(%)	Test of significance
Current Cs Non-elective	94(100.0)	92(100.0)	
Previous CS	74(78.7)	78(96.3)	χ^2=2.37 p=0.12
Breech presentation	4(4.3)	2(2.2)	FET P=0.68
Labor pain	85(90.4)	85(92.4)	χ^2=0.23 P=0.63
Acute fetal distress	2(2.1)	3(3.3)	**FET P=0.68**
Rupture membrane	27(28.7)	23(25.0)	χ^2=0.57 P=0.62
Anesthesia(spinal)	94(100.0)	92(100.0)	

χ^2:Chi-Square test FET: Fischer exact test *statistically significant p: probability of error

Table 3: Comparison between endometritis, wound infection needing antibiotic and re-admission during puerperium among studied groups.

	Test group N=94(%)	Control group n=92(%)	Test of significance
Endometritis needing antibiotic	4(4.3)	15(16.3)[#]	χ^2=7.36 p=0.006*
Wound infection needing antibiotics	3(3.2)	11(11.9) [$]	χ^2=5.13 p=0.02*
Re-admission during puerperium	0(0.0)	6(6.5)	χ^2=6.34 p=0.01*

[#]of them 2 cases with pelvic collections & need surgical intervention
[$]:4 of them need also admission for 2 weeks
χ^2:Chi-Square test *statistically significantp: probability of error

Table 4: Endometritis distribution among studied groups.

Endometritis	Test group N=94(%)	Control group n=92(%)	Test of significance
Puerperal Fever >38^{O}c due to endometritis	4(4.3)	14(15.2)	χ^2=6.39 p=0.01*
Uterine Tenderness	3(3.2)	11(11.9)	χ 2=5.13 p=0.02*
Abdominal Pain	1(1.1)	13(14.1)	χ^2=11.41 p=0.0007*
Offensive Vaginal discharge	2(2.1)	12(13.0)	χ^2=7.96 p=0.004*
Purulent drainage from uterus	2(2.1)	9(9.8)	χ^2=4.89 p=0.02*
WBCs at 7th day postpartum(K/UL) mean±SD	10.08±2.42	11.54±3.82	t=3.13 p=0.002*
Need for further antibiotic during puerperium	4(4.3)	15(16.3)	χ^2=7.36 p=0.006*
Re-admission during puerperium	0(0.0)	2(2.2)	FET P=0.24

χ^2:Chi-Square test *statistically significant FET: Fischer exact test
p: probability of error

Table 5: Wound infection distribution among studied groups.

Wound infection	Test group N=94(%)	Control group n=92(%)	Test of significance
Puerperal fever >38^{o}c due to wound infection	2(2.1)	6(6.5)	χ^2=2.18 P=0.13
Erythema around incision	2(2.1)	8(8.7)	χ^2=3.94 P=0.04*
Induration around incision	3(3.2)	11(12.0)	χ^2=5.13 P=0.02*
Purulent discharge from incision site	1(1.1)	9(9.8)	χ^2=6.95 P=0.008*
WBCs at 7th day postpartum(K/UL) mean±SD	10.08±2.42	11.54±3.82	t=3.13 p=0.002*
Need for further antibiotic during puerperium	3(3.2)	11(11.9)	χ^2=5.13 P=0.02*
Post-operative hospital stay(48 hours)	94(100.0)	92(100.0)	
Re-admission during puerperium	0(0.0)	4(4.3)	FET P=0.057

χ^2:Chi-Square test *statistically significantFET: Fischer exact test
p: probability of error

Table 6: Neonatal Outcome distribution among studied groups..

Neonatal Outcome	Test group N=94(%)	Control group n=92(%)	Test of significance
Death	0(0.0)	0(0.0)	
NICU admission	11(11.7)	13(14.1)	χ^2=0.24 P=0.62
Causes of NICU admission **Meconium aspiration** **Jaundice**	n=11 4(36.4) 7(63.6)	n=13 5(38.5) 8(61.5)	χ^2=0.01 P=0.91
Respiratory distress	2(2.1)	3(3.3)	FET P=0.68
Necrotizing enterocolitis	0	0	

χ^2:Chi-Square test FET: Fischer exact test p: probability of error

Maternal Vitamin D level and Early Pregnancy Loss A Nested Case Control Study

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Abstract

Objective: To assess the role of vitamin D deficiency and early pregnancy loss.

Patient and Method: A nested case control study conducted in outpatient antenatal care clinic under supervision of Ain Shams University Maternity hospital from the period of March 2020 to June 2020. Pregnant ladies in the first trimester were screened for eligibility criteria.Blood samples were taken from the participants at the time of presentation. All participants were followed till the end of the first trimester to report cases of miscarriage. Vitamin d was assessed for the 40 women who suffered from miscarriage (cases) and for 40 selected controls.The primary outcome was the relation between vitamin d level and early pregnancy loss. Secondary outcome was the relation between vitamin d deficiency and obesity.

Results: Our findings showed that the miscarriage groupwas significantly olderthan control group as $p<0.001$ and BMI was significantly higher as $p<0.007$.The mean value of 25(OH)D was significantly lower among miscarriage group (21.0±8.5) than control group (26.5±8.3) as $p=0.005$. And the majority of miscarriage group (42.5%) had 25(OH)D deficiency while (40.0%)&(17.5%) of cases had either 25(OH)D insufficiencyor sufficiency which significantly different than control group ($p=0.049$).25(OH)D ≤24.5 (ng/mL) was a significant factor that increased the likelihood of first-trimestric miscarriage with sensitivity 80%.No significant differences according to BMI grades regarding 25(OH)D grades.

Conclusion: Vitamin d deficiency is one of modifiable risk factors for first trimestric abortion. . Preconceptional vitamin D supplementation is an easy method for decreasing incidence of early pregnancy loss.

Keywords: vitamin D deficiency, early pregnancy loss, risk factors for miscarriage.

INTRODUCTION

Miscarriage is one of the most common pregnancy complications. Its prevalence ranges from 12 to 20%. Several risk factors are implemented in miscarriage including both genetic and acquired factors. Early detection and modification of these acquired risk factors is necessary. [1]

Pregnancy is considered as a challenge for the immune system. The human foetus is a semi-allograft, maternal immune tolerance is crucial for its survival. In the first trimester invasion of foetalcytotropho-

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blast into the maternal decidua and myometrium is crucial in placentation process and maintenance of pregnancy. [2]

Modification of maternal immune response in pregnancy is vital for normal pregnancy. It is believed that vitamin d has a significant role in its modification. Both vitamin D deficiency and insufficiency are very common among pregnant women. Several adverse pregnancy outcomes are associated with vitamin d deficiency such as Preeclampsia, gestational diabetes, and recurrent miscarriage, which are characterized by an exaggerated immune response. [3]

In addition to its well-known role in regulating calcium metabolism and maintain bone health, there is an increasing interest towards vitamin d immunomodulatory function. It has shown that vitamin d is synthesized by the tissues of the female reproductive system and to affect the reproductive events such as fertlisation and implantation by modulating the immune system function. Important constituents for vitamin d synthesis and activation are expressed in the endometrium, dicedua, placenta and ovaries. Low vitamin d level is associated with repeated failure of implantation, pregnancy loss and some obstetric complications such as preeclampsia and gestational diabetes.[4]

Up to 90% of daily requirement of vitamin D is absorbed through the skin via sunlight with minimal contribution from food. It is essentially required for everyone especially pregnant women to stay for 20 minutes in sunlight daily with more than 40 percent of skin exposed in order to avoid vitamin d deficiency.[5]

I t was suggested by some studies that obesity is accompanied with vitamin d defeceincy. As vitamin dis a fat soluble vitamin, it is accumulated in fat cells leading to decrease in its serum blood levels in obese persons.[6]

Although Egypt is a country with abundant sunshine all year round, majority of pregnant females had vitamin D deficiency and insufficiency. UnfortunatelyVitamin D deficiency is associated with many adverse effects in pregnancy.This nested case control study aimed to assess the role of vitamin D deficiency and early pregnancy loss.

PATIENTS AND METHODS

Study Design: A nested case control study.

Study setting: Outpatient clinics antenatal care clinicunder supervision of Ain Shams University Maternity hospital.

Study time: From March 2020 to June 2020

Study population: Pregnant ladies in the first trimester seeking antenatal care.

Inclusion criteria: Singleton pregnancy, spontaneous pregnancy and gestational age between (7-10 weeks).

Exclusion criteria: Ectopic pregnancy, molar pregnancy, thyroid disorders ,autoimmune disorders ,uterine malformation ,severe liver and kidney diseases ,diabetes mellitus,Pregnancy with assisted reproductive techniques,women consuming drugs interfering with Vitamin D metabolism and women who refused to participate.

Sample size: This study is conducted on 80 women.

Sample Justification: Sample size was calculated using the online PASS, setting the power at 0.80 and the type-1 error at 0.05. data from previous study byAndersen et al(7),showed that serum Vitamin D levels in women with miscarriage and women without miscarriage were 55.6 nmol/L, 66.0 nmol/L, respectively. Calculation according to these values produces a minimal sample size of 33 women.

Ethical Considerations: This study was done after approval of the ethical committee of the department of obstetrics and gynecology, faculty of medicine, Ain Shams University.

Study interventions and procedures:

All participating women were subjected to the following:

A) Detailed history (personal, present, obstetric, menstrual, family, medical and surgical).

B) Examination (general, abdominal and local).

C) Body mass index (BMI) calculation (weight/ height kg/ cm^2).

Gestational age was measured by first day of the last menstrual period and confirmed with ultrasound. Blood samples were taken from the participants. Venous samples were withdrawn from pregnant women at the time of presentation in 1st trimester and stored after centrifugation and serum separation.All participants were followed till the end of the first trimester to report the number of females who suffered miscarriage. After a number of 40 women with unexplained miscarriage have reached, recruitment was stopped. A control group of 40 women with ongoing pregnancy who passed the 13th week of pregnancy were selected by simple random method as a control group. Vitamin d was assessed for the 40 women who suffered miscarriage (cases) and for 40 selected controls.

Vitamin D status was determined by measuring serum levels of 25(OH) D (25 hydroxy vitamin d) using an enzyme immunoassay. Levels of 25(OH) D were measured by using 25-OH Vitamin D ELISA kit (Euroimmun, Luebeck, Germany) according to manufacturer's instructions. The serum vitamin d level is categorized depending on clinically accepted ranges for Vitamin D into : deficiency (<20 ng/ml), insufficiency (20–30 ng/ml) and replete (>30 ng/ml).

Outcomes: 1ry outcome: The relationship between vitamin d level and early pregnancy loss. **2ry outcome:** The relation between vitamin d deficiency and obesity.

Statistical Analysis:

The data was labelled, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013 and Microsoft Office Excel 2007. Descriptive statistics was done for quantitative data as minimum& maximum of the range as well as mean±SD (standard deviation) for quantitative normally distributed data, median and first& third inter-quartile range for quantitative non-normally distributed data, while it was done for qualitative data as number and percentage. Inferential analyses was done for quantitative variables using Shapiro-Wilk test for normality testing, independent t-test in cases of two independent groups with normally distributed data, Mann whiteny U in cases of two independent groups with non-normally distributed data, ANOVA test and Kruskal Wallis test. In qualitative data, inferential analyses for independent variables was done using Chi square test for differences between proportions and Fisher's Exact test for variables with small expected numbers. While correlations was done using Pearson correlation for numerical normally distributed data, and using spearman rho test for numerical non normally distributed and qualitative data. ROC curve was used to evaluate the performance of different tests differentiate between certain groups. Logistic regression model was used to find out independent factors affecting certain conditions. The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant.

RESULTS

After excluding 30 ineligible cases, it was needed to recruit 298 cases to collect 40 (13.4%) cases with first-trimestric miscarriage. From the 258 cases that passed first trimester without miscarriage 40 cases were randomly included as a control group.

Table (1): Comparison between the studied groups regarding maternal age.

Variables		Miscarriage (N=40)	No Miscarriage (N=40)	P-value
Age (years)	**Mean±SD**	32.0±6.5	26.9±5.4	**^<0.001***
	Range	20.0–46.0	17.0–37.0	
	Range	7.0–10.0	7.0–10.0	

^Independent t-test. *Significant

Table (1) shows that: Age was significantly higher among miscarriage group.

Table (2): Comparison between the studied groups regarding gravidity and parity.

Variables		Miscarriage (N=40)	No Miscarriage (N=40)	P-value
Gravidity	**Median (1st–3rd IQ)**	3.0 (2.0–4.0)	3.0 (2.0–3.8)	#0.363
	Range	1.0–5.0	1.0–6.0	
Parity	**Median (1st–3rd IQ)**	2.0 (1.0–2.0)	1.0 (1.0–2.0)	#0.541
	Range	0.0–4.0	0.0–3.0	
	Range	0.0–3.0	0.0–3.0	

IQ: Interquartiles. #Mann Whitney test

Table (2) shows that: No significant differences between the studied groups regarding parity and gravidity.

Table (3): Comparison between the studied groups regarding body mass index (BMI)

Variables		Abortion (N=40)	No abortion (N=40)	P-value
BMI (kg/m^2)	**Mean±SD**	28.0±4.5	25.4±3.8	^ 0.007*
	Range	21.9–39.8	18.0–33.6	
Grade	**Normal**	12 (30.0%)	23 (57.5%)	# 0.045*
	Over weight	16 (40.0%)	9 (22.5%)	
	Obese	12 (30.0%)	8 (20.0%)	

^Independent t-test. #Chi square test. *Significant

Table (3) shows that: BMI was significantly higher among miscarriage group. Overweight and obese grades were significantly more frequent amongmiscarriage group.

Table (4): Comparison between the studied groups regarding 25(OH)D

Variables		Miscarriage (N=40)	No Miscarriage (N=40)	P-value
Level (ng/mL)	**Mean±SD**	21.0±8.5	26.5±8.3	^ **0.005***
	Range	7.0–38.4	11.2–46.1	
Grade	**Sufficiency**	7 (17.5%)	16 (40.0%)	# **0.049***
	Insufficieny	16 (40.0%)	15 (37.5%)	
	Defficiency	17 (42.5%)	9 (22.5%)	

^Independent t-test. *Significant

Table (4) show that: 25(OH)D was significantly lower among miscarriage group

Table (5): Comparison according to 25(OH)D grades regarding BMI grades.

