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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

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

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Contents :

Letter from the Editor:

Dear colleagues,

Warm greeting & Happy new year. Excellent subjects are included in this issue. Preeclampsia has a higher incidence among women had H pylori (HP) infection, which might be considered as precipitating factor for PE development. Platelet rich plasma (PRP) infusion increased endometrial thickness and pregnancy rates, but the difference didn't reach to clinical significance. Insulin sensitizers could improve PCOS-associated disturbances. However, omega-3 adjuvant therapy significantly augmented the effects of insulin sensitizers, minimized the cardiac risk factors, and decreased the risk of probable cardiac events. Management of patients with placenta accrete spectrum (PAS) should be individualized according to PAS grade and patient wishes. The purse-string suture technique can help in reducing blood loss and decrease the cesarean hysterectomy rate. Endoscopic ovarian drilling had a significant decreasing effect on serum AMH, AFC, and Ovarian Doppler indices in PCOD patient not responding to clomiphene citrate.

Best regards.

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Preconception H pylori infection might be a risk factor for Development of Early-onset Preeclampsia

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Abstract

Objectives: Estimation of serum levels of Tumor necrosis factor-α (TNF-α), interleukin-1β and -6 at booking time and at time of diagnosis of preeclampsia (PE) in women infected and un-infected by H pylori (HP).

Patients & Methods: 232 primigravida gave blood samples for ELISA determination of HP IgG positivity and were categorized as HP+ and HP- groups. At booking time, systolic (SBP) and diastolic (DBP) blood pressure measures were recorded and serum levels of TNF-α, IL-1β and IL-6 were ELISA estimated (S1 sample). During the 4-weekly visit, BP was recorded to diagnose PE according to the American Society of Hypertension. At time of PE diagnosis, S2 blood sample was obtained for re-estimation of studied cytokines.

Results: There were 108 HP+ women and 54 women developed PE; 33 HP+ and 21 HP- women. The incidence of early-onset PE was significantly higher, while the incidence of severe PE was non-significantly higher among HP+ women. Positivity for HP IgG showed positive significant correlation with development of early and severe PE. At booking serum levels of studied cytokines were significantly higher in PE than Non-PE women and in HP+ women than in HP- women and showed positive significant correlation with BP measures at time of diagnosis of PE. Automatic Linear Modeling analysis defined high at booking serum TNF-α as the most important predictor for PE.

Conclusion: PE has a higher incidence among women had HP infection, which might be considered as precipitating factor for PE development. The positive correlation between severity of PE, HP-IgG positivity and increased serum levels of inflammatory cytokines represent a dangerous circle entrapping the pregnant women.

Keywords: H pylori infection, Preeclampsia, Primigravida, Inflammatory cytokines.

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Introduction

Helicobacter pylori (HP) infection is still one of the most prevalent infections worldwide [(1)]. The prevalence of HP infection varies in different parts of the world and most of the infected adults are asymptomatic [(2)], but bacterial eradication is necessary to prevent precancerous conditions especially with its increased resistance to antibiotics, which has made management more challenging [(1)].

Pregnant women are a category of population most vulnerable to H. pylori infection [(3)], which affect pregnant women with a global incidence of 46% [(4)]. Maternal HP infection was found to be associated with undesirable effects during pregnancy [(5)]; iron deficiency anemia during pregnancy [(6)], hyperemesis of pregnancy [(7)], gestational diabetes mellitus [(8)] and metabolic syndrome [(9)].

HP infection results in a variety of gastrointestinal and extra-gastrointestinal complications [(10)]. Arteriosclerosis, dyslipidemia, diabetes, obesity, hypertension, and cardiovascular disease are the common extra-gastrointestinal complications of HP infection [(11)].

Vascular lesions associated with HP infection occurs via activation of endothelium, stimulation of formation of macrophage derived foam cell and vascular lesion instability mostly through Toll-like receptors (TLR) as a continuous cascade [(12)]. This cascade starts with activation of TLR2 by lipopolysaccharide of H. pylori, which enhanced the expression of TLR4 through MAP/ERK 1/2 kinase pathway [(13)]. Activated TRL4/MyD88 signaling by H. pylori induces IL-10 and IL-12 secretion, TLR9 stimulated by H. pylori could induce pre-inflammatory cytokines such as IL-6 and IL-12 [(14)]. Additionally, H. pylori-flagellin A is recognized by TLR5 which induces IL-8 secretion through p38-map kinase signal [(15)].

Hypothesis

The study supposed a possible relation between preconception HP infection and development of PE during pregnancy.

Objectives

Estimation of serum levels of tumor necrosis factor-α (TNF-α), interleukin-1β and -6 at booking time and at time of diagnosis of PE in women infected and un-infected by HP.

Design

Prospective comparative single center clinical trial.

Settings

Departments of Obstetrics & Gynecology, and Medical Biochemistry, Faculty of Medicine, Benha University

Patients & Methods

After approval of the study protocol by the Local Ethical Committee (RC: 15-4-19), all primigravida who attended the Antenatal Care Unit (ACU) of Benha University Hospital for assurance of being pregnant, were evaluated for eligibility for inclusion in the study.

Exclusion criteria

History of gastric diseases, peptic ulcer, congenital heart diseases, valvular diseases, cardiomyopathy, myopathies, coagulopathy, infectious diseases, inflammatory states, manifest diabetes, endocrinopathy, essential hypertension (HTN), renal, hepatic or cardiac diseases, family history of essential HTN, metabolic syndrome, or body mass index (BMI) >35 kg/ m2. Also, women presenting after the 20th gestational week (GW) or refused to sign the written consent were excluded from the study.

Inclusion criteria

Primigravida had a singleton fetal sac as assured by ultrasonography, free of exclusion criteria and signed the written consent to participate in the study and attend the ACU 4-weekly till delivery for follow-up.

Clinical evaluation

After assurance of pregnancy, gestational age was determined and women were clinically evaluated to determine baseline measurements of systolic and diastolic blood pressures (SBP, DBP), baseline body mass index (BMI), and underwent routine investigations including complete blood count, kidney and liver function tests and urine analysis with special regard to presence of protein.

Diagnosis and categorization of preeclampsia (PE)

According to the American Society of Hypertension [16] PE was defined as development of gestational HTN in a previously normotensive (NT) woman and is associated with proteinuria quantified as 1+ on dipstick. PE was categorized as mild PE (MPE) if SBP and DBP were <160 and <110 mmHg, respectively with proteinuria of <2+ and absence of systemic manifestations and as severe PE (SPE) if elevated BP measures were associated with systemic manifestations or if SBP was ≥160 mmHg and DBP was ≥110 mmHg with proteinuria >2+ on a voided random urine [17]. Furthermore, PE was re-classified according to timing of development in relation to gestational age as early-onset (EPE) if diagnosed prior to 34 GW and late-onset (LPE) if diagnosed after the 34th GW [18, 19].

Diagnosis of HP infection

Infection by HP was diagnosed depending on the presence of positive serum test for H pylori IgG

Grouping

The enrolled women were grouped into HP+ and HP- according to the positivity for HP IgG in serum. Patients who developed PE within each group were sub-grouped as PE and those who did not as Non-PE groups.

Sampling and Investigations

Sampling

Blood samples (S1 & S2) were obtained at the start of the 12th GW and at time of diagnosis of PE. Blood sample (5 ml) was withdrawn under complete aseptic conditions, allowed to clot and then centrifuged at 3000 rpm for 10 minutes to separate serum that was collected in sterile Eppindorff tube and stored at -80oC till be assayed. Blood samples were collected and numbered by an assistant who was blinded about groups. Also, midstream urine sample was obtained for evaluation of the presence of proteinuria using dipstick.

Investigations

Estimated blood variables were measured using enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions and were read using a 96 well microplate ELISA reader (Dynatech. MR 7000)

1. Human TNF-α was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab179886, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique [20].
2. Human IL-1β was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46052, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique [21].
3. Human IL-6 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46027, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique [22].
4. Human anti-Helicobacter pylori IgG using ELISA kit (catalogue no. ab108736, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique [23].

Statistical analysis

Data are presented as mean, standard deviation (SD), numbers, percentages, median and interquartile range (IQR). Parametric results were analyzed using paired t-test for comparisons of estimated BP and serum cytokines at booking and diagnosis of PE times and non-parametric results were analyzed using Chi-square test and Mann-Whitney test. Receiver operating characteristic curve was used for analysis of predictors for PE development. Automatic Linear Modeling analysis for serum levels of estimated cytokines as predictors for high SBP during pregnancy. Statistical analysis was conducted using IBM® SPSS® Statistics (Version 22, 2015; Armonk, USA) for Windows statistical package. P value <0.05 was considered statistically significant.

Results

Evaluation for eligibility included 251 primigravida; 19 were excluded for not fulfilling the inclusion criteria and 232 women gave blood samples for evaluation for positivity for HP IgG. One hundred and eight women were HP+, while 124 women were HP-. Collected clinical data showed non-significant (p>0.05) difference between HP+ and HP- women (Table 1).