Variables		Normal	Over weight	Obese	p-value
Miscarriage group					
Number		12	16	12	
25(OH)D (ng/mL)		22.3±8.9	21.9±9.1	18.6±7.5	^0.513
25(OH)D grade	**Sufficiency**	3 (25.0%)	3 (18.8%)	1 (8.3%)	#0.874
	Insufficieny	4 (33.3%)	6 (37.5%)	6 (50.0%)	
	Deficiency	5 (41.7%)	7 (43.8%)	5 (41.7%)	
Control group					
Number		23	9	8	
25(OH)D (ng/mL)		27.2±8.2	26.4±10.6	24.2±6.0	^0.678
25(OH)D grade	**Sufficiency**	12 (52.2%)	3 (33.3%)	1 (12.5%)	#0.298
	Insufficieny	6 (26.1%)	4 (44.4%)	5 (62.5%)	
	Defficiency	5 (21.7%)	2 (22.2%)	2 (25.0%)	

^ANOVA test. #Fisher's Exact test.

Table (5) shows that: No significant differences according to BMI grades regarding 25(OH)D grades

Table (6): Diagnostic performance of age, BMI and 25(OH)D in predictingmiscarriage

Factors	AUC	SE	P-value	95% CI	Cut off
Age	0.712	0.057	0.001	0.600–0.823	≥32.0
BMI	0.647	0.062	0.024	0.525–0.768	≥26.0
25(OH)D	0.682	0.061	0.005	0.562–0.801	≤24.5

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

Table (6) and figure (1): Age, BMI and 25(OH)D had significant moderate diagnostic performance in predicting miscarriage

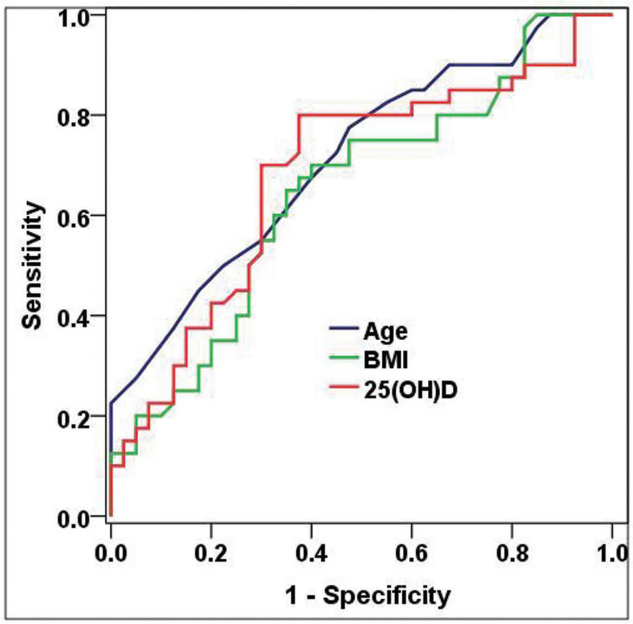

Figure (1): ROC curve for age, BMI and 25(OH)D in predicting miscarriage

Table (7): Diagnostic charactersitcis of age, BMI and 25(OH)D cutoff points in predicting miscarriage.

Characters	Age ≥32.0 (years)		BMI ≥26.0 (kg/m2)		25(OH)D ≤24.5 (ng/mL)	
	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	50.0%	33.8%–66.2%	65.0%	48.3%–79.4%	80.0%	64.4%–90.9%
Specificity	77.5%	61.5%–89.2%	65.0%	48.3%–79.4%	60.0%	43.3%–75.1%
DA	63.8%	52.2%–74.2%	65.0%	53.5%–75.3%	70.0%	58.7%–79.7%
YI	27.5%	7.3%–47.7%	30.0%	9.1%–50.9%	40.0%	20.4%–59.6%
PPV	69.0%	49.2%–84.7%	65.0%	48.3%–79.4%	66.7%	51.6%–79.6%
NPV	60.8%	46.1%–74.2%	65.0%	48.3%–79.4%	75.0%	56.6%–88.5%
LR+	2.22	1.16–4.27	1.86	1.15–3.00	2.00	1.33–3.01
LR-	0.65	0.45–0.92	0.54	0.33–0.87	0.33	0.17–0.65
LR	3.44	1.31–9.06	3.45	1.38–8.64	6.00	2.21–16.31

CI: Confidence interval, DA: Diagnostic accuracy, YI: Youden's index, PPV: Positive Predictive value, NPV: Negative Predictive value, LR+: Positive likelihood ratio, LR-: Negative likelihood ratio, LR: Diagnostic odd ratio

Table (7) show that: Age ≥32.0 (years), BMI ≥26.0 (kg/m2) and 25(OH)D ≤24.5 (ng/mL) had moderate diagnostic charactersitcis in predicting miscarriage

Table (8): Logistic regression for factors affecting first-trimestric miscarriage

Factors	B	SE	P-value	OR (95% CI)
Age ≥32.0 (years)	1.25	0.56	**0.026***	3.49 (1.16–10.53)
BMI ≥26.0(kg/m2)	1.11	0.53	**0.035***	3.04 (1.08–8.54)
25(OH)D ≤24.5 (ng/mL)	1.80	0.56	**0.001***	6.05 (2.04–17.98)
Constant	-2.10	0.58	**<0.001***	

β: Regression coefficient. SE: Standard error. OR; Odds ratio. CI: Confidence interval. *significant

Table (8) shows that: Age ≥32.0 (years), BMI ≥26.0 (kg/m2) and 25(OH)D ≤24.5 (ng/mL) were significant factors that increased the likelihood of first-trimestricmiscarriage .

DISCUSSION

Most pregnancy loss cases occur early in pregnancy before the end of the first trimester and pathophysiology is therefore sufficient to be of great importance. Multiple etiologies, such as chromosomal anomalies, infections, hormonal abnormalities, uterine malformation, autoimmune diseases and coagulopathies, were identified for the pathogenesis of repeated pregnancy loss, while about half of pregnancy loss cases still have no identifiable etiology.[8]

Vitamin d is thought to make important immunological modifications that help maintaining successful pregnancy. Several studies found that vitamin d defeceincy is associated with unfavorable pregnancy outcomes.[7]

Despite the extensive use of vitamins prior to pregnancy, vitamin D deficiency is prevelant among pregnant women. Low serum Vitamin Dlevel during pregnancy has been associated with adverse pregnancy effects such as gestational diabetes, preeclampsia, and foetal growth restriction.[9]

This nested case control study aimed to assess the role of vitamin D deficiency and early pregnancy loss in the first trimester. From the 258 cases that passed first trimester without abortion, 40 cases were randomly included as a control group.

There was no significant differences between the studied groups regarding gestational age at enrollment, parity, gravidity (P= 0.245, 0.363, 0.541& respectively).

Our findings showed that the mean value of 25(OH) D was significantly lower among miscarriage group (21.0±8.5) than control group (26.5±8.3) as p=0.005. The majority of miscarriage group (42.5%) had 25(OH) D deficiency while (40.0%) & (17.5%) of cases had either 25(OH) D insufficiency or sufficiency which significantly different than control group (p=0.049).25(OH) D ≤24.5 (ng/mL) was a significant factor that increased the likelihood of first-trimestricmiscarriage with sensitivity 80%.No significant differences according to BMI grades regarding 25(OH)D grades.

The present study is in harmony with a cohort study byAndersen et al.,performed on 1683 pregnant women before 22 weeksto whom serum 25-hydroxyvitamin D [25(OH)D] withdrawn. They reported that the miscarriage group had lower maternal serum concentration of 25(OH)D with an increased risk of miscarriage in the first but not in the second.(7)

Our findings agreed with ***Gonçalves et al***., who systematically reviewed 11 articles investigating relationship between recurrent miscarriage and vitamin d. This systematic review showed high incidence of both vitamin D insufficiency (VDI) or vitamin D deficiency (VDD) in cases of recurrent pregnancy loss (RPL).(10)

Another cross-sectional study by, ***Hou et al.***, found that pregnancy loss was significantly association with low vitamin D levels (OR= 1.71; 95% C.I: 1.2–2.4, P0.001).(11)

Moreover, in acase control study by ***Hamad et al***.included250 pregnant females during the 1st twenty weeks of gestation, with ages varying from 20 to 35 years showed that vitamin D deficiency was more common in pregnant ladies and was considered as one of the as one modifiable risk factors for pregnancy loss mostly among recurrent losses.(12)

Another retrospective case control study by***Alya et al.***, in Iraq reported that vitamin D deficiency was associated with 1st trimester miscarriages.(13)

In contrary to our study results a recent meta-analysis and systematic review by ***Amegah et al***., found that low vitamin D levels were not accompanied with increased risk of spontaneous recurrent miscarriage.(14)

As regard relationship between obesity and vitamin d deficiency similarly to our results, ***Woon et al.***, revealed that, there were no associations between pre-pregnancy BMI with vitamin D deficiency.(15)

On the opposite side, a study by***Agarwal et al***., demonstrated a significant relation between increased body mass index (BMI) and low vitamin D serum levels. All women with BMI ≥30 were found to have low vitamin d levels (100% prevalence).(16)

In addition another study by ***Shen et al***., didnot support the current results as they found that, among pregnant women, maternal vitamin D values during first trimester of pregnancy have been positively associated with maternal BMI.(17)

And by comparing deficient and optimal groups, ***Hamad et al***., was found that obesity, was a possible risk factors for VDD.(12)

CONCLUSION

Although Egypt is a country with abundant sunshine all year round, majority of pregnant females had vitamin D deficiency and insufficiency. Vitamin D deficiency had a role in spontaneous abortion. Vitamin D level not related to the BMI.

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Endometrial Volume as a Predictor of Endometrial Pathology in Perimenopausal Uterine Bleeding

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Abstract

Background: Transvaginal ultrasonography (TVS) imaging is a routine, non-invasive procedure in initial evaluation of patients with abnormal uterine bleeding in perimenopausal age. However, there is no yet a cutoff value to discriminate benign form malignant cases.

Aim of the Work: Is to assess the potential value of endometrial volume measurements by 2D TVS compared with that of endometrial thickness in prediction of different endometrial pathologies in women with perimenopausal uterine bleeding.

Patients and Methods: Ninety-two perimenopausal women presented with uterine bleeding were enrolled to the study after exclusion of general and local caused of bleeding as polyps and fibroids. TVS (2D) was done to measure endometrial thickness and volume using a specific formula. Endometrial biopsy was taken and the pathological results was correlated with the endometrial thickness and volume.

Results: There was a statistically noticeable difference between benign endometrial pathology compared to endometrial hyperplasia with atypia or endometrial carcinoma as regard age as well as body mass index. There was a high statistically significant difference between patients with benign endometrium pathology and malignant endometrial pathology regarding endometrial thickness and volume. That endometrial volume had a high predictive value as proved by an area under the ROC curve (AUC) of 0.826. The best cut-off value was an endometrial volume >11.675 cm3. While (ROC) curve analysis for differentiation of patients using endometrial volume and endometrial thickness into women with benign endometrial pathology or hyperplasia and those with endometrial carcinoma; Endometrial volume also had a higher predictive value than endometrial thickness as proved by an area under the ROC curve (AUC) of 0.871. The cut-off value was an endometrial volume >13.105 cm3. While using endometrial thickness cut-off value was an endometrial thickness > 14.5mm.

Conclusion: endometrial volume measured with inexpensive 2D TVS has a higher predictive value than measuring endometrial thickness in correspondence to the pathological results in patients with perimenopausal bleeding.

Keywords: Perimenopausal bleeding, endometrial volume, 2D transvaginal ultrasound.

INTRODUCTION

Persistent changes to the bleeding patterns during the perimenopausal period may be dangerous and careful evaluation should be done. (Klein NA, 1998).The increasing prevalence of obesity and metabolic syndromeyielded to higher incidence of endometrial cancer. Between 1992-1994 and 2009-2011, the European age-standardized rates of uterine cancer in the UK have risen by 48% **(CRUK, 2014)**.

Transvaginal ultrasound (TVS) is an inexpensive, non-invasive way to indirectly visualize the endometrial cavity. Therefore, it is recommended as a first diagnostic tool to assess uterine pathology in any woman presenting with AUB. There is continuing interest in the role of spectral and color Doppler imaging for the endometrium **(Kotdawala, et al.2013).**

Measuring of the endometrial thickness for determination of the cause of abnormal uterine bleeding in the perimenopausal women is not easy in absence of anatomical lesions. Anovulatory cycles are a common cause, but hyperplasia, submucous-myomas, and carcinoma are of the concern to the patients (**Merce, et al. 1991).**

Volumes are usually measured by 3D where data are retrieved and presented in multi-planer display mode which simultaneously displays three perpendicular planes on the screen for the volume calculation which done by built in computer program using VOCAL. The endometrial volume was measured in plane A by delineating the endometrial margin at the endometrial-myometrial interface from the fundus to the internal cervical in a number of parallel slices which are 1-2 mm apart (Elsokkary M, 2016).Endometrial volume also can be measured by 2D transvaginal sonography with easy and applicable procedure by 2D TVS ellipse help to predict endometrial pathology. The formula for determining the volume of the endometrium by using one diameter and ellipse or three diametrs of the endometrium.

The basic limitation of all imaging techniques has been the diagnosis of cellular changes in the endometrium. As of now the best use of ultrasonography along with saline infusion sonography in pre-menopausal AUB is to rule out organic pathologies such as a fibroid, adenomyosis, polyp etc., It has been proposed to use the 'thick endometrium' as a screening tool to select patients for histological assessment **(Tinelli R et al., 2008)**.

The cut off value for endometrial thickness is >12 mm is the most common used for screening but its use is not recommended as this has been found to be inaccurate and a blind adherence. In contrast, endometrial thickness >4 mm in post-menopausal bleeding has a better predictive value in diagnosis of about 99% patients with endometrial cancer. However, repeated episode of postmenopausal bleeding with a endometrial thickness <4 mm, a biopsy is done to exclude that 1% chance of endometrial cancer **(Litta P et al., 2005)**.

Endometrial pathology should be evaluated in women with abnormal uterine bleeding that not responding to a course of therapy for three months. **(Brand A et al., 2000)**.

Aim of the Work:

Is to assess the potential value of endometrial volume measurements by 2D TVS compared with that of endometrial thickness in prediction of different endometrial pathologies in women with perimenopausal uterine bleeding.

PATIENTS AND METHODS

A prospective observational study was conducted on perimenopausal women with abnormal uterine bleeding who attended to Obstetrics & Gynecology department at Mansoura university hospital.

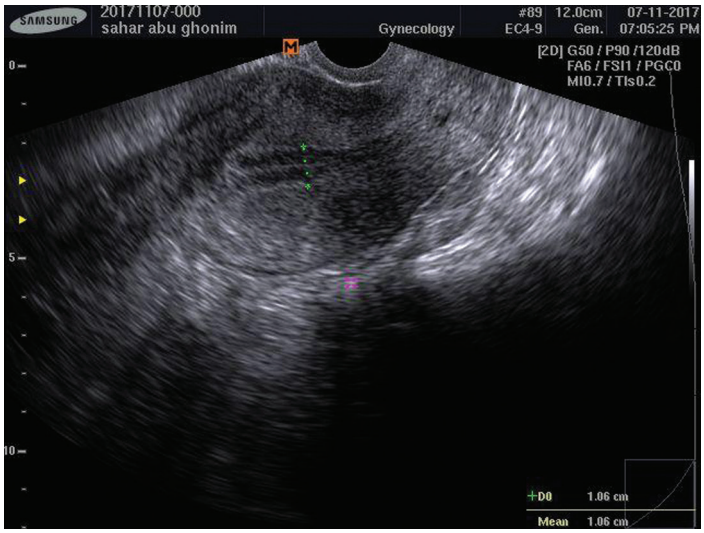
Patients: From August 2017 till August 2018,ninety two patients who were admitted to Obstetrics & Gynecology department at Mansoura university hospital suffering from perimenopausal uterine bleeding were enrolled into the study. Age ranged from 45-55 years with symptoms of abnormal bleeding e.g. heavy menstrual bleeding, intermenstrual bleeding and polymenorrhea within the last six months. General causes of bleeding e.g. liver cell failure, coagulopathy, hereditaryhemorrhagictelengectasia patients were excluded. Moreover; local causes of bleeding e.g. fibroid, polyp and cervical lesion were also excluded as well as patients with history of drug intake like antiplatelets, anticoagulant or recent hormonal contraception.

Ethical consideration: Study protocol was submitted for approval by IRB.Informed verbal and written consent was obtained from each participant sharing in the study.

Methods: All patients were exposed to the following

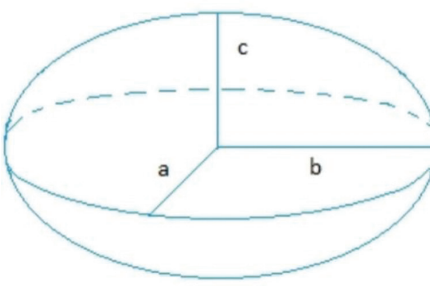
- Full history taking, General, abdominal and pelvic examination

2D-TVS measuring endometrial thickness by Sonoace R3medisone Samsung South Korea device) endometrial thickness is measured at its thickest part in the longitudinal plane. The surrounding more echo lucent layer (or ‘‘halo’’) is considered to be the inner myometrium and is not included.



- Fig (1): 2D endometrial thickness by TVS SAMSUNG MEDISONE sonoace R3 South Korea device
- 2D-TVS measuring endometrial volume by the same device. The formula for determining the volume of the endometrium by using one diameter and ellipse reads as follow :

$$V = \frac{4}{3} \times \pi \times a \times b \times c$$



Volume of an ellipsoid

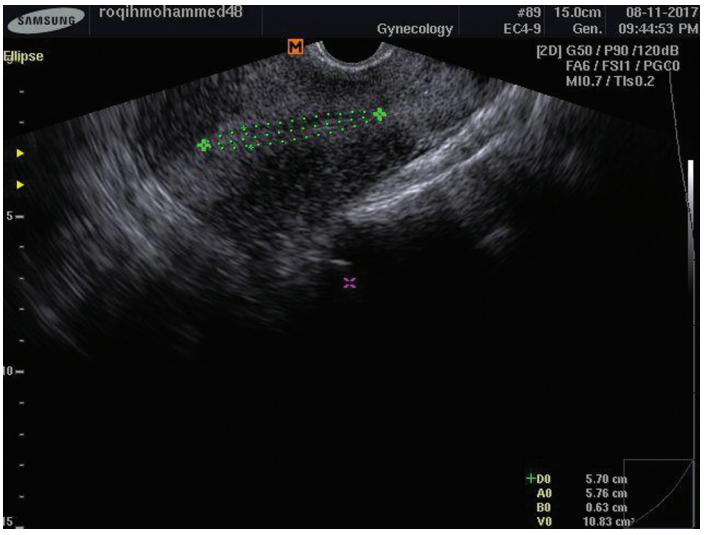


Fig (2): 2D endometrial volume by TVS SAMSUNG MEDISONE sonoace R3 South Korea device

- Patients prepared for dilatation and curettage(one to two days following ultrasonic assessment) using sedating agents e.g. Propofol 2%, Midazolam, Fentanyl.
- Endometrial samples sent to Pathology department at Mansoura university forhistopathological examination.
- Comparison of the endometrial thickness and volume obtained by 2D- TVS with the results of the histopathological examination of endometrial tissue.

Results:

The mean age of the patients was 50.53 ± 3.64 years. About third of them were diabetic patients, half of them were hypertensive, and 2.2% have had viral hepatitis.

Table (1): Distribution of the pathological results in women under study:

Result of pathology	No	%
Endometrial hyperplasia without atypia	37	40.2
Disordered proliferative endometrium	23	25.0
Endometrial carcinoma	13	14.1
Endometrial hyperplasia with atypia	7	7.6
Proliferative endometrium	3	3.3
picture of irregular endometrial shedding	1	1.1
Scanty endometrial tissue with occasional proliferative glands	1	1.1
Atrophic endometritis	6	6.5
Endometritis	1	1.1

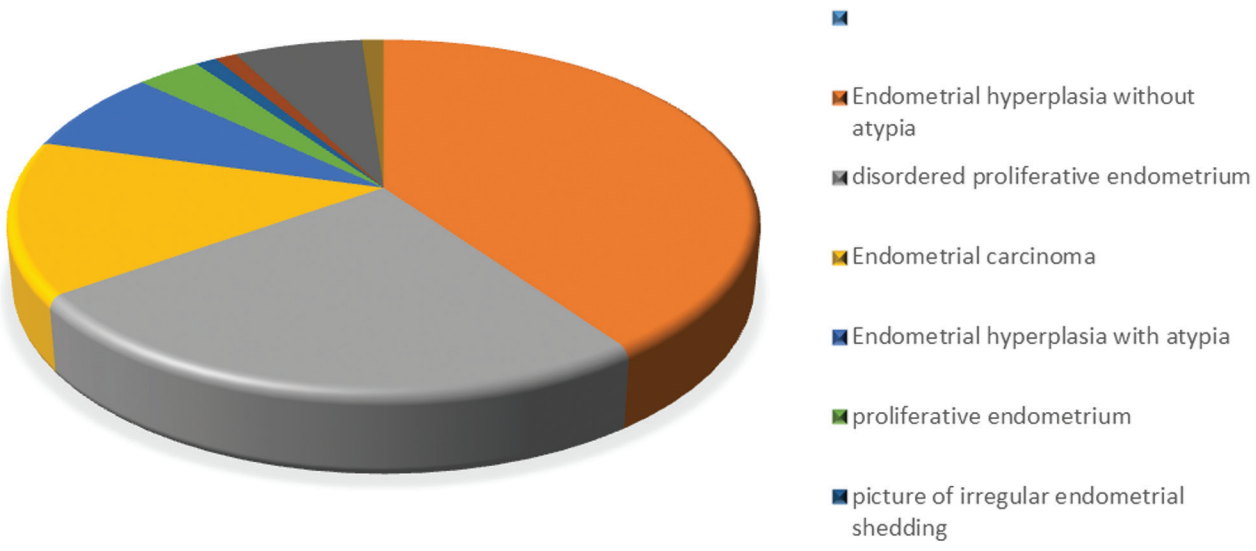


Figure (3): Chart for the patients histopathological results

There was a statistically noticeable difference between benign endometrial pathology compared to endometrial hyperplasia with atypia or endometrial carcinoma as regard age [(49.93 ± 3.58 and 52.70 ± 3.05) respectively], parity [3.74 ± 1.48, 3.30 ± 1.59respectively] and body mass index [35.49 ± 2.34, 37.95 ± 1.61respectively] using independent sample t-test.

Table (3): Endometrial volume and thickness in patients with benign endometrial pathology, endometrial hyperplasia and endometrial carcinoma ranging from the 1stand 3rdquartiles (interquartile range) using Kruskall-Wallis test.

	N	Endometrial thickness (mm) [median(range)]	Endometrial volume (cm3) [median(range)]
Benign endometrial pathology	35	9 (6 – 10)	9 (7.2 – 11.7)
Endometrial hyperplasia	44	10 (8 – 12)	12.2 (10.1 – 18.3)
Endometrial carcinoma	13	17 (13.5 – 30)	26 (16 – 52.5)
Kruskall-Wallis test	□2	23.222	26.682
	P	< 0.001*	< 0.001*

As regarding to endometrial volume and endometrial thickness there was a high statistically significant difference between patients with benign endometrium pathology and malignant endometrial pathology. That endometrial volume had a high predictive value as proved by an area under the ROC curve (AUC) of 0.826. The best cut-off value was an endometrial volume >11.675 cm3. This had a sensitivity of 95%, a specificity of 62.5%, +ve predictive value 41.3% and -ve predictive value 97.8% and endometrial thickness cut-off value was an endometrial thickness>10.5mm, evidenced by an area under the ROC curve (AUC) of 0.809 This had a sensitivity of 85%, a specificity of 69.4%, +ve predictive value 43.6% and -ve predictive value 94.3%.

Table (4): ROC curve analysis for differentiation of patient's pathology results into those with benign endometrial results and those with endometrial hyperplasia with atypia or carcinoma using measured endometrial volume

Variable	Volume
Cut off point	11.675
Area under the curve	0.826
Sensitivity	95%
Specificity	62.5%
Positive predictive value	41.3%
Negative predictive value	97.8%
Accuracy	69.6%
P value	< 0.001*

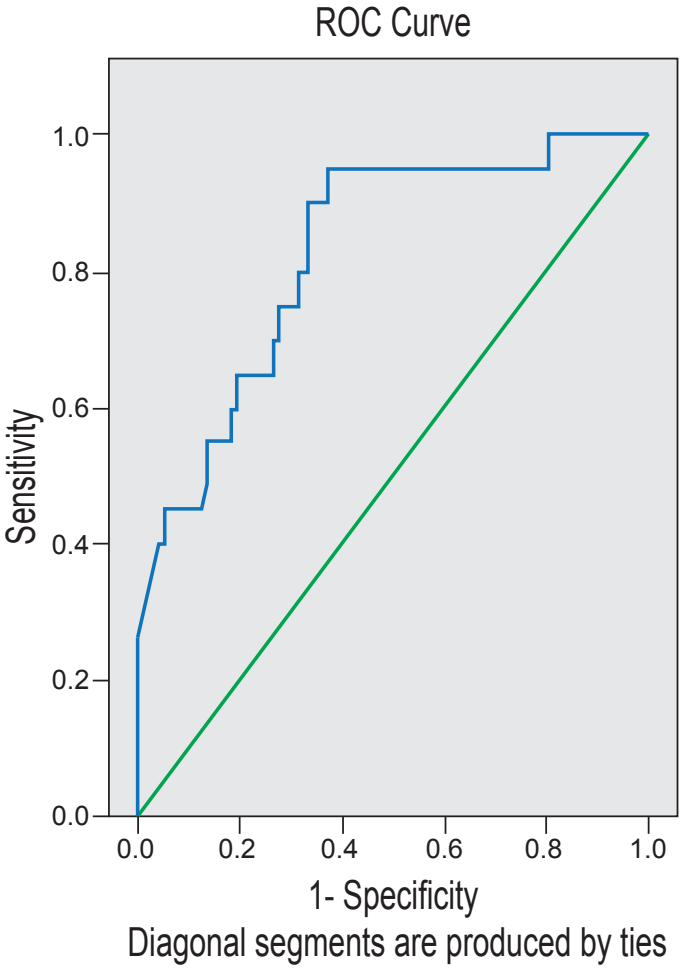
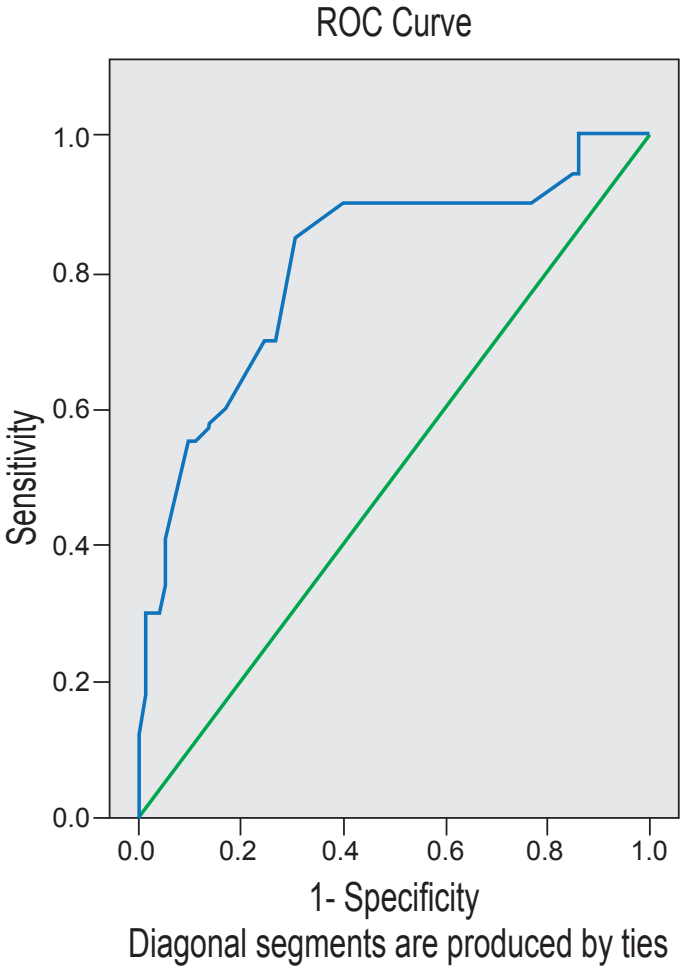


Table (5): ROC curve analysis for differentiation of patient's pathology results into those with benign endometrial pathology and those with endometrial hyperplasia with atypia or carcinoma using endometrial thickness.

	Thickness
Cut off point	10.5
Area under the curve	0.809
Sensitivity	85%
Specificity	69.4%
Positive predictive value	43.6%
Negative predictive value	94.3%
Accuracy	72.8%
P value	< 0.001*



The results of the ROC curve analysis for differentiation of patient's pathology into benign endometrial pathology or hyperplasia and endometrial carcinoma using endometrial volume had shown also that endometrial volume had a good predictive value as proved by an area under the ROC curve (AUC) of 0.871. The best cut-off value was an endometrial volume >13.105 cm3. This had a sensitivity of 92.3% , a specificity of 73.4%, Positive predictive value 36.4% and negative predictive value 98.3%, using endometrial thickness cut-off value was an endometrial thickness>14.5mm, proved by an area under the ROC curve (AUC) of 0.867This had a sensitivity of 76.9%, a specificity of 89.9%, +ve predictive value 55.6% and -ve predictive value 96%.