Table (1): Booking data of studied women categorized according to serum positivity for HP IgG

Parameter	HP+ women (n=108)	HP- women (n=124)	P value
Age (years)	26.3±4.4	26.8±3.8	0.407
Body mass index (kg/m^2)	27.6±2.3	27±2.9	0.069
SBP (mmHg)	119.4±4	118.4±4.3	0.059
DBP (mmHg)	81.2±4.9	81.7±5.2	0.474

Data are presented as mean, standard deviation, HP+: H pylori IgG positive; HP-: H pylori IgG negative; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; p value indicates the significance of difference between both groups; P<0.05: indicates significant difference; P>0.05: indicates non-significant difference

During course of pregnancy, all women showed follow-up blood pressure higher than the measure recorded at booking visit. However, 54 women developed PE; 33 HP+ women and 21 HP- women with non-significantly (p=0.197) higher frequency of PE among HP+ women. Nineteen women developed EPE; 17 HP+ and only two HP- women, while 35 women developed LPE; 16 HP+ and 19 HP- women with significantly (p=0.0016) higher frequency of EPE among HP+ women. Fifteen women developed SPE; 11 HP+ and 4 HP- women, while 39 women developed MPE; 22 HP+ women and 17 HP- women with non-significantly (p=0.282) higher frequency of SPE among HP+ women (Fig. 2).

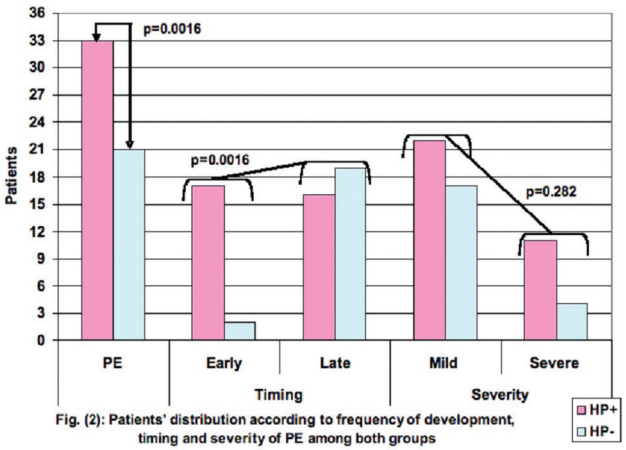

Fig. (2): Patients' distribution according to frequency of development, timing and severity of PE among both groups

At time of PE diagnosis, mean BP measures were significantly (p<0.0001) higher in comparison to measures recorded at booking time with significantly higher measures in patients of HP+ group in comparison to HP- group.

DBP measures at time of diagnosis of PE were significantly higher in women of PE+ group in comparison to women of PE- group, both who developed PE (p=0.0317) or not (p=0.0021). Interestingly, BP measures of non-PE women of HP+ group were significantly (p<0.0001) higher than that of non-PE women of HP- group, while BP measures of PE women of HP+ group were non-significantly (p=0.310) higher than that of PE women of HP- group (Table 2, Fig. 3).

Table (2): BP measures of studied women categorized according to serum positivity for HP IgG at time of diagnosis of PE

Variables		Time Group	HP+ women	HP- women	P value
SBP (mmHg)	Total	Booking	119.4±4	118.4±4.3	0.059
		Diagnosis of PE	137±14.8	128.6±12.8	<0.0001
		P1	<0.0001	<0.0001	
	Diagnosis of PE	PE	156.9±10.5	154±10.3	0.310
		No PE	128.2±3.6	123.4±4.2	<0.0001
DBP (mmHg)	Total	Booking	81.2±4.9	81.7±5.2	0.474
		Diagnosis of PE	94.2±11.4	88.5±9.2	0.00003
		P1	<0.0001	<0.0001	
	Diagnosis of PE	PE	108.8±8.2	103.7±8.7	0.0317
		No PE	87.8±4.9	85.4±5.4	0.0021

Data are presented as mean, standard deviation, HP+: H pylori IgG positive; HP-: H pylori IgG negative; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; p value indicates the significance of difference between both groups; P<0.05: indicates significant difference; P>0.05: indicates non-significant difference

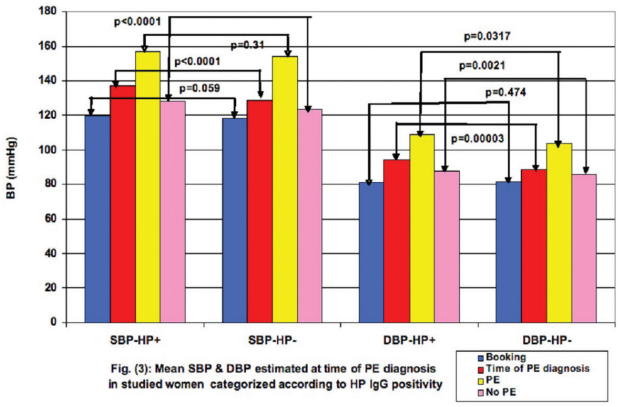

Fig. (3): Mean SBP & DBP estimated at time of PE diagnosis in studied women categorized according to HP IgG positivity

There was positive significant correlation between positivity for HP IgG and earlier development of PE (Rho: 0.183, p=0.005) and severity of PE (Rho: 0.168, p=0.010). ROC curve analysis defined positive HP IgG at booking time for development of SBP>132 mmHg during pregnancy (Fig. 4), irrespective of progression to PE or not with AUC= 0.776 (SE: 0.054; p=0.013; 95% CI: 0.67-0.881)

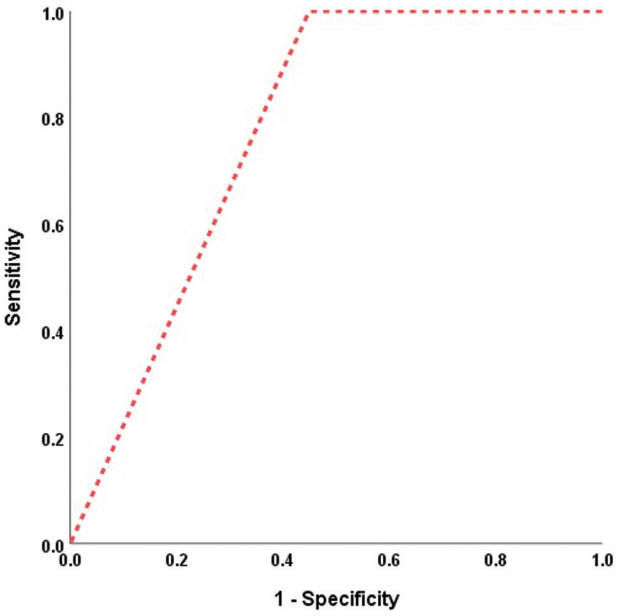

Fig. (4): ROC curve analysis for the cutoff point of SBP predicted to be achieved during pregnancy of primigravida with HP+

Serum levels of TNF-α, IL-1β and IL-6 estimated at booking time were significantly higher in PE women in comparison to Non-PE women and in HP+ women than in HP- women, irrespective of development of PE (Table 3).

Table (3): Mean levels of estimated serum cytokines at booking time in women categorized according to serum positivity for HP IgG and development of PE

Parameters	Groups	HP+	HP-	PE	No PE
TNF-α (ng/ml)	Level	2.835±1.09	2.305±0.79	3.76±0.96	2.1±0.62
	P1	<0.0001		<0.0001	
IL-1β (ng/ml)	Level	39.2±20	21.1±11.7	54.3±20.8	17.3±4.6
	P1	<0.0001		<0.0001	
IL-6 (ng/ml)	Level	26.5±11	16.6±12.3	38.7±13.2	12.2±4.9
	P1	<0.0001		<0.0001	

Data are presented as mean, standard deviation; HP: H pylori; PE: Preeclampsia; TNF-α: Tumor necrosis factor-α; IL: Interleukin; p value indicates the significance of statistical analysis; P<0.05: indicates significant difference; P>0.05: indicates non-significant difference

Moreover, serum levels of studied cytokines showed positive significant correlation with SBP and DBP measures estimated at time of diagnosis of PE. Moreover, serum levels of cytokines estimated at booking time were positively correlated with both SBP and DBP estimated at time of development of PE. ROC curve analysis showed that all of the three cytokines are predictors for development of PE (Table 4, Fig. 5). However, evaluation of importance of predictors for high SBP at time of PE diagnosis using the Automatic Linear Modeling defined high at booking serum TNF-α as the most important, followed by serum IL-6 levels and lastly serum IL-1β levels (Fig. 6).