Table (6): ROC curve analysis for differentiation of the patient's pathology into benign endometrial pathology or hyperplasia and endometrial carcinoma using endometrial volume at a cut off value of 13.105 cm^3.

	Volume
Cut off point	13.105
Area under the curve	0.871
Sensitivity	92.3%
Specificity	73.4%
Positive predictive value	36.4%
Negative predictive value	98.3%
Accuracy	76.1%
P value	< 0.001*

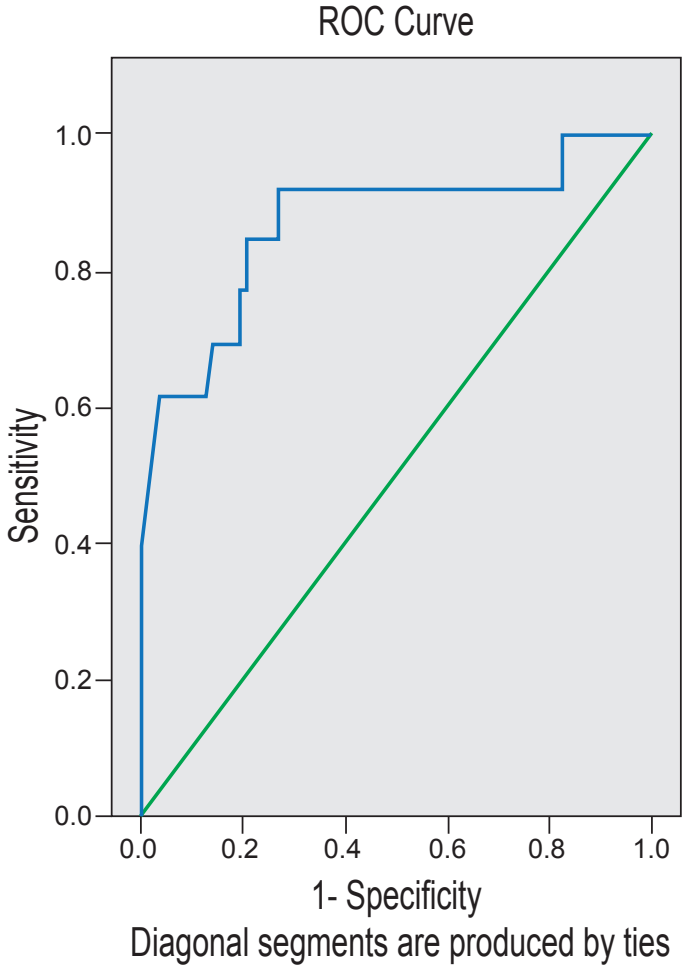
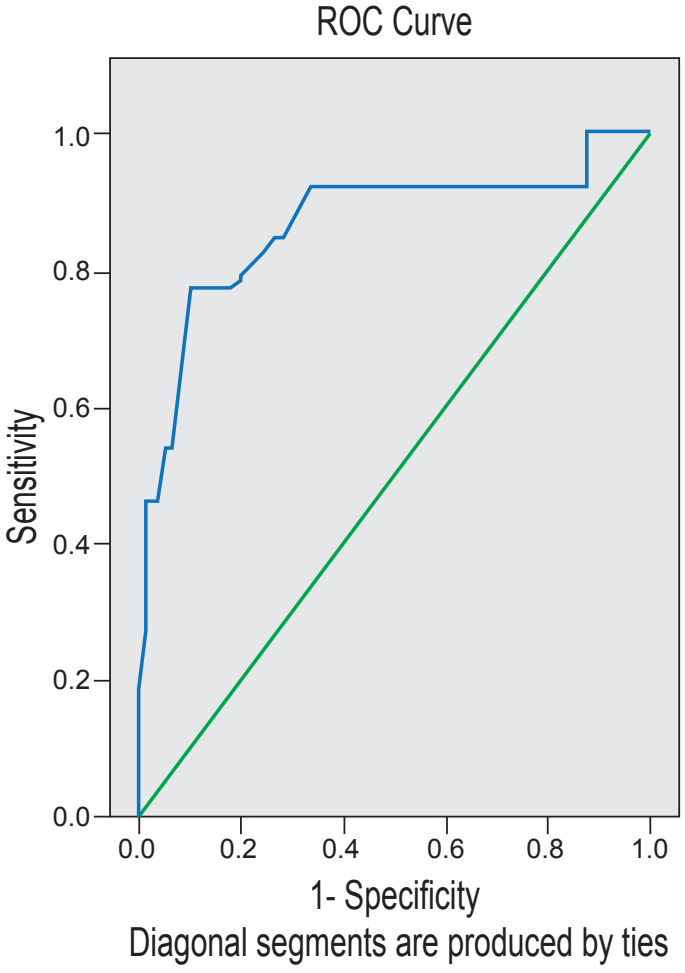


Table (7): ROC curve analysis to differentiation of patient's pathology into benign endometrial pathology or hyperplasia and endometrial carcinoma using endometrial thickness at a cut off value of 14.5mm

	Thickness
Cut off point	14.5
Area under the curve	0.867
Sensitivity	76.9%
Specificity	89.9%
Positive predictive value	55.6%
Negative predictive value	96%
Accuracy	88%
P value	< 0.001*



Discussion

Menstrual disorders affect the lifestyle in otherwise healthy women. Once bleeding occurs, investigation should be done to exclude benign and malignant causes (Munro M, 2014).In most studies about 43-66% of cases with hyperplasia were missed by D&C when comprising both women with perimenopausal and those with postmenopausal bleeding. (Karampl E et al., 2001).

Three-dimension ultrasound has been used to explore anatomical sections of the uterine cavity; the relations of myomas to the cavity, endometrial polyps can be diagnosed through it and endometrial volume measurement rather than thickness in women with AUB are feasible (Fleischer AC et al., 2003).

The current study aimed at assessment of the potential value of endometrial volume measurements by 2D transvaginal sonography compared with that of endometrial thickness in foretelling of different endometrial pathologies in women with perimenopausal uterine bleeding. The resultsshowed that there is higher predictive role of endometrial volume in prediction of different endometrial pathologies like hyperplasia or carcinoma that affect women with perimenopausal uterine bleeding and this comes consistent with findings from other studies(M. Odeh et al., 2007, Elsokkary M et al., 2016 and Wael .S. Nossair, 2017).

However, to the best of our knowledge, all studies concerned with assessment endometrial volume were done by 3D ultrasound machine. However,the financial burden and the limitation of the resources in many areas in the world made the idea of using 2D and getting accurate results looks glory and reasonable.

The histopathological results in the current studyshowed that 6.5% of specimen was atrophic endometritis, 25% was disordered proliferative endometrium, 40.2% was endometrial hyperplasia without atypia, 7.6% was with atypiaand 14.1% was endometrial carcinoma.This is different to another study that showed that myomas found in 32% of specimens, endometrial hyperplasia in 22% and endometrial carcinoma in only 4% of them(Pyari JW, et al., 2006).Low incidence of endometrial atrophy may be explained by the group

of enrolled patients that usually take hormonal replacement therapy in the perimenopausal period. There is similarity in the prevalence of endometrial carcinoma in the present study (14.1%) and that reported in another study (Khare A, 2012).

Regarding to endometrial volume there was a difference between cases with benign endometrial pathology, endometrial hyperplasia and endometrial carcinoma, the medians (IQR) were [9 (7.2 – 11.7), 12.2 (10.1 – 18.3) and 26 (16 – 52.5) cm3 respectively, using Kruskal-Wallis test. Similarly using the same test as regards endometrial thickness there was noticeable difference between them; the medians (IQR) were [9 (6 – 10), 10 (8 – 12) and 17 (13.5 – 30) mm respectively.

Results were consistent with other study done many years ago, (Kupesic S, et al., 1991), the mean value of the endometrial volume in hyperplasia was 7.82 ± 7.60 cc and was higher than the volume of patients with polyps (mean 2.63 ± 2.12 cc). Correspondingly; in a study done byM. Odeh et al. in 2007the endometrial volume in patients with normal endometrium and pathologic endometrial were 6.87± 6.3 cc and 13.79 ± 13.2 cc respectively.

Endometrial volume in women with endometrial cancer was 18.1 cc and 11.2 cc in women with hyperplasia; both values were higher than in the normal. Their results showed difference than the current study results that may be explained by the heterogeneity in the group studied and the type of endometrial cancer present.

Discrimination of the results using ROC curve for endometrial volume and thickness into those with benign endometrium and those with endometrial hyperplasia with atypia or carcinoma; endometrial volume had a better predictive value than endometrial thickness as proved by an area under the ROC curve (AUC) of 0.826. The cut-off value was an endometrial volume >11.675cm3. On the other hand,endometrial thickness cut-off value was 10.5mm.

While (ROC) curve analysis for differentiation of patients using endometrial volume and endometrial thickness into women with benign endometrial pathology or hyperplasia and those with endometrial carcinoma; Endometrial volume also had a higher predictive value than endometrial thickness as proved by an area under the ROC curve (AUC) of 0.871. The cut-off value was an endometrial volume >13.105 cm3. While using endometrial thickness cut-off value was an endometrial thickness > 14.5mm.

In another study done to predict the carcinoma of the endometrium in women with postmenopausal bleeding by measuring the endometrial volume through vocal. Patients were classified into two groups of postmenopausal Women with and without bleeding the later was used for control. 50% of the patients had benign disease, 35% with atypia and 15% with cancer in the study group. Whereas in cases with atypia or cancer endometrial thickness was 9.61+/-5.12 mm but endometrial volume was 3+/-1.1 ml, in cases with benign endometrium they were 4.87+/-3.43 mm and 1.52±0.82 ml, respectively. While in the control group, endometrial volume was 1.15+/-0.14 ml Endometrial volume with cutoff value of 1.35 ml was found to be with higher sensitivity than endometrial thickness in prediction of malignancy (Mansour GM et al., 2007).

Ebrashy et al., 2004; showed that 3Dtransvaginal sonography has higher accuracy than 2dtransvaginal sonography in detection of endometrial pathology.While other studies assessing the value of ultrasound and hysteroscopy in diagnosis of intrauterine lesions showed there is higher sensitivity of combined 2D US and hysteroscopy than 2D US alone in diagnosis of perimenopausal bleeding. (Pasqualotto EB, et al., 2000, Bonnamy et al. 2002, Dery et al. in 2007).

Conclusion

Our study confirms that endometrial volume is a better diagnostic tool than endometrial thickness in foretelling carcinoma of the endometrium and hyperplasia in women with perimenopausal bleeding.

Recommendations:

- Endometrial volume is more sensitive than endometrial thickness for predicting malignancy in perimenopausal women
- The commonest pathology of the endometrium in women with perimenopausal bleeding is endometrial hyperplasia.
- Endometrial volume of 13.105 cm3 or greater may predict malignancy in women with perimenopausal bleeding.This has a sensitivity of 92.3% , a specificity of 73.4%, Positive predictive value 36.4% and negative predictive value 98.3%,
- Endometrial thickness cut-off value of benign rather malignant lesion in perimenopuasal women is >14.5mm,This has a sensitivity of 76.9%, a specificity of 89.9%, +ve predictive value 55.6% and -ve predictive value 96%.

Limitation of the study

This study has some limitations. First, it may be, untilthe date submitting this work, the first observational study to assess the effect of 2D endometrial volume in foretelling of the endometrial pathology in the Mansoura university hospitals. Second, recruitment of a larger group of patients, multicentric studies may show different relations with different pathologies

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Paracervical Block for Oocyte Retrieval: Experience at a Public Health Facility in Nigeria

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Abstract

Background: Transvaginal Ultrasound Guided Oocyte Retrieval (TUGOR) for in vitro fertilization is one of the most common minor surgical procedures. Despite this, it is stressful and painful for the patient and thus requires some form of analgesia with or without sedation. The effects of various anesthetic techniques used for TUGOR on reproductive outcomes remain controversial.

Aims: This study assessed patients' perception of pain using paracervical block and its effect on IVF outcomes.

Methods: A cross sectional study of 66 eligible patients that underwent assisted reproduction program in our facility. All clients were treated with antagonist protocol for Controlled Ovarian Hyperstimulation. Self- administered questionnaires was used as the research instrument. Pain was assessed using a 10cm Visual Analogue Scale while clients' overall satisfaction was rated using Likert scoring system.

Results: Client's aged 32.8 ±3.4. More than half had primary infertility with mean duration of 4.6 ± 2.4. Female factor infertility was the commonest cause of infertility. The pregnancy rate per embryo transfer was 36.4%, miscarriage rate was 9.1%, while the live rate was 27.3%. The mean VAS scores at 1hour, 6 hours, 24 hours and at embryo transfer were 7.1 ± 2.8, 4.6 ±1.4, 2.8 ±1.2 and 1.0 ± 0.9 respectively. The mean Likert score was 2.4 ± 0.9.

Conclusion: Paracervical block is a safe and effective anaesthesia/ analgesia option for TUGOR.

However a multimodal approach of analgesia/anaesthesia for TUGOR is recommended to further improve on clients' satisfaction and acceptance.

Keywords: In vitro fertilization, oocyte retrieval paracervical block,anaethesia, Nigeria,.

INTRODUCTION

In vitro fertilization (IVF) requires the harvesting of mature oocytes from the ovaries of infertile patients which are subsequently fertilized in vitro and allowed to develop into embryos that are finally transferred into the uterus of these patients. [1], [2], [3]

Oocyte retrieval is reported to be the most painful step of the IVF procedure, and various methods of analgesia are in use.Pain during oocyte retrieval is caused by the puncture of the vaginal skin and ovarian capsule by the aspirating needle as well as manipulation

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within theovary during the entire procedure. Here it becomes customary for the anaesthetist to provide adequate pain relief to immobilise the patient and eliminate the danger of piercing any vessel during the process of oocyte retrieval. [4], [5]

A good analgesic method for oocyte retrieval has to give satisfactory pain relief with rapid onset, rapid recovery and ease of administration and monitoring. It is also important that it is safe and has no toxic effects on the oocytes. [6] We therefore present our experience with paracervical block for TUGOR on clients' pain perceptions and IVF outcomes

PATIENTS AND METHODS

This is a cross-sectional study of 66 patients that underwent IVF-ET at the Assisted Reproductive Technology (ART) unit of the University of Ilorin teaching hospital (UITH), Ilorin between 1st January, 2013 and 31st December, 2017. Client's folder was assessed for information such as demography, cause of infertility, duration of infertility, type of stimulation protocols, duration of FSH used/ dosage and endometrial thickness . Paracervical block was used as anaesthesia for all 66 patients based on clients' and or clinicians' preference. Informed consent was obtained from the clients and protection of personal data and confidentiality were priortized. Inclusion criteria were normo-responders (age less than 40 years), clients' with normogonadotrophic normogonadism, first attempt at TUGOR and those consented to participate in the study while the exclusion criteria included clients' who are allergic to anaesthetic agents, with cardiopulmonary compromise, thyroid dysfunction, whose TUGOR exceeded more than 30 minutes and those required other forms of analgesia for pain relief during TUGOR.