Table (4): Statistical analyses of laboratory findings as predictors for development of PE

Methods Variables	Pearson's correlation				ROC curve analysis			
	HP IgG positivity		Development of PE					
	"r"	P	"r"	P	AUC	SE	P	95% CI
HP IgG positivity			0.161	0.014	0.595	0.044		0.509-0.681
TNF-α	0.271	<0.001	0.684	<0.001	0.978	0.015	<0.001	0.914-0.972
IL-6	0.414	<0.001	0.769	<0.001	0.943	0.010	<0.001	0.959-0.998
IL-1β	0.492	<0.001	0.740	<0.001	0.942	0.017	<0.001	0.909-0.975

"r": Pearson's correlation coefficient; AUC: Area under curve; SE: Standard error; CI: Confidence interval; HP: H pylori; PE: Preeclampsia; TNF-α: Tumor necrosis factor-α; IL: Interleukin; p value indicates the significance of statistical analysis; P<0.05: indicates significant difference; P>0.05: indicates non-significant difference

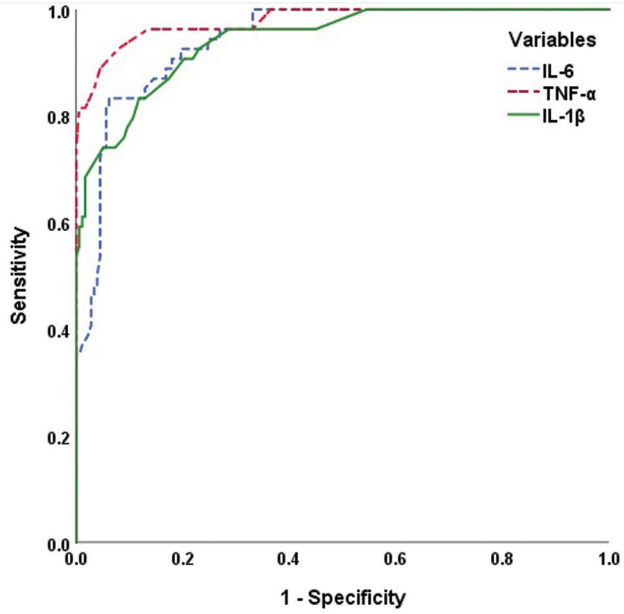

Fig. (5): ROC curve analysis for the studied cytokines as predictors for development of PE

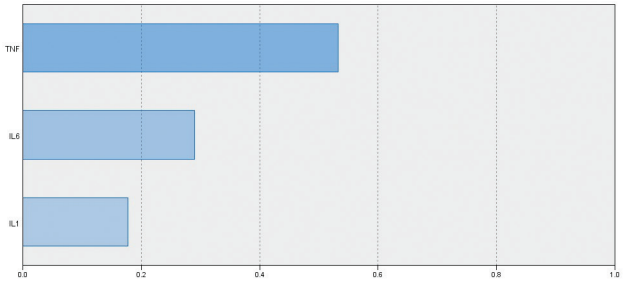

Fig. (6): Automatic Linear Modeling analysis for serum levels of estimated cytokines as predictors for high SBP during pregnancy

Discussion

At booking time, women of HP+ group had non-significantly higher blood pressure measures than HP- women; a finding points to a certain relation between HP infection and elevation of BP measures. In support of this assumption, during pregnancy course, NT women of HP+ group had significantly higher BP measures in comparison to their booking time BP and to NT women of HP- group. These findings spot light on the effect of pregnancy on maternal BP and this effect was magnified by HP infection. Regarding development of PE, 54 women developed PE; 33 of HP+ and 21 of HP- groups with significantly higher frequency of PE women among those had HP infection. Moreover, frequency of EPE was significantly higher, while the frequency of SPE was non-significantly higher among HP+ women in comparison to HP- women.

The obtained data go in hand with **Bellos et al.** [24] who after literature review detected more significant prevalence of HP IgG seropositivity and anti-CagA antibodies among pre-eclamptic than healthy pregnant women and concluded that HP infection doubles the risk of developing PE. Recently, **Li et al**. [12], **Su et al.,** [25] and **Ahmed et al.** [26] found HP infection significantly increased the incidence of pregnancy-induced hypertension and PE.

These data indicated a possible role of HP infection that may underlie development of early-onset severe PE. In support of this assumption, there was a positive significant correlation between development and severity of PE and HP infection and statistical analyses defined preconception HP infection as an early significant predictor for the possibility of development of PE and of SPE. Similarly, previous literature reviews documented a positive association between HP infection and PE and Cag-seropositivity was a substantial risk factor for PE and concluded HP infected women especially those infected with Cag A positive strains are more likely to have PE than uninfected women [26, 27, 28].

The results of the current study showed significantly higher serum levels of inflammatory cytokines in PE women than in NT women, in HP infected than uninfected and in PE women in HP+ than in HP- group. These findings indicated a possible pathogenic role for inflammatory cytokines in development of PE. In line with these findings, **Shiadeh et al.** [29] suggested that inflammatory responses against infections shift immunological cytokine profile of Th2 toward Th1 with high levels of pro-inflammatory cytokines, increased oxidative stress, anti-angiogenic proteins, vascular endothelial growth factor receptor 1 and complement C5a leading to enhancement of PE development. Recently,

Spence et al. [30] in literature review reported that pro-inflammatory cytokines are significantly elevated in PE, but TNF-α levels increases as pregnancy progresses, and lower levels of IL-10 concentrations during the 2nd trimester may be an early predictor for PE development.

Moreover, the obtained results suggested that HP infection led to increased inflammatory cytokines and these in turn lead to placental affection with subsequent development of PE; thus suggesting a vicious circle between HP infections, increased inflammatory cytokines and PE. In support of this vicious circle, an experimental study documented that HP membrane protein-1 is a member of TNF-α-inducing protein gene family [31]. Also, animal studies detected significantly higher levels of pro-inflammatory mediators, NF-κB expression and apoptotic cells in HP infected rats than control animals [32] and sodium butyrate intake by HP infected mice had reduced the production of virulence factors, inhibited the IκBα/NF-κB pathway by reducing the expression of Toll-like receptors and reduced the production of TNF-α and IL-6 [33].

On the other hand, **Michalczyk et al.** [34] documented that oxidative stress; altered angiogenic/antiangiogenic balance and impaired inflammatory response triggered by inflammasomes are significant factors responsible for PE development. Using animal model of PE, **Travis et al.** [35] found TNF-α blockade reduced mean arterial pressure and uterine artery resistance index, improved fetal growth, and increased NO bioavailability, so suggested that TNF-α regulation of NO bioavailability is a potential mechanism that contributes to PE pathophysiology and **Ji et al.** [36] detected that the use of a novel peptide attenuated the upregulation of antiangiogenic factors and the reduction in TNFα-induced mitochondrial potential and decreased numbers of THP-1 monocytes. Also, **Oda et al.** [37] found the use of recombinant thrombomodulin reduced levels of IL-6 and TNF-α; the well-known key inflammatory mediators in PE pathogenesis. Moreover, **Yin et al.** [38] found miR-138 improves LPS-induced inflammation on trophoblasts and decreased levels of IL-6 and TNF-α through targeting RELA and affecting NF-κB signaling, and concluded that down-regulation of miR-138/RELA axis might be involved in PE pathogenesis. Review of clinical

Conclusion

Preeclampsia has a higher incidence among women had HP infection. HP infection might be considered as precipitating factor for development of early-onset and/or severe PE. The positive correlation between severity of PE, HP-IgG positivity and increased serum levels of inflammatory cytokines represent a dangerous circle entrapping the pregnant women

Limitations

The study is a single-center study evaluating population of certain locality.

Recommendation

Multicenter studies evaluating the same topic and the trial of preconception eradication of HP infection as a method to break this dangerous circle

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Intrauterine infusion of autologous Platelet-rich plasma before frozen embryo transfer in patients with prior implantation failure: A randomized controlled study

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Abstract

Objectives: To evaluate whether intrauterine infusion of autologous platelet rich plasma (PRP) in patients with prior implantation failure (RIF) improves pregnancy rate or not.

Materials and methods: This randomized controlled study was conducted at 4 fertility centers in the period from December 1, 2018 to October, 2021. Patients with repeated implantation failure were recruited and randomly allocated into either study group who received PRP intrauterine infusion or control group who received intrauterine saline infusion. The primary outcome was occurrence of pregnancy. Secondary outcome was increase in endometrial thickness.

Results: Basal demographic data of enrolled patients were comparable. The endometrial thickness on the 11th day did not differ between control and treatment group, 8.5± 1.34mm and 8.18± 1.35mm respectively, P=0.23, while the thickness was significantly increased in the study group at FET P=0.04. Clinical pregnancy rate was 10.92% in the control group versus 16.81% in the study group (P= 0.22). Implantation rate was 15.97% in the control group versus 21.24% in the study group (P= 0.36). Endometrial thickness at FET was correlated to high pregnancy rate.

Conclusion: Although PRP infusion increased endometrial thickness and pregnancy rates in the treatment and control group, but the difference didn't reach to clinical significance.

Keywords: Repeated implantation failure, PRP, Frozen embryo transfer, implantation rate, Clinical pregnancy rate.

Introduction

Repeated implantation failure (RIF) is one of the frustrating conditions in assisted reproductive technologies. RIF means failed conception following at least 3 transfers of good quality embryos (≥4 embryos) in women aged ≤40 years.(1) Implantation requires optimally receptive endometrium, good thickness of endometrium ≥ 7 mm at start of progesterone supplementation, tolerance of maternal immune system to implanted embryo and finally implantation of euploid embryos.(2)

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Endometrial receptivity and success of implantation represent an obstacle in reproductive medicine and until now are unsolved secrets. Hostile environment interferes with implantation. The hostile environment includes submucosal myomas, endometrial polyps, chronic endometritis (CE), and intrauterine adhesions. Research on RIF is running and focusing on improvement of implantation environment. Many drugs and procedures will examined including Antibiotics, Sildenafil, Granulocyte colony stimulating factor, endometrial scratching (3)

PRP is recently studied in enhancing endometrial receptivity. The rationale for PRP administration is that PRP contains high concentrations of platelet-derived growth factors and cytokines that improve endometrial thickness and receptivity. These growth factors include platelet-derived growth factor (PDGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and transforming growth factor beta (TGF β). (4)

Zadehmodarres et al studied the effect of PRP intrauterine infusion in frozen embryo transfer in combination with hormonal therapy. PRP injections were done twice, starting on days 11-12 and repeated on days 13-14). Patients had endometrial thickness ≤7. Their results will improvement of endometrial thickness ≥7 mm and 50% of patients became pregnant. (5)

Recent studies showed that PRP not only improved endometrial thickness but also increased implantation rates when applied in patients with RIF. These studies recommended the use of PRP as a potential therapy in endometrial preparation for successful implantation. (6-,8)

The main objective of this study was assessment of PRP efficacy when infused inside uterine cavity prior to frozen embryo transfer (FET) in patients with repeated implantation failure.