Seminal Fluid Analysis was conducted for male partners. The criteria for men were a sperm count of at least 20 million cells per milliliter of semen and progressive sperm motility of 50% or greater. Male partners with semen count and/or motility less than the cut-off values were offered Intracytoplasmic Sperm Injection (ICSI) unless the sperm count was zero after centrifugation; therefore donor sperm was used for in vitro fertilization.

All clients had a Body Mass Index (BMI) (calculated as weight in kilograms divided by the square of height in meters) ranging between 18 and 30 with a mean of 24 ± 4 Kg/m2. All had antagonist protocol for Controlled Ovarian Hyperstimulation. Their infertility evaluation results were normal. Also, all had oral contraceptive pills (OCP) for menstrual cycle synchronization and pre-cervical assessment (trial/dummy transfer) on day 2/3 of menses prior to commencement of stimulation.

Stimulation Protocol

Clients was commenced on 150IU (2vials) of recombinant FSH Gonal F (Gonal F(R); Merckserono, Germany) and 75 IU (1 vial) highly purified FSH (Folliculin®; Barrat pharmaceutical, India) on day 3 of menstrual cycle for 11-14 days. Transvaginal ultrasonographic scan was also done at interval from day 5/6 of stimulation to determine the numbers, size of follicles and endometrial thickness. Subcutaneous 2.5mg daily GnRH antagonist (Cetrotide ®; merckserono, Germany) was administered whenever the follicles have grown to 14mm size usually around day 6/7 of stimulation and was continued till the day of trigger to prevent premature LH surge. Eight three micrograms (83µg [2000IU]) of recombinant Human Chorionic gonadotrophin (hCG: Ovitrelle; merckserono, Germany) and 0.25mg of buserelin (Supricure ®; Aventis Pharm, West Malling, UK) were administered subcutaneously for trigger whenever 2 or more follicles have grown to 18mm or more and oocyte retrieval was carried out at 35.5hours thereafter.

Anaesthesia/analgesia for Oocyte retrieval

All clients were counseled to fast overnight and 1mg of Atropine was administered intravenously as pre-anaesthetic medication. Paracervical block (PCB) was carried out with the aid of a specially designed needle of 0.9 mm diameter and 120 mm length (Mediplast AB, Malmö, Sweden) and a total of 100mg (10 ml of 1% lidocaine, Xylocain™ 10 mg/ml, AstraZeneca Sverige AB) was injected into lateral fornices between three and four o'clock and eight and nine o'clock position [2.5ml (25mg of 1% lidnocaine) at each position] after which TUGOR was commenced within 5 minutes of administration.

Oocyte retrieval, insemination,embryo transfer and luteal phase

Oocyte retrieval was done at 35.5 hours of hCG injection by TUGOR with the aid of 17G needle (Origio®, Denmark) and the aspirate in a test tube was transferred immediately to the laboratory for oocyte screening and pickup. Mature oocytes were inseminated with prepared sperm after six (6) hours of oocyte pickup and incubated. ICSI was done in cases of severe male factor infertility. Best cleavage embryos were transferred on day 5 of oocyte retrieval usually at the blastocyst stage under trans-abdominal ultrasound guidance and the transfer catheters were checked to ensure all the embryos were transferred. The number of embryo transferred was individualized, 2 or 3 in most cases. The luteal phase support was conducted with progesterone (800mg twice daily [cyclogest pessaries®; Cox, Brarnstaple, UK] and Intramuscular 100mg twice weekly [Gestone ®;Ferring, pharmaceutical, Mumbai, India]). Serum pregnancy test was carried out two weeks after embryo transfer and subsequently transvaginal ultrasound at 6th week for detection of gestational sac and /or viability of the fetus.

Visual Analog Scale (VAS) and Likert score

Following TUGOR, clients had self administered questionnaire administered to assess their perception of pain using VAS scoring system [6] on a scale of (0-10cm) at 1hr, 6hrs, and 24hrs and on the day of embryo transfer respectively and their responses were properly documented. VAS scoring was graded as 0- no pain, 1-3 – mild pain, 4-6 – moderate pain and 7-10 – severe pain. Also overall clients' satisfaction were assessed through Likert scoring system [7] on a scale of (1-5) categorized as 1- poor, 2- fair, 3- satisfactory, 4- very good and 5- Excellent.

Statistical analysis

Statistical analysis was done using Microsoft excel version 2007 and Epi-info version 7.1.3.0 (Centres for Disease Control and Prevention-CDC, Atlanta, USA), Categorical data were expressed as numbers and percentages while numerical data were expressed as mean and standard deviation. Associations of categorical variables were tested using Chi square test, while statistical significance was set at $p \leq 0.05$. Results were presented in tables.

Results

The characteristics of the women who underwent IVF-ET cycles and their spouses are shown in Table 1. The mean age of the women and their spouse were 32.8 ± 3.4 and 38.7 ± 4.4 respectively. The mean duration of infertility was 4.6 ± 2.4, most of the couple presented with primary infertility (53%), while female factor infertility was the predominant cause of infertility (40.9%).

The mean duration of FSH, Mean FSH ampoule used and endometrial thickness were 14.3 ± 1.9, 32.7 ± 4.8 and 8.8 ± 2.1 respectively. The mean number of oocyte retrieved and mean number of oocyte fertilized were 10.0 ± 4.3 and 6.1 ± 3.2 respectively. (Table 2)

The clinical pregnancy rate per embryo transferred was 36.4%, miscarriage rate was 25%, while live birth rate was 27.3%. (Table 3).

Table 4 shows patient perception of pain and satisfaction. The mean VAS score at 1hour, 6hours, 24 hours and at embryo transfer were 7.1 ± 2.8, 4.6 ± 1.4, 2.8 ± 1.2 and 1.0 ± 0.9 respectively. The mean Likert score was 2.4 ± 0.9.

Discussion

In this study, the mean age of the clients and the mean number of oocytes retrieved were 32.8 ± 3.4 and 10.0 ± 4.3. This is comparable with the mean age of 33.7 ± 4.9 and means number of oocytes retrieved of 13.31 ± 9.04 obtained in a similar study in Nigeria. [3] Also, female factor infertility was the commonest cause of infertility in our series. On the contrary combined male and female factors predominate in an earlier study at Tamil, India. [7] This could be attributed to dissimilar geographic locations of both studies.

The clinical pregnancy rate per embryo transfer of 36.4% is comparable to 29.6% documented in a previous study. [8]However, there exits controversies regarding the effects of anaesthetic agents administered during TUGOR on fertilization, embryonic development and conception rate. [9-11]. A possible risk associated with PCB is the potential toxicity of absorbed lidocaine. [12], [13] Follicular fluid lidocaine concentrations as low as 1.0 µg/ml were associated with toxic effects on fertilization and embryo development in a mouse model. [13]

On the contrary there is no evidence of adverse events associated with lidocaine PCB usage in human. [13] Mean follicular fluid lidocaine concentration was 0.36 ± 1.1µg/ml after PCB with 50 mg of lidocaine. [12] Undoubtedly, exposure to high concentrations of different local anesthetic agents adversely affects fertilization and embryonic development. [14] However, given that much lower concentrations of lignocaine (100mg) was administered in this study and that oocytes were washed after retrieval, the clinical effects of using local anesthetics should be limited and probably no adverse effects should occur.[7], [12]

These could be responsible for the `clinical pregnancy rate of 36.4% reported in our series. However, no single method of anaesthesia/analgesia for TUGOR appeared superior for pregnancy rates and pain relief as observed in a randomized controlled trial. [15] Thus the need for a multimodal approach to anaesthesia /analgesia for oocyte retrieval.

The mean VAS score at 1hour, 6hours, 24 hours and at embryo transfer of 7.1 ± 2.8, 4.6 ± 1.4, 2.8 ± 1.2 and 1.0 ± 0.9 obtained in our study is higher than 2.83 ± 1.67, 0.78 ± 1.04, 0.39 ± 1.09, and 0.14 ± 0.58 reported in a similar study in Tamil, India that used conscious sedation for anaesthesia/analgesia during TUGOR. [16]This is in keeping with findings from a previous study that shows that patients who received only paracervical block during oocyte collection experienced 2.5 times higher levels of vaginal and abdominal pain than those who received conscious sedation and or paracervical block. [12] However paracervical block for pain relief during oocyte aspiration compared with placebo was associated with lower pain scores for oocyte retrieval process. [17]

The overall clients' satisfaction was assessed with Likert scoring system. The mean Likert score of (2.4 ± 0.9) ranging between fair and satisfactory obtained in this study is lower compared with mean Likert score of 3.65 ± 0.82 reported in a previous study in Tamil Nadu, India where conscious sedation (Pethidine and Midazolam combination) was employed for anaesthesia/analgesia during TUGOR with high client satisfaction and acceptance. [7] This underscores the need to compliment paracervical block with other forms of anaesthesia/analgesia to improve on its effectiveness and acceptance for pain relief during TUGOR.

Conclusion:

This study showed that paracervical block has no detrimental effects on fertilization and embryonic development although its effectiveness and acceptance for pain relief during TUGOR is restricted. Hence the need for patient selection for paracervical block during TUGOR. We recommend a multimodal approach to anaethesia/analgesia during TUGOR to further enhance clients' satisfaction. Also, randomized prospective studies with larger sample sizes are advocated to validate our findings.

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Table 1: Sociodemographic characteristics

Variables	Frequency	Percentage
Age(years)		
25-29	12	18.2
30-34	35	53.0
35-39	15	22.7
40-44	4	6.1
Mean= 32.8 ± 3.4	**range= 27-40**	
Age (spouse)		
30-34	11	16.7
35-39	29	44.0
40-44	17	25.8
45-49	7	10.6
50-54	2	3.0
Mean= 38.7 ± 4.4	**range=31-50**	
Parity		
P0	34	51.5
P1	15	22.7
P2	6	9.1
P3	8	12.1
P4	3	4.5
Duration of infertility		
1-5	46	69.7
6-10	18	27.3
11-15	2	3.0
Type of infertility		
Primary	35	53.0
Secondary	31	47.0
Cause of infertility		
Male Factor	14	21.2
Female Factor	27	40.9
Male/Female Factor	16	24.2
Unexplained	9	13.6

Table 2: Stimulation cycle characteristics of the clients

Variables	Mean
Duration of FSH	14.3 ±1.9
FSH ampoule used	32.7 ± 4.8
Endometrial thickness	8.8 ± 2.1
No. of oocytes retrieved	10.0 ±4.3
No. of oocyte fertilized	6.1 ±3.2

Table 3: Clinical outcome

Outcome	Frequency n=66	Percentage
Clinical Pregnancy per ET	24	36.4
Miscarriage rate	6	9.1
Live birth rate	18	27.3

Table 4: clients' perception of pain and satisfaction

VAS score	Mean
VAS at 1hour	7.1 ± 2.8
VAS at 6 hour	4.6 ± 1.4
VAS at 24 hour	2.8 ± 1.2
VAS at ET	1.0 ± 0.9
Likert	2.4 ±0.9

Duration of stimulation in patients with polycystic ovarian syndrome undergoing ICSI: Does it affect the outcome?

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Abstract

Back ground: Oocyte and embryo quality are affected by the duration of ovarian stimulation in different categories of patients undergoing ICSI specially for those having polycystic ovarian syndrome as they undergo ovarian stimulation usinglow dose step up antagonist protocol.

Methods: Retrospective analysis of 139 patient underwent ovarian stimulatiaon using antagonist protocol and freeze all policy

Results: Patients were divided into 3 groups according to the duration of ovarian stimulation (group A ≤ 8 days, group B 9-10 days, group C ≥ 11 days). We found that in spite of having no statistically significant difference in the total number of retrieved oocytes, there was statistically significant difference in the median number of germinal vesicle oocytes between group A and group C (3 (1-13) & 1(0-5) P value= 0.01). Also this difference is present between group B and group C (3 (1-11) & 1(0-5) P value= 0.01). For metaphase II oocytes there was a statistically significant difference in the median number between group A and group B (3 (0-8) & 5 (1-14) P value= 0.001). Also this difference is present between group A and group C (3 (0-8) & 3 (3-12) P value= 0.001).There was no statistically significant difference in the total number of embryos in between the 3 groups.

Conclusion: We found that it seems to be safe to extend the duration of stimulation more than 8 days when treating patients with polycystic ovarian syndrome undergoing IVF/ICSI, as it was associated with more increase in mature oocytes (MII)

Key words: polycystic ovarian syndrome, antagonist protocol, freeze all, ovarian hyperstimualtion syndrome, intracytoplasmic sperm injection

Introduction

Polycystic ovary syndrome (PCOS) affects 8-13% of subfertile females in the reproductive-age (1). A gonadotrophin releasing hormone (GnRH) antagonist protocol is recommendedfor women having PCOS and will undergo an in-vitro fertilization or intracytoplasmic sperm injection (IVF / ICSI) cycle. As it is known to reduce the duration of stimulation, total gonadotrophin dose and incidence of ovarian hyperstimulation syndrome (OHSS) (2). Human chorionic gonadotropin (HCG) administration for final oocyte maturation is the standard trigger of ovulation in controlled ovarian hyperstimulation (COH). It is administered when at least1-2 leading follicles reach ≥18 mm or 3 folliclesreach ≥17 mm in diameter. However, GnRH agonist is recommended to be used for final oocyte maturation in cases predicted to be at high risk of developing OHSS as PCOS patients.

The number of dominant follicles areconsidered as important determinants for HCG administration. Obviously, it is not suitable for all patients with different ovarian reserve specially in PCOS with a high ovarian reserve (3).It is also known that the number of mature oocytes and good quality embryos are related to the proportion of mature follicle on the day of humanchorionic gonadotropin trigger(4).

Consequently, the quality of retrieved oocytes and the fertilized embryos are correlated with pregnancy outcomes (5). Complete maturation of the oocyte determines oocyte quality. Complete maturation of oocytes includes both nuclear maturation and cytoplasmic maturation. For oocyte nuclear maturation, resumption and progression of meiosis to MII cannot be used as the only determinant of an oocyte's developmental competence (6). Formation of good quality embryos and occurrence of pregnancyshould be also considered after excluding other factors which are involved in the process of implantation. Pregnancies were reported after 7–12 days of controlled stimulation of ovarian cycles (7). We aimed to determine whether the duration of ovarian hyperstimulation affects the quality of embryos on post-retrieval day 3.