Patients and methods

Study design and settings: This multicenter study is a double armed, non-blinded, randomized controlled trial conducted from December, 2018 October 2021 at Egyptian Consultants fertility center, Adam & Hawa fertility center, Ingab fertility center and Al-Yasmin fertility center.

Patients: One hundred and ten patients with history of prior ICSI failure were recruited from 4 fertility centers. Eligible patients were enrolled according to fulfillment of inclusion. The inclusion criteria were (a) patients with prior implantation failure prepared for FET (b) age of 20-35 years, (c) thin endometrium with thickness ≤ 7 mm at day 11. The exclusion criteria were (a) hepatic disorders, (b) platelet count of <150 000/mm3, (c) patients with abnormal uterine cavity septate uterus, bicornuate uterus or uterine myoma, (d) patients wishing fresh embryo transfer (e) patients with abnormal embryos grade B or C (f) patients with immunological disorders as autoimmune thyroiditis or systemic lupus or rheumatoid arthritis.

Sample size: The current study included independent cases and controls with 1:1 ratio. Previous studies showed that failure rate was 0.1 among controls. Assumption was made that true failure rate among study group to be 0.33. In that case, the appropriate sample required was 49 patients for study and similar 49 patients for control group. This sample is just enough to reject null hypothesis (N0) denoting equal failure rates for study and control groups with probability (power) 0.8 and Type I error of 0.05. Uncorrected chi-squared statistics were used to evaluate this null hypothesis.

Randomization and allocation: Randomization was done using computer based program with letter P denoting PRP group and letter S denoting control group receiving saline infusion. These letters were enveloped and patients were allocated into either group

after opening of the envelope. Envelope opening didn't change allocation. Allocation was with equal ratio 1:1.

Interventions

Endometrial preparation: Hormonal preparation of endometrium was done in all patients in both groups with estradiol oral tablets (white tablets of Cycloprogynova) with 2 mg estradiol in each white tablet. The endometrial preparation started on day 3 of menstrual cycle and continued in fixed hormonal protocol. The dose was one tablet three times daily. Endometrial thickness was measured on days 3, 7, 11, 13 and at day of embryo transfer. Vaginal progesterone 400µg twice daily was given on day 13 continuously along with estradiol oral tablet.

PRP preparation: Fifteen CC of venous blood was taken from patients allocated into PRP study group. The blood is collected in specified tubes for PRP (Acti-PRP tube, Taiwan). The blood is better collected at a temperature between 21-24 C and first centrifugation was performed at 1200 rpm for 12 minutes. The upper layer is transferred into another sterile tube and second centrifugation at 3300 rpm for 7 minutes was done. The upper 2/3 of the tube is discarded while the remaining 1/3 is homogenized and a total of 1 mL of PRP will obtained. (9) The platelet concentration in each 1 ml will 400×10^3 to 900×10^3/ml.

In study group, PRP (1 ml) was infused inside the uterine cavity immediately after preparation with embryo transfer catheter (Kitazato Medical Co., Ltd.) under ultrasound guidance at day 11^{th} of menstrual cycle and may be repeated at day 13 if endometrial thickness is still<7mm. In the control group 1 ml of normal saline 0.9% will performed on day 11 using the same embryo transfer catheter.

Embryo transfer: Good quality (2-3) grade (A) blastocyst stage were transferred on the 18^{th} day. Serum pregnancy test was done 2 weeks after embryo transfer.

Outcomes of the study: The primary outcome was occurrence of pregnancy evidenced by positive hCG, 14 days following Embryo transfer. The secondary outcomes included endometrial thickness on FET day, implantation evidenced by presence of gestational sac at 6 weeks by ultrasound examination. Clinical pregnancy rate (CPR) is obtained by dividing sum of viable fetal poles at 6-week ultrasound by the sum of transferred embryos. Implantation rate is obtained by dividing the sum of gestational sacs at 6-week ultrasound by the sum of transferred embryos.

Data collection: The demographic data, basal hormonal profiles, number of prior failed ICSI cycle, endometrial thickness at day of embryo transfer, pregnancy rate, implantation rate and any complications.

Study registration and ethical issues: Patients were provided through explanation about objectives, benefits and risks of the study and a written consent was obtained from all participation. The study was conducted according to principles of the Declaration of Helsinki. This study was approved by Tanta University ethical committee (Approval No. 34803), registered on clinicaltrials.gov, and given the unique ID: NCT03734042 and is available on the following link: https://register.clinicaltrials.gov/prs/app/action/ViewOrUnrelease?uid=U000404W&ts=18&sid=S0008G0D&cx=-qthfns

Statistical analysis: Continuous data distribution was evaluated using the Shapiro-Wilk test, and normally distributed variables were analyzed by descriptive statistics as mean, standard deviation and tested with the Student t-test for independent pairs. Non-normally distributed data were expressed as median (25th and 75th percentiles). Categorical data were analyzed and expressed as numbers and percentages. Comparison of categorical data was done by Chi-square test or Fisher exact test. Univariable logistic regression analysis was used to identify factors affecting the occurrence of pregnancy. Stata 16.1 (STATA

Corp, USA) was used for statistical analyses. P-value ≤ 0.05 was considered statistically significant.

Results:

A total of (n=117) patients were assessed for eligibility. Patients who were excluded were (13) either not fulfilling inclusion criteria (n=8) or declined to participate (n=5). The consort flow chart is presented in figure 1.

Baseline data:

We compared patients who had PPR (treatment group) to a control group. Baseline data were nearly similar with no significant difference between both groups. (Table 1)

Outcome data:

Regarding duration of endometrial preparation, there was no significant difference in between both groups. The endometrial thickness on the 11th day did not differ between groups, while the thickness was significantly higher in the treatment group at FET day. The clinical pregnancy rate was 10.92% in the control group versus 16.81% in the treatment group (P= 0.22). The implantation rate was 15.97% in the control group versus 21.24% in the treatment group (P= 0.36). (Table 2)

Factors affecting the occurrence of pregnancy:

Pregnancy was lower in patients with tubal factors [OR=0.15 (0.03- 0.79)] p-value=0.02. Endometrial thickness at FET also was positively correlated to occurrence of pregnancy. No other factors affected the occurrence of pregnancy. (Table 3)

Discussion

Repeated implantation failure (RIF) remains a big frustrating condition facing reproductive medicine specialists. There are no definite causes in most cases and the proposed treatment plans are unable to solve this problem in many cases. (1,2) Endometrium had a built-in program for reception or rejection of embryos and this endometrial receptivity allows an appropriate environment for implantation and placenta development. Endometrial receptivity is the main player in (RIF) and has a crucial role in implantation. [10]

Appropriate assessment of endometrial receptivity was studied and several markers were investigated in a large meta analysis including 88834 women. The studies included in that meta analysis advocated the use of certain markers and others didn't, so no marker is superior to others. Endometrial receptivity array (ERA) and endometrial thickness (ET) could denote appropriate endometrium or window of implantation (WOI). (11-13)

Several treatment strategies were introduced for solving RIF.[14] Away from embryo quality and stimulation protocols, the investigated strategies were antibiotics, [15] immunosuppressive drugs eg. Tacrolimus, [16] hCG-activated human peripheral mononuclear cells, [17] hysteroscopic endometrial injury [18] vitamin D, [19] IVIG, [20] G-CSF, [21] salpingectomy, [22] Intravenous intralipid infusion [23], LMH, [24] and Platelet rich plasma [25,26].

In the current study, autologous PRP injection was infused in the uterine cavity on day 11 and repeated at day 13 if endometrial thickness is still<7mm. The control group were infused by 1 ml saline. Regarding endometrial thickness, it was significantly increased in the treatment group on day of FET with P-value=0.04. Our results are in agreement with Nazari et al [25], Zamaniyanet al [26], Chang et al [27], Kusumi et al,[28], Obidniak et al.[29], Mehrafza et al.[30], Coksuer et al.[31], Nazari et al [1, 32] and Eftekhar et al [33].

On the other hand, Allahveisi et al conducted a study on 50 infertile patients with RIF and found that PRP infusion didn't increase endometrial thickness (ET) in study or control group where ET was 9.36 ± 0.27mm in con-

trol and 9.6 ± 0.27mm in study group with p-value= 0.54.[34] and similarly Madhavan et al.[35]

In the current study, although rates of chemical pregnancy, implantation and clinical pregnancy were higher in treatment group with PRP rather in control group but didn't reach to clinical significance with p-value 0.40, 0.49, and 0.38 respectively.

Our data are in agreement with Allahveisi et al who reported that pregnancy rate in control group was 36% and was 28% in treatment group (p-value= 0.83). They concluded that PRP had no significant role on improving pregnancy following FET. [34] Also Madhavan et al conducted a cohort study on ninety-eight patients with 42 patients allocated in PRP group and 56 patients allocated in the control group. The authors reported no difference in clinical pregnancy rate (CPR) in patients with primary infertility while in patients with secondary infertility the difference didn't reach to clinical significnace.[35]

Similar to our results, Aghajanzadeh et al conducted a study on 30 cases with RIF. They infused PRP 48 hours prior to embryo transfer. They found that implantation rate was 6.7% in PRP group. They also found that no significant differences regarding implantation rate, CPR, and miscarriage rates following FET with and without PRP infusion. [36]

On the other hand Chang et al found that pregnancy rate was 44.12% in study group while it was 20% in control group with p value= 0.036. Chang et al concluded that PRP has beneficial role in endometrial proliferation, and improving implantation and pregnancy rates for women with thin endometrium in FET cycles. Nazari et al reported 90% pregnancy rate in PRP group. [25]

Zamaniyan et al. conducted a randomized clinical trial on 98 patients with RIF. Patients were allocated in PRP group (n=55) and in the control group (n=43). They found that clinical pregnancy was significantly higher in study group than in control group (48.3% versus 23.26) respectively with p=0.001. The implantation rate was 58.3% versus 25%; p=0.001) in study and control groups respectively. They concluded that rates of implantation, clinical pregnancy and ongoing pregnancy were improved following PRP use. [26]

Kusumi et al conducted a randomized self-controlled study on 39 patients. They found that endometrial thickness was improved significantly and on the same side clinical pregnancy rate was improved following PRP infusion (15.6%) in patients with FET. They reported no adverse events and concluded that PRP therapy was safe and increased both endometrial thickness and clinical pregnancy rate.[28]

The strengths of this study were the randomized controlled design and blinding of participants while the weakness point was the small sample size.

Conclusion:

Autologous PRP is a cheap office procedure and doesn't require great experience in its handling and preparation with high safety. It is a new modality of treatment in the field of reproductive medicine which was investigated in many conditions. In Repeated implantation failure, although it significantly increased endometrial thickness at FET day, but the increase in clinical pregnancy rate, and implantation rated didn't reach to clinical significance. A larger studies are required to reach to an evidence based solid conclusion regarding the use of PRP in RIF.

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Conflict of interest:

no conflicts do exist for all authors

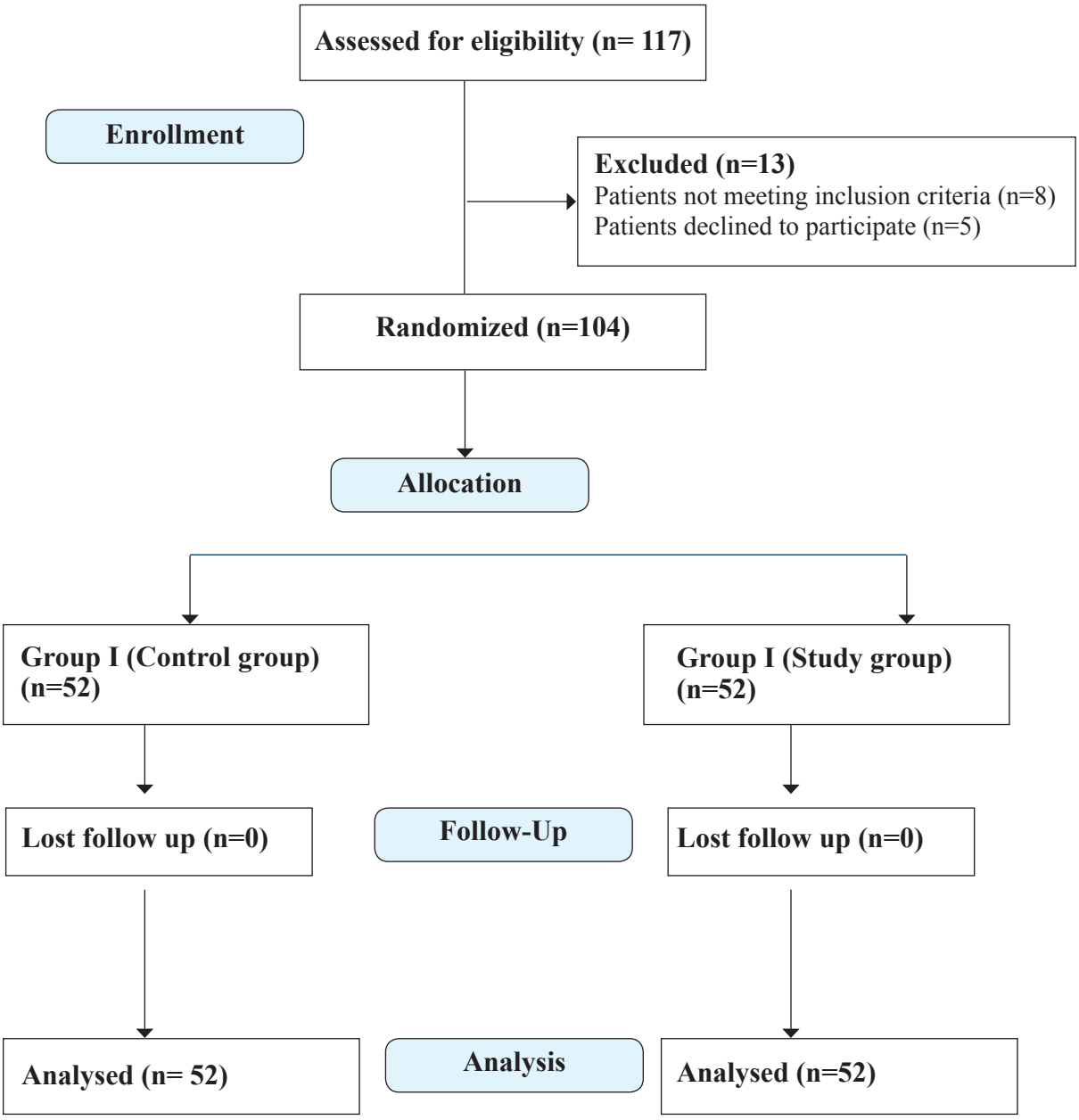

Figure 1: CONSORT Flow chart of cases through the study

Table 1: Baseline data of enrolled patients

Variables	Control group (n= 52)	Treatment group (n= 52)	P
Age (years)	30.52± 3.381	29.60± 2.99	0.14
Gravidity Zero One Two Three	 27 (51.92%) 14 (26.92%) 7 (13.46%) 4 (7.69%)	 28 (53.85%) 15 (28.85%) 8 (15.38%) 1 (1.92%)	0.66
Parity Zero One	 40 (76.92%) 12 (23.08%)	 34 (65.38%) 18 (34.62%)	0.19
BMI (kg/m2)	25.2 (23.5- 28.35)	27.65 (25- 29.65)	0.06
Type of infertility Primary Secondary	 27 (51.92%) 25 (48.08%)	 28 (53.85%) 24 (46.15%)	>0.99
Duration of infertility (years)	6.65 (5- 8.5)	5.25 (4.5- 7)	0.08
Indication of ICSI Endometriosis Male factor PCOS Tubal factor Unexplained	 6 (11.54%) 20 (38.46%) 12 (23.08%) 11 (21.15%) 3 (5.77%)	 6 (11.54%) 19 (36.54%) 13 (25%) 9 (17.31%) 5 (9.62%)	0.95
Number of failed ICSI One Two Three Four	 23 (44.23%) 19 (36.54%) 8 (15.38%) 2 (3.85%)	 32 (61.54%) 10 (19.23%) 6 (11.54%) 4 (7.69%)	0.16
FSH	8 (6.9- 8.6)	8 (6.65- 8)	0.66
AMH	2.8 (2.25- 3.5)	3 (2.85- 3.6)	0.08

AMH: Antimullerian hormone; BMI: body mass index; FSH: follicular stimulating hormone; ICSI: Intracytoplasmic sperm injection; PCOS: polycystic ovary syndrome

Table 2: Comparison of the fertilization and outcomes between both groups.

Variables	Control group (n= 52)	Treatment group (n= 52)	P
Days of endometrial preparation	12.56± 1.16	12.46± 1.29	0.69
Endometrial thickness day 11 (mm)	8.5± 1.34	8.18± 1.35	0.23
Endometrial thickness at FET (mm)	12 (11.55- 13.4)	12.5 (12- 14)	0.04
Difference in endometrial thickness	3.71± 1.65	4.60± 1.43	0.004
Number of embryos Two Three	 37 (71.15%) 15 (28.85%)	 43 (82.69%) 9 (17.31%)	0.16
Number of sacs One Two	 9 (64.29%) 5 (35.71%)	 12 (66.67%) 6 (33.33%)	>0.99
Chemical Pregnancy rate	14 (26.92%)	18 (34.62%)	0.40
Clinical pregnancy rate	10.92%	16.8 %	0.38
Implantation rate	15.97%	21.24%	0.49

Table 3: Univariable logistic regression analysis for factors affecting pregnancy

	(OR (95% confidence interval	P-value
Treatment	1.44 (0.62- 3.32)	0.40
Age	0.95 (0.84- 1.09)	0.47
Gravidity	0.80 (0.48- 1.31)	0.37
Parity	0.95 (0.38- 2.39)	0.91
BMI	1.03 (0.89- 1.20)	0.69
Type of infertility	0.68 (0.29- 1.59)	0.38
Duration of infertility	0.97 (0.77- 1.23)	0.82
Tubal factor	0.15 (0.03- 0.79)	0.02
Number of failed ICSI	0.94 (0.59- 1.50)	0.80
FSH	1.34 (0.90- 2.00)	0.14
AMH	1.04 (0.76- 1.42)	0.82
Endometrial thickness (11 day)	0.83 (0.60- 1.13)	0.23
Endometrial thickness at FET	0.92 (0.69- 1.22)	0.03
Difference in thickness	1.07 (0.82- 1.40)	0.01

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Combination of Insulin Sensitizer and Omega-3 Fatty acids might minimize the risk for Cardiac events in PCOS women

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Abstract

Objectives: Evaluation of the effect of 3 months therapy of metformin/omega-3 (M/O) combination on body mass index (BMI), insulin resistance (IR), and oxidative and inflammatory milieu in PCOS women at probable cardiac risk (CR) as predicted by the atherogenic index of plasma (AIP).