Material and methods

We conducted a hospital based retrospective study to evaluate the effect of duration of ovarian stimulation on embryo quality in patients diagnosed to have polycystic ovarian syndrome underwenttrial of ICSI cycles using the GnRH antagonist protocol with the use of GnRHagonist as a trigger of ovulation and freeze all policy as a preventive methods of OHSS . Participants in this study were group of sub fertile females attended to Mansoura university hospital fertility care unit and werediagnosed to have polycystic ovarian syndrome according to Rotterdam criteria (8). We investigated patients' hospital files in the past five years (January 2014 till January 2019).

Inclusion criteria

a) Patient age > 20 years and < 40 years

b) Patients diagnosed to have polycystic ovarian

syndrome according to Rotterdam criteria(8)

c) Patients underwent IVF or ICSI due to one or more of the following indications:

a. Clomiphene citrate resistant patients

b. Unilateral or bilateral Fallopian tube obstruction, salpingectomy or tubal ligation

c. Semen analysis showed: oligoasthenozoospermia or investigation results showed obstructive azoospermia

Exclusion criteria

a) Patients known to have chromosomal anomalies

b) Male factor subfertility due to having non-obstructive azoospermia

c) Patient with body mass index › 30 kg/m^2

d) Patients underwent ovarian stimulation using the long agonist protocol

e) Patients underwent final maturation of oocytes using the HCG trigger

f) Patients poor ovarian reserve according to Bolognia criteria(9)

After revising patients' files all base line demographic data were collected. We selected patients underwent ovarian stimulation according to the following protocol; Pre cycle treatment with oral contraceptive pills (as a method of follicular synchronization) then a wash out period of 5 days then started HMG ovarian stimulation in the second day of menstruation according to BMI and the ovarian response (starting dose was 75-150 IU) for 5 days. GnRH antagonist protocol was used in a fixed manner was given in the 6th day of HMG stimulation(Cetrorelix 0.25 mg subcutaneous injection). HMG administration was continued in a step up manner. Triggering of ovulation was done when at least 3 dominant follicles of ≥ 17 mm diameter were reached. We selected only patients underwent final maturation of oocytes using GnRH agonist and freeze all policy as a preventive method of OHSS.Oocyte retrieval was performed 34-36 hours after triggering of ovulation. Oocyte quality was assessed by embryologist and classified into (atretic - germinal vesicle (GV) – MI –MII -post mature)(10).Intracytoplasmic sperm injection (ICSI) was done for all mature oocytes. Embryo quality was assessed morphologically at the 3rd day and it was classified into (grade A- grade B –grade C- discarded embryos) according to the degree of asymmetry and fragmentation(11).The data wereanalysed after classifying patients into 3 groups according to the duration of ovarian stimulation. Group A consisted of patientswho spent 8 days or less duringovarian stimulation, Group B consisted of patients who spent 9 or10 days duringovarian stimulation and Group C patients who spent 11 days or more duringovarian stimulation.

Outcome measurements

Primary outcome

The primary outcome of this study is the number of grad A embryos in the 3 groups at the 3rd day after oocyte retrieval.

Secondary outcome

The secondary outcomes of this study will be the degree and severity of ovarian hyperstimulation syndrome(OHSS), the rate of mature and immature oocytes in relation to duration of stimulation and the number of grad B and grade C embryos in the 3 groups at the 3rd day after oocyte retrieval.

Statistical analysis

Statistical analysis and data interpretation:

Data were fed to the computer and analysed using IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Qualitative data were described as number and percent. Testing of normality was done using Kolmogrov-Smirnov test. Quantitative data were described as median (minimum and maximum) for non-parametric data and mean, standard deviation for parametric data. Significance of the obtained results was judged at the (0.05) level.

Data analysis

Qualitative data:

- Chi-Square test was used for comparison of 2 or more groups
- Monte Carlo test was used as correction for Chi-Square test when more than 25% of cells have count less than 5 in tables (>2*2).

Quantitative data between groups:

Parametric tests:

- Student t-test was used to compare 2 independent groups
- One Way ANOVA test was used to compare more than 2 independent groups with Post Hoc Tukey test to detect pair-wise comparison Non Parametric tests:
- Mann-Whitney U test was used to compare 2 independent groups
- KruskalWallis test was used to compare more than 2 independent groups with Mann Whitney U test to detect pair-wise comparison

Spearman's correlation:

The Spearman's rank-order correlation was used to determine the strength and direction of a linear relationship between two non-normally distributed continuous variables and / or ordinal variables.

Results

The mean ages of patients in the 3 groups were (30,31&28 years) respectively. Also the mean body mass indices were (28.1,27.2&27.9 kg/m2) respectively. There were no statistically significant differences between the 3 groups in the base line characteristics and demographic data (table1). The starting doses of FSH which wereadjusted according to BMI showed no statistically significant differences between the three groups (146.6,135.5,136.3 IU respectively).However,there werestatistically significant differences in the sum of total doses of HMG used during ovarian stimulation (table 1). As regard the endometrial response to ovarian stimulation there were no statistically significant differences in the means of endometrial thickness at time of GnRH agonist trigger administration or the grade of endometrial pattern in the 3 groups (table 1). We found also that there were no statistically significant differencesin the total number of retrieved oocytes nor the serum estradiol level at time of trigger administration between the 3 groups. Also there were no statistically significant differences in the markers of ovarian hyperstimulation syndrome (OHSS) (serum E2 level≥3500pg/dl & retrieval of ≥15 oocytes) between the 3 groups (table 1).On the other hand, when we compered the quality of post retrieval oocytes, we found that there werestatistically significant differences in the median number of germinal vesicle oocytes between group A and group C (3 (1-13) & 1(0-5) P value= 0.01). Also this difference is present between group B and group C (3 (1-11) & 1(0-5) P value= 0.01). There was a statistically significant difference in the median number of metaphase I oocytes between group A and group B (5 (2-11) & 4 (0-12) P value= 0.01). Also this difference was present between group A and group C (5 (2-11) & 3 (0-12) P value= 0.01). For median number of metaphase II oocytes, there was a statistically significant difference between group A and group B (3 (0-8) & 5 (1-14) P value= 0.001) (table 2). However, there was no statistically significant difference in the median number of atretic follicles between the 3 groups. For postmature oocytes, we found that there was a statistically significant difference in the median number between group A and group B also this difference is present between group A and group C (table 2). From the previously mentioned date we can conclude that the number of MI oocytes is likely to be higher when the duration of ovarian stimulation was 8 days or less. However, the number of MII oocytes was higher when duration of stimulation was between 9-11 days (table 2).

There were no statistically significant differences in the total number of embryos between the 3 groups. After comparing the good quality embryos between the 3 groups we found that there was statistically significant difference in the number of grade A embryo between group A and group B (26/262 (9.9%) & 168/683 (24.6%) P value= 0.04) and the same difference was present between group A and group C (26/262 (9.9%) & 42 /96 (43.75%) P value= 0.006). Also there were nostatistically significant differences in the number of grade B embryos between the 3 groups.However, there was a statistically significant difference between group A and group B if we compared the number of grade C embryos (123/262 (46.9%) & 167/683 (24.5%) P value= 0.009). After comparing the sum of grade A and grade B embryos in the 3 groups, we found that there was a statistically significant difference between group A and group B only (133/262 (50.8%) & 496/683 (72.6%) Pvalue =0.013) (table 3). We found that there was a positive correlation between number of grade A embryo(figure 1) or number of grade A plus grade B (figure 2)embryos and the duration of ovarian stimulation when not exceeding 11 days. Also we found that there was a negative correlation between the number of immature oocytes, number of postmature oocytes and the duration of stimulation when it was less than 8 days (table 4). There was no relation between the marker of OHSS in this study (serum estradiol level and number of retrieved oocytes) and the duration of ovarian stimulation (table 5).

Discussion

Data from our study suggest that the quality of retrieved oocytes in patients diagnosed to have polycystic ovarian syndrome underwent ICSI using antagonist protocol wasrelated to the duration of ovarian stimulation i.e: when the duration was 8 days or less the chances of having immature (GV, atretic) oocytes were increased. On the other hand, the embryo quality at the 3rd day after oocyte retrieval has positive correlation with duration of stimulation i.e. the chance of having grade C embryos is going to increase when the duration of ovarian stimulation is less than 8 days.The relation between type of stimulation protocol , quality of embryos and proportion

of dominant follicles(PDF) (the number of ≥18/≥10 mm follicles) at time of HCG or GnRH agonist trigger were studied(12) andit was found that there was no relation between clinical pregnancy rate and the degree of PDF however, it was found that postponing HCG or GnRH agonist trigger till the degree of PDF become between 20%-40% or more than 40% was associated with more risk of OHSS inspite of having more numbers of fertilized oocytes . In our study weinvestigated the effect of duration of ovarian stimulation after using GnRH antagonist protocol with administration of GnRH agonist as a trigger of ovulationand freeze all policy because it is considered to be the safest method of prevention of OHSS (13). The duration of stimulation while usingGnRH antagonist protocol also was studied in normal and poor responders(14)and it was found that less than 6 days of stimulation were not sufficient for oocyte maturation and extending the duration to 10-12 days of stimulation was considered to have positive correlation with MII oocytes in normal responders. But 6 days duration of ovarian stimulation may be enough duration for oocyte maturation for patients known to be poor responders(14) and this may be explained by the use of different stimulation protocols in such group of patients and staring stimulation with higher doses of HMG compared to patients having PCOS. The maturation rate of retrieved oocyte was found also to be higher with middle and high proportion when the follicles were divided according to their size (≥17 mm/≥10mm follicles ratio on the day of human chorionic gonadotropinadministration) into (Low proportion: _30%, Middle proportion: 30%–60%, High proportion: _60%)(4), these resultswere found in patients received ovarian stimulation with antagonist protocol only. However, the were no statistically significant differences in implantation rates in the three groups. Also, it was found that a duration less than 9 days of ovarian stimulation was associated with more embryos and with more than 10% fragmentation on post-retrieval day 3(5).However, this was studied in a group of patients received the long agonist protocol. In our study we analysed cases treated with oral contraceptive pills before ovarian stimulation with antagonist protocol to avoid the effect of asynchronous growth of the cohort of follicles on the maturity of oocytes as it is one of the main disadvantages during the use of antagonist protocol(15). Also nowadays with the improvement of in vitro culture for oocytes there is an increasing use of in vitro maturation IVM of oocytes specially in patients with polycystic ovarian syndrome to eliminate the risk of OHSS in this category of patients(16).For the fertilized oocytes we evaluated the embryo quality and all good quality embryos were cryopreserved at day 3 post retrieval. However, nowadays there is an increasingorientation toward keeping the embryos in vitro till day 5 after retrieval. A systematic review and meta-analysis showed comparable results in the live birth rate between embryo transfer at day 3 and day 5 after oocytes retrieval (17). As blastocyst transfer is considered as a method of natural selection of good quality embryos.in our local protocol we used cryopreservation of embryos at the cleavage stage (day 3) and if we have more than 4 grade A embryos we allow in vitro culture after thawing till day 5 and we do embryo transfer only for the best one or 2 blastocyst according to the morphological scoring (18). It was also found that there was a positive association between the number of retrieved oocytes and the number of top quality embryos either at the cleavage stage or at the blastocyst stage (19).In our study we found that most of patients had elevated markers of OHSS(E2 level≥3500pg/dl-retrieval of ≥15 oocytes) showed mild clinical manifestations and achieved rapid clinical improvement with such protocol and there were no reported ICU or hospital admission of any of them. This protocol is considered to be applicable toward the trend of decreasing or eliminating the critical effects of OHSS(20).

In conclusion we found that it seems to be safe to extend the duration of stimulation more than 8 days when treating patients with PCOS underwent IVF/ICSI using antagonist protocol and GnRH agonist trigger and freeze all policy, as it was associated with more increase in mature oocytes(MII) and good quality embryos (grade A). Limitation of the study

It is a retrospective study and used the assessment of embryo quality at day 3 of oocyte retrieval than day 5 assessment of blastocyst. We recommend conducting a prospective study using blastocyst assessment of in comparison to day 3 assessment of blastomere.

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Disclosure

All authors disclose no conflict of interest.

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Table (1) Demographic data and base line characteristic of the 3 groups

	Group A ≤ 8 days of stimulation n=45	**Group B 9--10 days of stimulation n=83**	**Group C ≥ 11 days of stimulation n=11**	**Test of significance**
Age/years Mean±SD	30.18 ± 4.8	31.2 ± 6.03	28.9 ± 5.6	F=1.08 P=0.34
BMI (Kg/m^2) Mean±SD	28.12 ± 2.5	27.2 ± 2.9	27.9 ± 2.01	F=1.68 P=0.191
Type of infertility N (%)				
primary	33 (73.3)	59 (71.1)	10 (90.9)	MC
secondary	12 (26.7)	24 (18.9)	1 (9.1)	P=0.376
Duration of infertility /years Median(Min-Max)	7.0 (2.0-18.0)	6.0 (1.0-20.0)	6.0 (4.0-9.0)	KW P=0.749
Cycle rhythm N (%)				
Oligomenorrhea	41 (91.1)	75 (90.4)	11 (100.0)	MC
Regular	4 (8.9)	8 (9.6)	0 (0.0)	P=0.563
AMH / ng/ml	3.8 (1.0-10.6)	3.1(1.2-13.0)	4.09 (2.5-16.0)	KW P=0.076
Basal FSH / mIU/mL	5.8 (1.9-10.6)	5.2 (1.2-10.1)	4.2 (2.7-8.1)	KW P=0.165
Number of ICSI trials Median (min-max)	1.0 (0.0-6.0)	0.0 (0.0-7.0)	1.0 (0.0-5.0)	KW P=0.114
Starting dose of HMG / IU/ day mean±SD	146.6 ± 15.6	135.5 ± 29.7	136.3 ± 30.3	F=2.72 P=0.069
Endometrial thickness at time of trigger /mm mean±SD	11.80±1.97	11.81±1.66	12.91±3.11	F=1.69 P=0.187
Endometrial shape at time of trigger n (%)				
Triple	34 (75.6)	57 (68.7)	8 (72.7)	MC
Homogenous	11 (24.4)	26 (31.3)	3 (27.3)	P=0.709

BMI: body mass index, FSH:Follicle-Stimulating Hormone, AMH:anti-mullerian hormone, HMG:human menopausal gonadotropin, ICSI:intracytoplasmic sperm injection

MC: Monte Carlo test, F: One-way ANOVA test, KW: Kruskal Wallis test
*statistically significant (if $p<0.05$)
Similar superscripted letters denote significant difference between groups

Table (2) Differences in number of retrieved oocytes , oocytes quality and serum estradiol level between 3 groups