Patients and methods: 90 PCOS women were randomly allocated into the M group received metformin (500 mg bi-daily) and M/O group received metformin (500 mg bi-daily) and Omega3 (950 mg active omega-3 once daily). Pre- and Post-treatment BMI, Homeostasis model assessment for IR (HOMA-IR), AIP and serum tumor necrosis factor-α (TNF-α), interleukin (IL)-β, superoxide dismutase (SOD), and malondialdehyde (MDA) levels were evaluated. The primary outcome is the effect of provided 3-m therapy AIP.

Results: Pre-treatment AIP defined 15.6% and 58.9% of studied women had high or intermediate cardiac risk (CR), 59 women were obese, 16 women were morbidly obese, and 52 women were insulin resistant with elevated serum levels of TNF-α, IL-1β, MDA, and lower serum SOD levels. Combined therapy allowed a significant decrease in HOMA-IR score, serum TNF-α, IL-1β, and MDA levels with significant elevation of serum SOD. Combination therapy significantly reduced the AIP in comparison to pre-treatment AIP and to that of women of the M group. Moreover, no woman still had high CR after M/O therapy and the frequency of women who had low CR was increased by about 107%.

Conclusion: Insulin sensitizers could improve PCOS-associated disturbances. However, omega-3 adjuvant therapy significantly augmented the effects of insulin sensitizers, minimized the cardiac risk factors, and decreased the risk of probable cardiac events.

Keywords: PCOS, Omega 3, Metformin, Atherogenic index of plasma, Cardiac risk.

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Introduction

Polycystic ovarian syndrome (PCOS) is a complex disorder that affects around 5-10% of women of childbearing age worldwide [1]. PCOS is associated with traits including hyperandrogenemia, irregular menstrual periods, obesity, and insulin resistance [2]. The progress of PCOS women to metabolic syndrome is an important bridge for the development of other diseases especially diabetes mellitus and coronary heart disease [3].

PCOS women frequently have an increased risk for adverse cardiac events secondary to progress to hypertension, atherosclerosis, and vascular disease [3], which gradually lead to endothelial dysfunction and coronary artery calcification with subsequent cardiac events [1].

Obesity is a major risk factor for cardiovascular (CV) disease in the general population and is highly prevalent in PCOS, but the role of hyperandrogenemia is still unclear (4). Moreover, antiandrogenic drugs as combined oral contraceptives usually used in adult women with PCOS carry a low risk of CV or thromboembolic events [5].

Dietary bioactive as omega-3 fatty acids, flavonoids, lutein, and zeaxanthin are food substances that promote health but are not essential to preventing typical deficiency conditions [6]. Omega-3 long-chain, polyunsaturated fatty acids (n-3 PUFA) are essential and had to be provided through the diet due to their limited biological synthesis [7]. The n-3 PUFA can protect against inflammation-related diseases including heart disease [8].

Cardiovascular diseases are defined as conditions involving decreased cardiac muscle blood flow that can lead to heart attacks, stroke, or other disorders [9]. Animal studies showed enhanced cardiac contractile efficiency with attenuation of dysfunction attributable to ischemia on supplementing animal diets with fish oil, in dose equivalent to regular consumption of fish in the human diet, for its high content of omega-3 docosahexaenoic acid [10]. Moreover, an epidemiological study suggested the importance of n-3 PUFAs in preventing ischemic heart disease [11]. Moreover, n-3 PUFA therapy in patients with virus-induced myocardial injury significantly regulated the expression levels of mRNA of and protein synthesis of Toll-like receptor 3 and 4, increased antioxidant gene expression, reduced the secretion of inflammatory factors, alleviated myocardial injury, and improved cardiac function [12].

Hypothesis

This study suggests that combined therapy with 3-n PUFA and metformin might act synergistically to improve PCOS-associated metabolic problems that most probably pave the way for PCOS-associated cardiac diseases.

Objectives

Evaluation of the effect of metformin/omega-3 (M/O) combination therapy on body mass index (BMI), insulin resistance (IR), lipid profile, and oxidative and inflammatory milieu in PCOS women at probable cardiac risk (CR) as predicted by the atherogenic index of plasma (AIP).

Design

Prospective interventional comparative study

Setting

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Patients & Methods

This study was conducted from Jan 2020 till Feb 2021 after approval of the study protocol by the Local Ethical Committee to include all women attending the infertility clinic at Benha University Hospital with a picture

suggestive of PCOS for evaluation and those eligible for inclusion were enrolled in the study after signing a written fully informed consent to participate the study and donate blood samples for assigned investigations.

Evaluation Parameters

1. Diagnosis of PCOS

Patients were considered to have PCOS if there were at least two of Rotterdam criteria[13]. Rotterdam criteria included the following items [14]:

(a) Menstrual history: amenorrhea or oligomenorrhea. Oligomenorrhea was defined as having <8 spontaneous menstrual cycles yearly for at least 3 years

(b) Lab findings: Hyperandrogenemia was defined as serum total testosterone level of >0.8 ng/ml

(c) US findings: ovarian volume of >10 ml per ovary on transvaginal ultrasound (TVU) imaging or ovaries containing >12 follicles of varied sizes and ranging between 2 and 9 mm

2. Diagnosis of obesity

Obesity was diagnosed and graded according to BMI which was calculated according to Bray [15] as body weight (in kg) divided by body height (in m^2). BMI was graded according to WHO guidelines [16] as underweight (BMI<18.5 kg/m^2), average weight (BMI=18.5-24.9 kg/m^2), overweight (BMI=25-29.9 kg/m^2), obese-1 (BMI=30-34.9 kg/m^2), obese-2 (BMI=35-39.9 kg/m^2) and obese-3 (BMI>40 kg/m^2).

3. Insulin resistance diagnosis and scoring

Insulin resistance (IR) was evaluated using the homeostasis model assessment (HOMA). The HOMA-IR score was calculated as (fasting serum insulin (μU/ml) x [fasting plasma glucose (mg/ml)/18])/22.5 [17] with HOMA-IR index of >2 indicates IR [18].

4. Atherogenic index of plasma (AIP)

The atherogenic index of plasma (AIP) is defined as the base 10 logarithms of the ratio of plasma triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) [19]. AIP was employed as a predictor of CR with values of -0.3 to 0.1 are associated with low, values of 0.1-0.24 are associated with the medium, and values above 0.24 with high CR [20].

Laboratory investigations

Blood Sampling

Blood sampling was conducted before starting treatment (Pre-T) and after the end of the 3-m treatment period (Post-T). All enrolled women were asked to attend the hospital lab fasting for 12 hours and gave a blood sample for estimation of blood lipids and to re-attend on the second day fasting 6 hours and gave another blood sample for estimation of fasting blood glucose (FBG) and other parameters. Blood samples were obtained under complete aseptic condition and divided into three parts:

1. The first part was put in a tube containing sodium fluoride (2 mg sodium fluoride/ml blood) to prevent glycolysis for estimation of FBG levels.
2. The second part was put in EDTA containing tube to determine the levels of TG and HDL-c and to calculate the AIP.
3. The third part was collected in a plain tube, allowed to clot, centrifuged at 1500×g for 15 min, and the serum samples were collected in a clean Eppindorff tube and stored at -20°C till be ELISA assayed.

Estimated parameters

Blood sampling was conducted before starting treatment

1. Blood glucose levels were estimated by the glucose oxidase method using

BT1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).

2. Plasma levels of triglycerides (TG) and high-density lipoprotein (HDL) were estimated by photoluminescence methods using BT1500 Automatic biochemistry analyzer (SPAN Diagnostics, Gujarat India).
3. Serum levels of insulin, testosterone (T), and sex-hormone-binding globulin (SHBG) using Automatic Immunoassay Analyzer (MAGLUMI 600, Snipe Diagnostic Co., Ltd., China).
4. ELISA estimation of serum levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-β, superoxide dismutase (SOD), and malondialdehyde (MDA).

Exclusion criteria

Menstrual disturbances and/or infertility due to causes other than PCOS, obesity inducing endocrinopathy, ovarian cysts for any cause, hyperprolactinemia, adrenal or ovarian tumor, thyroid dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, current or previous pregnancy within 1 year of enrollment, autoimmune disease, malignancy, chronic inflammatory disorders, current or previous use of oral contraceptives within 6 months of enrollment. Women younger than 30 years were also excluded from the study

Inclusion criteria

Women aged >30 years and had menstrual disorders and/or infertility secondary to PCOS who were free of exclusion criteria and signed fully-informed written consent to participate in the study, receive medications and follow-up visits.

Randomization & Masking

Women who fulfilled the inclusion criteria were randomly divided into two study groups using cards carrying group labels and put in a closed dark envelope. Cards were prepared by an assistant who was blinded about the significance of the label and was chosen by the patient herself. Collection of baseline data and prescription of medications was the duty of one of the authors. Blood samples were obtained and numbered by code numbers and the clinical pathologist was blinded about the baseline data and indications for investigations. Post-treatment data were collected by the 2nd author who was blinded about both the baseline data and results of laboratory investigations.

Groups

Twenty age and BMI-matched fertile females with regular menstrual cycle and free of exclusion criteria were collected as a control group. The enrolled PCOS women were divided into two equal groups:

1. Metformin group (M group): included women who received metformin HCl (Glucophage, Minapharm Pharmaceuticals, Amyria, Egypt) 500 mg film-coated tablets twice daily for three months.
2. Combination group (M/O group): included women who received metformin HCl (Glucophage, Minapharm Pharmaceuticals, Amyria, Egypt) 500 mg film-coated tablets twice daily and 3-n PUFA (Omega 3 Fish oil,) containing 625 mg eicosapentaenoic acid (EPA), 244 mg of docosahexaenoic acid (DHA) with mixed natural tocopherols and fish ingredients. The 3-n OMEGA was provided as soft gel tablets 950 mg active omega-3 and was taken once daily with a meal for three months.

Study outcomes

1. The primary outcome is the effect of provided 3-m M/O therapy in comparison to 3-m metformin therapy on AIP and level of CR in PCOS women.
2. Secondary outcomes included the effects of therapy on BMI, IR, and serum levels of studied biomarkers

Results

During the duration of the study, 127 women were eligible for evaluation, but 37 were excluded for not fulfilling the inclusion criteria and 90 women with a mean age of 35.7 (±2.7) years, were randomly divided into two groups (Fig. 1). Twenty control women of mean age of 35.9 (±2.4) were also included in the study.

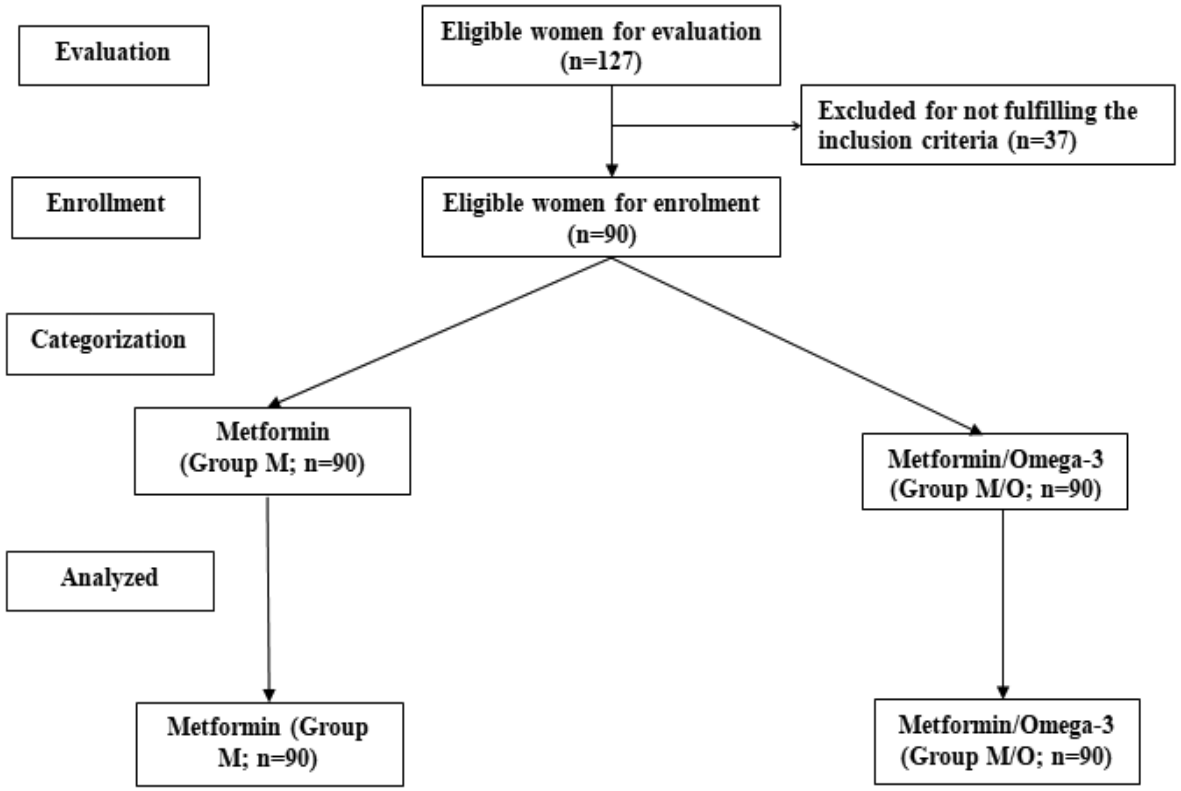

Fig. (1): Study Flow Chart

Pre-treatment BMI (BMI-1) showed a non-significant ($p>0.05$) difference between women of both groups and was non-significantly ($p>0.05$) higher in comparison to BMI of control women. Post-treatment BMI (BMI-2) of women of group M was non-significantly ($p=0.076$) lower in comparison to their BMI-1 with a mean decrease of 2.77 (1.4%), but was still non-significantly higher in comparison to BMI of control women. On contrary, BMI-2 of women of the M/O group was significantly ($p=0.0016$) lower in comparison to their BMI-1 with a mean percentage of decreased BMI of 4.8 (2.4%) and was non-significantly lower than BMI of control and M group women. The percentage of decreased BMI-2 of women of the M/O group was significantly ($p<0.0001$) higher in comparison to that of women of the M group. The distribution of women among BMI strata showed a decreased frequency of morbidly obese and increased frequency of women with average BMI with the non-significant difference between both groups (Table 1, Fig. 2).

Pre-treatment HOMA-IR score was significantly ($p<0.0001$) higher in women of study groups in comparison to control women with non-significant ($p=0.509$) difference between both study groups. Unfortunately, the post-treatment HOMA-IR score of women of both study groups was still significantly ($p<0.0001$) higher in comparison to the score of control women. However, the post-treatment HOMA-IR score was significantly low-

er in both group M (p=0.0002) and M/O (p<0.0001) in comparison to their pre-treatment score with significantly (p=0.0146) lower score of women of M/O group in comparison to that of women of M group. The frequency of IR women was decreased non-significantly (p=0.089) in women of group M but was decreased significantly (p=0.011) in women of group M/O after treatment in comparison to before treatment, despite the non-significant difference between the frequencies of IR women between both groups on both pre-and post-treatment evaluations (Table 1, Fig. 3).

Table 1: BMI and IR data of women of study and control groups and percentage of change after treatment

Variable \ Group		C (n=20)	M (n=45)	M/O (n=45)	P value		
					C vs. M	C vs. M/O	M vs. M/O
BMI (kg/m²)							
Pre-treatment		32 (1.6)	33.3 (2.4)	32.6 (2.4)	0.066	0.57	0.431
Post-treatment			32.4 (2.4)	31 (2.2)	0.911	0.171	0.073
P1 value			0.076	0.0016			
% of change			2.77(1.4%)	4.8 (2.4)			<0.0001
Pre	Av: Ob: MO	4:16:0	6:29:10	9:30:6	0.070	0.222	0.446
Post			9:29:7	12:29:4			0.529
P1 value			0.561	0.673			
HOMA-IR score							
Pre-treatment		0.76±0.19	2.17±0.42	2.07±0.39	<0.0001	<0.0001	0.509
Post-treatment			1.84±0.37	1.59±0.4	<0.0001	<0.0001	0.0146
P1 value			0.0002	<0.0001			
% of change			15.1±5.7	23.3±10.5			0.0001
Pre	IR: IS		29:16	26:19			0.517
Post			21:24	14:31			0.13
P1 value			0.089	0.011			

Data are shown as mean, standard deviation, percentages, and ratios; C: control group; M: Metformin group; M/O: Metformin/Omega 3 group; BMI: Body mass index; AV: Average weight; Ob: obese; MO: morbid obese; HOMA-IR: Homeostasis model assessment of insulin resistance; IR: Insulin resistant; IS: Insulin sensitive; P-value indicates the statistical significance of the difference between the three groups; P1 value indicates the statistical significance of the difference between pre-and post-treatment values; P<0.05 indicates a significant difference; P>0.05 indicates a non-significant difference

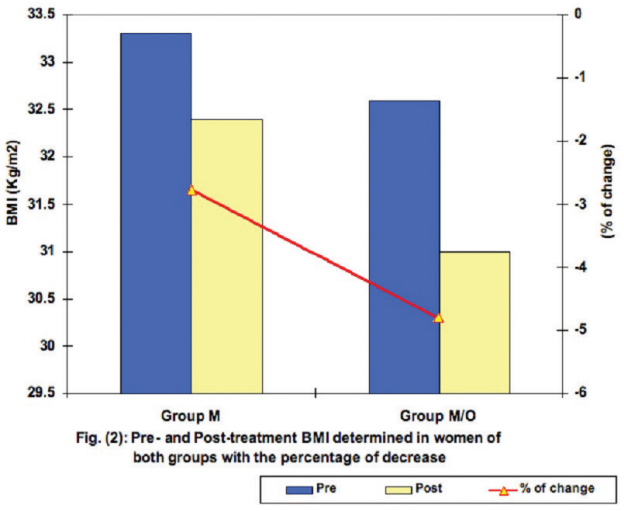

Fig. (2): Pre- and Post-treatment BMI determined in women of both groups with the percentage of decrease