	Group A ≤ 8 days of stimulation n=45	**Group B 9--10 days of stimulation n=83**	**Group C ≥ 11 days of stimulation n=11**	**Test of significance**
Number of oocytes median (min-max)	16 (7-30)	15 (8 -32)	12 (8 -25)	KW P=0.266
≥15 N (%)	22 (48.9)	50 (60.2)	8 (72.7)	MC
<15	23 (51.1)	33 (39.8)	3 (27.3)	P=0.264
Number of GV oocytes median (min-max)	3 (1.0-13.0)[a]	3 (1.0-11.0)[b]	1 (0.0-5.0)[ab]	KW P=0.01*
Number of M1 oocytes median (min-max)	5 (2.0-11.0)[ab]	4 (0.0-12.0)[a]	3 (0.0-12.0)[b]	KW P=0.01*
Number of M2 oocytes median (min-max)	3 (0.0-8.0)[ab]	5 (1.0-14.0)[a]	5 (3.0-12.0)[b]	KW P=0.001*
Number of Atretic oocytes median (min-max)	1 (0.0-8.0)	0.0(0.0-4.0)	1 (0.0-5.0)	KW P=0.096
Number of Post mature oocytes median (min-max)	3 (1.0-8.0)[ab]	0.0(0.0-5.0)[a]	0.0(0.0-2.0)[b]	KW P<0.001*
Serum E2 level / pg/mL median (min-max)	3017 (1041-8560)	2434 (1078-8166)	1869 (1224-7558)	KW P=0.068
<3500pg/mL N (%)	28 (62.2)	58 (69.9)	8 (72.7)	MC
≥3500pg/mL	17 (37.8)	25 (30.1)	3 (27.3)	P=0.630

GV: Germinal vesicle, MI: metaphase I, MII: metaphase II, E2: estradiol
MC: Monte Carlo test, F: One-way ANOVA test, KW: Kruskal Wallis test
*statistically significant (if p<0.05)
Similar superscripted letters denote significant difference between groups

Table (3) difference in the quality of embryos between the 3 groups

	Group A ≤ 8 days of stimulation n=45	**Group B 9--10 days of stimulation n=83**	**Group C ≥ 11 days of stimulation n=11**	**Test of significance**
Number of embryos median (min-max)	5 (2.0-13.0)	7 (2.0-17.0)	6 (2.0-17.0)	KW P=0.248
Number of Grade A embryos (Number/total number)	26/262 (9.9%)	168/683 (24.6%)	42 /96 (43.75%)	p1=0.04* p2=0.006* p3=0.17
Number of Grade B embryos (Number/total number)	107/262 (40.8%)	328/683 (48.0%)	27/96 (28.13%)	p1=0.435 p2=0.441 p3=0.21
Number of Grade C embryos (Number/total number)	123/262 (46.9%)	167/683 (24.5%)	27/96 (28.13%)	p1=0.009* p2=0.258 p3=0.795
Number of Grade A + B embryos (Number/total number)	133/262 (50.8%)	496/683 (72.6%)	69/96 (71.88%)	p1=0.013* p2=0.207 p3=0.96

MC: Monte Carlo test KW: Kruskal Wallis test

*statistically significant (if p<0.05)
P1 denotes difference between group A and group B
P2 denotes difference between group A and group C
P3 denotes difference between group B and group C
Similar superscripted letters denote significant difference between groups

Table (4) Correlation between oocytes quality and duration of ovarian stimulation

Quality of oocytes		Duration of ovarian stimulation
Mature oocytes (MII,MI)	r	.037
	p	.664
Immature oocytes	r	-.287**
	p	.001
Post matureoocytes	r	-.751**
	p	<0.001

MI: metaphase I MII: metaphase II
r: Spearman correlation co-efficient *statistically significant (if p<0.05)

Table (5) Correlation between markers of ovarian hyper stimulation syndrome (Serum estradiol (E2) level and-number of retrieved oocytes) and duration of ovarian stimulation

		Duration of ovarian stimulation
Serum estradiol (E2) level	r	-.148
	p	.083
Number of retrieved oocytes	r	-.093
	p	.277

r: Spearman correlation co-efficient *statistically significant (if p<0.05)

Figure (1): The correlation between grade A embryos and duration of stimulation

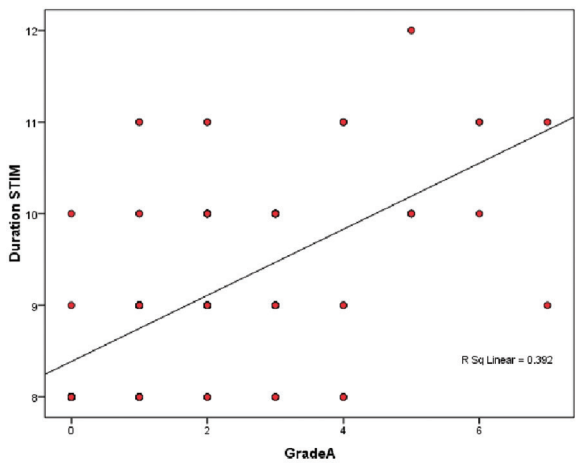
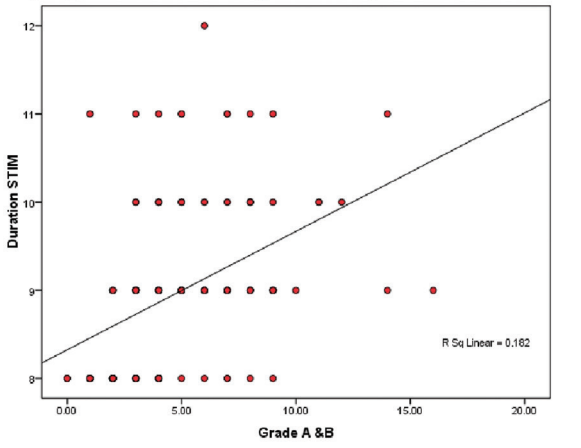


Figure (2): The correlation between grade A+B embryos and duration of stimulation



Legends of figures

Figure 1:There is a positive correlation between formation of grade A embryos at day 3 after oocyte retrieval and the duration of ovarian stimulation in patients having PCOS undergoing IVF/ICSI using GnRH antagonist protocol and GnRH agonist trigger for final oocyte maturation
Figure 2:There is a positive correlation between formation of grade A + grade B embryos at day 3 after oocyte retrieval and the duration of ovarian stimulation in patients having PCOS undergoing IVF/ICSI using GnRH antagonist protocol and GnRH agonist trigger for final oocyte maturation

Neonatal and Maternal Outcome after Conservative Management of Preterm Premature Rupture of Membranes (PPROM) between 24-28 Weeks Gestation

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Abstract

Background: The Incidence of preterm pre mature rupture of membranes ranges from 3.0-10.0% of all deliveries and leads to one third of preterm births. There are several risk factors for PPROM, such as intrauterine infection at early gestational age, sexually transmitted infections, vaginal bleeding, and smoking during pregnancy.

Methods: An observational prospective study included 100 pregnant females with PPROM between the 24th and 28th week's gestation that were admitted to Mansoura University Hospital (MUH) between April 2018 and December 2019. Pregnancies with known fetal malformations, multiple fetuses, stillbirths, placenta previa, pre-eclampsia & Eclampsia and diabetes mellitus were excluded from this study group. Women who deliver within 24 hours of PPROM were also excluded.

Results: Out of 100 pregnant females were eligible for the study only 88 patients continued the study. Data from our study showed that the median gestational age at delivery after conservative management of patient diagnosed to have PPROM was 33 weeks. 85.2% of patients delivered by cesarean section. Recurrent urinary tract infection and Vulvo-vaginitis are the most common associated risk factors (28 %, 24 %) respectively.The percentage of live born neonates was (65.9%) while still birth occurred in (15.9%) and miscarriage occurred in (18.2%) of the females who completed the study. The main neonatal complications reported in the study was 55 out of 58 live birth cases were admitted to NICU (62.5%). Twenty-nine cases developed respiratory distress syndrome (32.9 %), 17 cases developed bronchopulmonary dysplasia (19.3%), 6 cases developed pulmonary hypoplasia (6.8%),47 casesdeveloped neonatal sepsis (53.4%) and 34 cases developed perinatal death after admission to NICU (38.6%). Fourteen neonates were discharged with different degrees of disability (15.9%). The overall survived neonates were 24 cases (27.3%). For maternal complications we noticed that 43 cases developed signs of chorioamnionitis (48.9%). Also cord prolapse was reported in 3 cases (3.4%). After delivery postpartum hemorrhage occurred in 14 cases (15.9%) and 10 cases (11.4%) were in need of blood transfusion. Signs of maternal sepsis developed only in 3 cases (3.4%).

Conclusion: Data from our study showed that after conservative management of patients with PPROM the overall neonatal survival rate was 27.3 %. 3.4% of neonates were discharged without need of NICU, 7.95% of neonates were healthy after being discharged form NICU and 15.9 % of neonates were discharged with different degrees of disability. Most of material complication were not fatal and showed improvement after giving the appropriate care.

Keywords: Preterm labor, neonatal outcome, chorioamnionitis, prematurity, maternal sepsis.

INTRODUCTION

Preterm premature rupture of membrane (PPROM) is defined as rupture of foetal membrane before onset of labour at less than 37 weeks of gestation. It was reported that 5 in 1000 women are at risk of developing PPROM (1). Unfortunately, when this happens it will be associated with poor neonatal outcome due to preterm delivery and due to a certain degree of pulmonary hypoplasia as a result of the reduction of amniotic fluid at a very early gestational age.

It is also known that it leads to about one third of preterm deliveries. many risk factors were associated with this condition as multiparity, low socioeconomic standards, smoking, malnutrition, and recurrent vulvo-vaginal infections (2). The mechanism by which PROM happens refers to release of collagenase and phospholipases enzymes that will lead to erosions in the amniotic membranes and leakage of amniotic fluid. These groups of enzymes could be execrated from microorganisms like bacteria or due to malnutrition of bad habits like active or passive smoking. (3).

Many maternal and neonatal complication could happen after PPROM and it differs according to the gestational age of the onset of ROM, the degree of oligohydramnios, the associated maternal comorbidities and the latency period till the onset of labour or delivery (4). The most common maternal complications are the risk of antenatal or postpartum infections. Multiple foetal and neonatal complications also could happen as prematurity, very low birth weight, respiratory distress syndrome, lung hypoplasia, intracerebral haemorrhage, patent ductus arteriosus, neurologic disorders and perineal mortality (2).

The proper management in the event of PPROM is controversial. Expectant management is feasible to decrease the risk of prematurity. Prophylactic antibiotic could help in decreasing the incidence of chorioamnionitis, also administration of corticosteroids could stimulate type II pneumocytes to excrete surfactant to enhance lung maturity and a short course of tocolytic drugs to achieves such target. On the other hand, extending the latency period "duration between the start of rupture of membranes and the onset of labour "may increase the risk of uterine infections, consequently this will lead to harmful neonatal complications. (5). The plan of management of such condition differs from on country to another according to the facility of neonatal care and it should be conducted in a tertiary health care centre to maximize the benefit of this plan. (6). It was generally recommended that if premature rupture of membranes (PROM) happened between 24–31 weeks' gestational age (GA) expectant management could be tried. Labour induction is suggested if lung maturation is confirmed at 32–33 weeks' gestational age (GA). After 33 weeks' GA, it is generally recommended to proceed to delivery (usually by induction) because of the decreased likelihood of respiratory complications (7). We conducted this study to evaluate the efficacy of conservative management of patients with PPROM at Mansoura university hospitals (MUH) and to assess safety of the management plan.

Methods

We conducted a prospective observational cross-sectional and analytical study for a group of pregnant females attending at outpatient clinic and were admitted at Obstetrics and Gynaecology Department, Mansoura University Hospital, Mansoura, Egypt. The study was conducted over the period of 20 months in the period from April 2018 till December 2019.

Study subjects

Participant in the study were 100 pregnant females with PPROM between the 24th and 28th weeks gestation. Diagnosis of PPROM was based on the patient's history of watery vaginal discharge and leakage of amniotic fluid from the cervical os during a sterile speculum examination and decrease in the amniotic fluid index (2) by transabdominal ultrasonic examination.

Exclusion criteria:

1. Confirmed Gestational age other than our inclusion group 24 – 28 weeks' gestation.
2. All pregnancies with known foetal malformations, multiple foetuses, stillbirths, placenta previa, pre-eclampsia & Eclampsia and diabetes mellitus.
3. Intrauterine growth restriction.
4. Women who present with abruptio placenta.
5. Maternal and/or foetal indications for immediate delivery after admission.
6. Clinical signs of chorioamnionitis at presentation with PPROM.
7. Women who deliver within 24 hours of PPROM are also excluded from the study group.

Protocol of management of PPROM:

A thorough clinical evaluation was done for every patient by complete history taking, and the following data were collected demographic data (age, sex and other demographic data, general medical history, associated comorbidities, obstetric history (gravidity, parity and complications of previous pregnancies if present), and accurate estimation of gestational age. Also clinical examination was done to document any signs of chorioamnionitis. Vaginal examination was done as well by inspection and sterile speculum examination to confirm the occurrence of rupture of membranes. Then trans-abdominal ultrasonic examination was done to document the viability of pregnancy, amniotic fluid index (2) and to ensure the gestational age. The following laboratory investigations were requested including complete blood count (CBC) and serum C-reactive protein (CRP). After patients' counselling all patients were put on our local management protocol. All patients were admitted to obstetrics and gynaecology department. A single course of corticosteroids (dexamethasone) was given in two divided doses (24 mg divided into two over 24 hours), simultaneously antibiotic therapy was started (Intravenous ampicillin 1 gm every 12 hours for 2 days, followed oral amoxicillin or erythromycin for another 5 days). Urine samples were taken upon admission for culture and sensitivity testing. Monitoring of patients' vital signs was done on daily basis, also estimation of any abdominal tenderness, recording of foetal movement count and foetal heart rate. Ultrasound

and laboratory assessment were done twice weekly.If patients developed regular and frequent uterine contractions (≥ 3 contractions every 10 min), then vaginal examination was done under complete aseptic conditions. A short term tocolysis was prescribed for 48 hours to gain the effect of enhancing lung maturity by corticosteroids. If 2 or more of the following signs were present (Maternal body temperature ≥ 38°C, Maternal heart rate (≥ 110 beats/min), Persistent increase in foetal heart rate (FHR) (> 160 beats/min) or decrease in FHR (< 110 beats/min), evidence of significant abdominal or uterine tenderness, rising serum levels of CRP, appearance of offensive vaginal discharge, increase in white blood cell count (WBCs) (≥ 15,000 cells/mm3) the diagnosis of chorioamnionitis was confirmed. The decision to deliver is based on the clinical assessment by the supervising obstetrician. Induction of labour is indicated in case of maternal infection or intrauterine death, and for all other cases, once the pregnancy reached 32-34 weeks' gestation. After delivery all patients continued on antibiotic therapy till discharge after at least 48 hours. If any signs of puerperal sepsis or pyrexia were present, the duration of treatment was extended till clinical and laboratory improvements were documented.An outpatient clinic visits were arranged for all patients to discuss their pregnancy events and outcome and to formulate a management plan for subsequent pregnancies.

Outcome measures:

A- Maternal outcome measures included:

- Estimation of gestational age at PPROM.
- Estimation of gestational age at delivery.
- Estimation of Latency period (period between onset of PPROM till the onset of labour or delivery).
- Mode of delivery (vaginal or abdominal).
- Maternal morbidity rates (e.g. postpartum haemorrhage, vaginal or cervical tears, chorioamnionitis and endometritis).

B-Neonatal outcomes included:

- Degree of neonatal distress.
- Foetal birth weight at delivery.
- Apgar scores at the 1 and 5 minutes.
- Rate of admission to neonatal intensive care unit (NICU).
- Rate and type of neonatal infection.
- Major neonatal conditions (including patent ductus arteriosus (PDA), respiratory distress syndrome (RDS), intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC) and sepsis).
- Congenital malformations.
- Perinatal mortality rate.

Statistical analyses:

The collected data were coded, processed and analysed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA).

Data were tested for normal distribution using the Shapiro Walk test. Quantitative data were expressed as mean ± SD (Standard deviation) or median (range) according to distribution (parametric and non-parametric respectively).

Logistic regression analysis was used to analyse the occurrence of categorical outcome by other variables. Univariate regression analysis was used to test the individual variables for prediction while multivariate regression analysis was used to determine the independent predictor factors.

Results

The study included 100 pregnant females presented with PPROM between 24 and 28 weeks' gestation. As shown in table 1 the mean age of the patients was 27.47 ± 6.17 years. About (54%) of patients had moderate degree level of socioeconomic standards. The majority of the females had no special habits of medical importance, but there were 31 females who were passive smokers. More than 50% of patients were multiparous. Among all patients, there were 11 patients with previous history of PROM, 5 patients with previous history of preterm birth in 7 patients with previous history of still birth. 33 % of patients had past history of intrauterine device (IUD) usage as a method of contraception. Regarding the associated chronic diseases among the females in this study, recurrent UTI and Vulvo-vaginitis were reported in 38 females and 34 females respectively. Diabetes mellitus (DM) was present in 11% of patients, SLE was present in 2% of patients. Also the median gestational age of rupture of membrane in this study was at the 25th

week. The majority of patients did not show significant signs of chorioamnionits either at clinical or laboratory levels at the onset of ROM.

Out of 100 pregnant females were eligible for the study, only 88 females completed the routine follow up till delivery. The majority of patients gave rise to live birth neonates (65.9%) while still birth occurred in 14 females (15.9%) and miscarriage occurred in 16 females (18.2%) (table 2). The median gestational age at delivery was 33 weeks. Regarding the mode of delivery, vaginal delivery was conducted in 13 females (14.8%) while caesarean delivery (CD) was conducted in 75 females (85.2%). The average duration of latency period (duration between the onset of ROM and delivery) was about 5 weeks.

For neonatal outcomes, the mean neonatal birth weight was 1406.22 ± 419.42 gm. The median 5-min Apgar score was 5. The following neonatal complications were reported as follows; 62.5% of neonates were admitted to NICU, 32.9 % developed different degrees of respiratory distress syndrome, neonatal sepsis was present in 53.4% of neonates, only one neonate showed signs of intrauterine growth restrictions (IUGR) and the perinatal mortality rate was 38.6% (table 3).

Table 4 showed the different maternal complications occurred for patients who completed the study. Clinical chorioamnionitis was diagnosed in 43 cases (48.9%), cord prolapse was reported in 3 cases (3.4%), postpartum haemorrhage was present in 14 cases (15.9%), and maternal sepsis occurred only in 3 cases (3.4%).

After doing logistic regression analysis, elevated CRP and amniotic fluid index were identified as determinants of development of maternal and neonatal complications, however, by multivariate regression analysis, elevated CRP was detected as an independent determinant of development of complications (table 5).

Discussion

The present study is being planned to describe the course of pregnancies and evaluate neonatal and maternal outcomes with PPROM between 24 and 28 weeks' gestation that are conservatively managed in Mansoura University Hospital (MUH). The overall neonatal survival to discharge rate was 27.3% and it was improved significantly with increasing gestational age at PPROM and gestational age at birth. There were no reported cases of maternal mortality.

In this study, the mean age of the included cases was 27.47 ± 6.2. This is comparable to other studies that found that the commonest age group among PPROM patients were 20-24 years with ranges between 17 to 35 years. (8-11).

In the present study increased cases of PPROM were observed in cases of multigravidas more than primigravida which is in agree with many of the studies, multiparity is a risk factor for PROM due to long standing infection, trauma to cervix and cervical incompetence (12).

The majority of patients developed ROM the 26th week of gestation. In spite of associated many risk factors with occurrence of PPROM we found that the most common associated conditions were recurrent UTI and Vulvo-vaginal infection. Also the laboratory markers of infections did not show a significant elevation with the start of the condition. Also maternal serum CRP concentration was found to be among the most commonly used clinical non-invasive markers to predict infectious-related and inflammatory complications in women with PPROM, in spite of the absence of strong evidence for its use in relation to these indications (13).

In our study, the onset of delivery ranged between 28 and 36 weeks' gestation with the median duration of 33 weeks. And the average duration of latency period was 5 weeks. The latency period between the occurrence of PPROM and delivery is considered an important factor to improve neonatal outcomes. As the managing team succeeded to increase the duration of latency period as the preferable neonatal outcomes are expected (14). On the other hand, this should be performed with caution to prevent any unwanted serious maternal or neonatal complications. Also the neonatal survival rate was found to be about 56% when the onset of PPROM happens in later gestational ages even when the latency period was short (15). In another study, it was found that the neonatal survival rate was about 86 %, in a group of patients who had PPROM between 14 + 0 and 32 + 0 weeks of gestation (16). To achieve this prolongation of la-

tency period, it was found that antibiotics are the key player in the management protocol (17). It was recommended that penicillin is the drug of choice in this situation. Intravenous therapy could be initiated the first 2 days, followed by maintenance therapy using amoxicillin or enteric coated erythromycin for the next 5 days (17).

In our study we found that 85.2% of patients delivered by caesarean section. The increase in Caesarean section rate was largely due to the increased incidence of foetal malpresentations and failed induction in the immediate induction group.

In spite of achieving high survival rates in some studies, it was found that the neonatal morbidity could reach up to 75% in certain situations. This was found to be due to the impact of medications used for treatment and lengthy admission period in the NICU (18). In our study, the majority of the females gave rise to live birth neonates (65.9%) while still birth occurred in 14 females (15.9%) and miscarriage occurred in 16 females (18.2 %). The mean birth weight of the live birth was 1406.2 ± 419.4 gm. 62.5% of neonates were admitted to NICU and the perinatal mortality rate was 38.6%. In another study, Al-Riyami et al. (2013) reported that the live birth rate was 55%. For those neonates Apgar scores at 1 and 5 minutes were found to be 8.7 and 10, respectively. Also the mean weight of neonates was 2.2 kg. Sixty-four percent of neonates were born having very low birth weight. The majority (79%) of the live born neonates developed different degrees of respiratory distress syndrome. Neonatal sepsis occurred in 50% of the NICU admitted neonates. On the other hand, the miscarriage rate was 20% and the stillbirth rate 9%. Seven percent of the live born neonates had early neonatal death in the first day after delivery (19).

A high perinatal mortality rate was reported (67.7%) among group of pregnant females who developed PPROM between 24 and 26 weeks of gestation. They reported also lower survival rate (13%) when the onset of PPROM was below 23 weeks. It was increased up to 50% if the onset of PPROM was between 24-26 weeks (20). In another study the live birth rate was 25%, and the rate of maternal complications was 58.5% (21). Also after studying a cohort of 73 pregnant females, it was found that the main determinant of the neonatal and maternal complications is the age of onset of PPROM (22). They found poor neonatal outcomes when the age of onset of PPROM was between 16-23 weeks of gestation. This was totally different when comparing the neonatal outcome when PPROM happened after 24 weeks of gestations. The most common neonatal complications in the previous studies were respiratory distress syndrome (79.2 %) and bronchopulmonary hypoplasia (68.4%). It was found that the neonatal complication rates varies among many studies (22-24). Also these variations were reported for the live birth rates. Verma and colleagues reported a live birth rate of 18.3 % when they followed a group of pregnant females had PPROM between 18-23 weeks of gestations (25). On the other hand, Loeb and colleagues reported an overall survival rate of 6.25 % when they studied a group of pregnant females had PPROM between 20-24 weeks of gestation (26). Different survival rates were reported by Farooqi and colleagues when PPROM happened at different gestational ages (40, 92 and 100 % for those who developed PPROM at 14–19, 20–25 and 26–28 weeks respectively). (27). Newman and colleagues reported higher perinatal mortality rate (98.8 %) in a group of pregnant females developed PPROM between 23–24 weeks and it was decreased up to 36.6 % when PPROM occurred between 25–27weeks (28). The neonatal mortality rate was found to be of 82 % when patients developed PPROM before 22 weeks of gestation (29). Margato and colleagues also reported very low live birth rate when PPROM occurred before 20 weeks of gestation (30). In spite of the heterogeneity of data a recent meta-analysis concluded that most of the adverse neonatal complications that are responsible for neonatal morbidity are related to the degree of oligohydramnios that happens after PPROM (31)

However, we did not report any case if maternal mortality in our study, clinical chorioamnionitis was the most common maternal complication as it was present in (48.9%) of cases. In our study, with the logistic regression analysis, elevated CRP and amniotic fluid index were identified as determinants of development of maternal and neonatal complications, however, by multivariate regression analysis, elevated CRP was detected as an independent determinant of development of complications.

Conclusion

Data from our study showed that after conservative management of patients with PPROM the overall neonatal survival rate was 27.3 %. 3.4% of neonates were discharged without need of NICU, 7.95% of neonates were healthy after being discharged form NICU and 15.9 % of neonates were discharged with different degrees of disability. Most of material complication were not fatal and showed improvement after giving the appropriate care. Clinical chorioamnionitis was the most common maternal morbidity followed by Bleeding after PROM

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Disclosure

All authors disclose no conflict of interest.

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Table (1): Demographic data and base line characteristics and initial laboratory investigations of the patients included in the study

Variables	Whole study group (N= 100)
Age (years) [Mean ± SD]	27.47 ± 6.17
Gravidity [Median (range)]	2 (1 – 5)
Parity[Median (range)]	1 (0 – 3)
Socioeconomic level [n (%)]	
High	21 (21%)
Moderate	54 (54%)
Low	25 (25%)
Special habits [n (%)]	
No	69 (69%)
Passive Smoking	31 (31%)
Previous PROM[n (%)]	11 (11%)
Previous preterm birth[n (%)]	5 (5%)
Use of contraception[n (%)]	
No	39 (39%)
Intrauterine device (IUD)	33 (33%)
Associated chronic condition[n (%)]	11 (11%)
Diabetes mellitus (DM)	2 (2%)
Systemic lupus erythematosus (SLE)	38 (28%)
Recurrent urinary tract infection (UTI)	34 (24%)
Vulvo-vaginitis	
GA at onset of ROM [Median (range)]	26 (24 – 28)
WBCs (× 10^3) [Mean ± SD]	10.91± 4.31
CRP (mg/L)[Mean ± SD]	17.36± 3.26

SD: slandered deviation PROM: premature rupture of membranes n: Number
WBCs: white blood cells CRP:C-reactive protein GA: gestational age
ROM: rupture of membranes

Table (2): Analysis of the outcomes of pregnancy after PROM

VariablesFemales completed the study	(N=88)
Outcome of pregnancy [n (%)]	
Miscarriage	16 (18.2%)
Still birth	14 (15.9%)
Live birth	64 (72.7%)
GA at delivery (weeks)	
Median (range)33 (28-36)	
Mode of delivery [n (%)]	
Vaginal	13 (14.8%)
CS	75 (85.2%)
Latency period (weeks)	
Median (range):	6 (1-9) weeks

n: Number GA: gestational age CS: caesarean section

Table (3): Analysis of the neonatal outcome in cases of live birth

Variables	Female with live birth (n=58)
Birth weight	
Mean ± SD 1406.22 ± 419.42	
5 min ARGAR score	
Median (min-max) 5 (3 - 7)	
NICU [n (%)]	55 (62.5%)
RDS [n (%)]	29 (32.9%)
BPD [n (%)]	17 (19.3%)
Pulmonary hypoplasia[n (%)]	6 (6.8%)
Neonatal sepsis [n (%)]	47 (53.4%)
Perinatal death [n (%)]	34 (38.6%)
ROP[n (%)]	5 (5.7%)
NEC[n (%)]	3 (3.4%)
HIE [n (%)]	2 (2.3%)
Limb contractures[n (%)]	1 (1.1%)
IUGR[n (%)]	1 (1.1%)
IVH[n (%)]	1 (1.1%)
PDA[n (%)]	1 (1.1%)
Healthy neonates who didn't need NICU[n (%)]	3 (3.4%)
Healthy neonates discharged from NICU[n (%)]	7 (7.95%)
Neonates discharged with morbidity[n (%)]	14 (15.9%)
Over all survived neonates [n (%)]	24 (27.3%)

SD: slandered deviation NICU: neonatal intensive care unit n: Number
RDS: Respiratory distress syndrome NEC: necrotizing enterocolitis
HIE: hypoxic ischemic encephalopathy IUGR: intrauterine growth restriction
ROP: Retinopathy of prematurity IVH: intraventricular haemorrhage
PDA: patent ductus arteriosus

Table (4): Analysis of the maternal complications:

Variables	Females completed the study (N=88)
Clinical chorioamnionitis [n (%)]	43 (48.9%)
Bleeding after PROM [n (%)]	27 (30.7%)
Cord prolapse [n (%)]	3 (3.4%)
Postpartum hemorrhage [n (%)]	14 (15.9%)
Blood transfusion [n (%)]	10 (11.4%)
Maternal sepsis [n (%)]	3 (3.4%)
DVT [n (%)]	1 (1.14%)

PROM: premature rupture of membranes n: Number DVT: deep vein thrombosis

Table (5): determinants of development of maternal and neonatal complications.

Variables	Univariate analysis	Multivariate analysis		
		B	95% CI	P value
Chronic diseases	0.763			
Previous PROM	0.706			
WBCs	0.706			
CRP	0.015*	3.26	2.93 – 3.72	0.043*
Amniotic fluid index	0.034*	1.054	0.756 – 1.375	0.534

* Statistically significant when P value less than 0.05
PROM: premature rupture of membranes CI: confidence interval
WBCs: white blood cells CRP:C-reactive protein