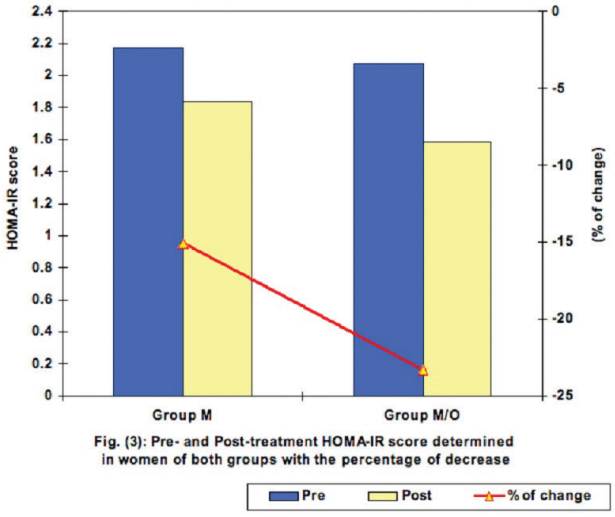

Fig. (3): Pre- and Post-treatment HOMA-IR score determined in women of both groups with the percentage of decrease

Pre-treatment serum TG levels were significantly (p <0.0001) lower, while serum HDL levels were significantly (p <0.0001) higher in control women in comparison to that of women of both study groups. Irrespective of the effect of treatment, post-treatment levels of women of study women were still significantly (p <0.0001) different in comparison to control women. Metformin therapy alone allowed a non-significant (p=0.215) reduction of serum TG with a mean percentage of decrease of 2.59 (±1.24), while it allowed a significant (p=0.038) increase of serum HDL with the percentage of increase of 4.62 (±1.66). On the other hand, combined therapy allowed a significant reduction of serum TG (p=0.0002) with significant elevation of serum HDL (p=0.0008) in comparison to pre-treatment levels. Moreover, post-treatment serum levels of TG and HDL in women of the M/O group showed significant differences (p=0.0194 & 0.031, respectively) in comparison to post-treatment levels estimated in women of the M group. Also, the percentages of change of serum TG and HDL in women of the M/O group were significantly higher in comparison to percentages of change in women of the M group (Table 2, Fig. 4).

Table 2: Atherogenic index of plasma of women of study and control groups and percentage of change after treatment

Variable \ Group	C (n=20)	M (n=45)	M/O (n=45)	P value		
				C vs. M	C vs. M/O	M vs. M/O
TG (mg/dl)						
Pre-treatment	89.8±14.4	122±8.2	123.6±12.3	<0.0001	<0.0001	0.838
Post-treatment		120.4±12	115.3±7.8	<0.0001	<0.0001	0.0194
P1 value		0.215	0.0002			
% of change		2.59±1.24	5.48±0.57			<0.001
HDL-c (mg/dl)						
Pre-treatment	45.4±4.6	38±4	39±3.3	<0.0001	<0.0001	0.552
Post-treatment		39.7±3.8	41.3±3	<0.0001	<0.0001	0.031
P1 value		0.038	0.0008			
% of change		4.62±1.66	6±2.62			0.004
AIP						
Pre-treatment	-0.07±0.11	0.152±0.08	0.136±0.062	<0.0001	<0.0001	0.337
Post-treatment		0.121±0.075	0.084±0.058	<0.0001	<0.0001	0.0085
P1 value		0.066	0.0001			

Cardiac risk							
Pre	Low	19 (95%)	8 (17.8%)	15 (33.3%)	<0.0001	0.00002	0.094
	Intermediate	1 (5%)	27 (60%)	26 (57.8%)			
	High	0	10 (22.2%)	4 (8.9%)			
Post	Low		16 (35.6%)	31 (68.9%)	0.00005	0.021	0.002
	Intermediate		24 (53.3%)	14 (31.1%)			
	High		5 (11.1%)	0			
P1 value			0.105	0.0014			

Data are shown as mean, standard deviation, percentages, and ratios; C: control group; M: Metformin group; M/O: Metformin/Omega 3 group; TG: Triglycerides; HDL-c: High-density lipoprotein cholesterol; P-value indicates the statistical significance of the difference between the three groups; P1 value indicates the statistical significance of the difference between pre-and post-treatment values; P<0.05 indicates a significant difference; P>0.05 indicates a non-significant difference

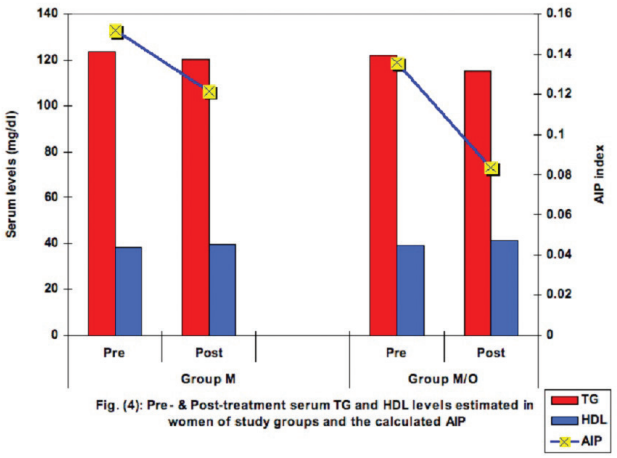

Fig. (4): Pre- & Post-treatment serum TG and HDL levels estimated in women of study groups and the calculated AIP

The calculated pre-and post-treatment AIP of women of study groups was significantly (p<0.0001) higher in comparison to AIP of control women. Pre-treatment AIP of women of group M was non-significantly higher, while post-treatment of group M was significantly higher in comparison to the corresponding AIP of women of group M/O (Fig. 4). Only one of the control women had intermediate CR, while 19 women had low CR. On contrary, pre-treatment CR was high, intermediate, and low in 14 (15.6%), 53 (58.9%), and 23 (25.5%) study women, respectively, with a non-significantly higher frequency of women, had high CR among women of group M. Both lines of therapy, significantly reduced the cardiac risk, but such effect was non-significant (p=0.103) with metformin alone, while was significant (p=0.0014) with combined therapy, in comparison to their respective pre-treatment frequencies. Moreover, at end of 3-m therapy, the frequency of women had mild, intermediate, and high CR was 16 women (35.6%), 24 women (53.3%), and 5 women (11.1%) in group M, while in group M/O, 31 women (68.9%) and 14 women (31.1%) had low and intermediate CR, respectively with a significantly higher frequency of women had low CR among women of group M/O (Table 2; Fig. 5).

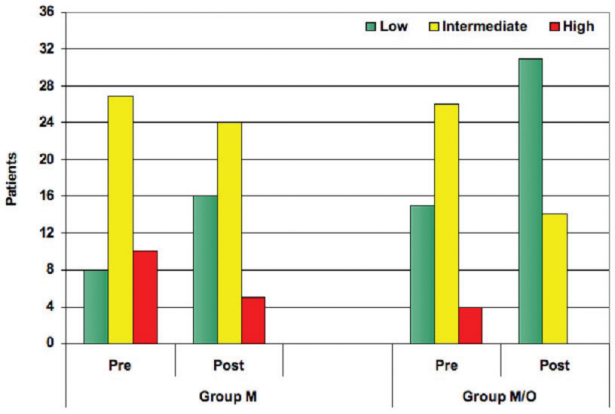

Fig. (5): Pre- and Post-treatment women' distribution according to the predicted cardiac risk by the calculated AIP

Both pre-and post-treatment serum TNF-α and IL-1β levels were significantly higher in comparison to levels estimated in control women with non-significant differences between women of study groups. Post-treatment serum TNF-α and IL-1β levels in women of group M were non-significantly lower in comparison to their pre-treatment levels with percentages of decrease of 3.83 (±2.62) and 3.9 (±2.2) for serum TNF-α and IL-1β levels. On contrary, post-treatment estimated serum TNF-α and IL-1β levels were decreased significantly (p=0.042 & 0.0488, respectively) in comparison to pre-treatment levels. The percentages of decrease of serum TNF-α and IL-1β levels in women of group M/O were significantly (p=0.0003 & 0.0004, respectively) higher in comparison to the percentages of decrease detected in women of group M (Table 3, Fig. 6).

Pre- and post-treatment serum MDA levels and SOD activity levels were significantly (p<0.0001) different in comparison to control levels. Post-treatment MDA levels were significantly decreased in women of groups M and M/O (p=0.0315 & <0.0001, respectively) with significantly (p=0.0019) lower levels in women of group M/O in comparison to women of group M. Moreover, the percentage of decreased MDA levels was (p<0.0001) higher in women of M/O group than in women of M group (Table 3, Fig. 7). Post-treatment SOD activity levels were significantly increased in women of both group M (p=0.0003) and group M/O (p<0.0001) with significantly (p=0.0376) higher activity levels in women of group M/O in comparison to women of group M. Moreover, the percentage of increased SOD activity levels was (p<0.0001) higher in women of M/O group than in women of M group (Table 3, Fig. 8).

Table 3: Pre- and Post-treatment serum levels of studied biomarkers in women of study and control groups and percentage of change after treatment

Variable \ Group	C (n=20)	M (n=45)	M/O (n=45)	P value		
				C vs. M	C vs. M/O	M vs. M/O
TNF-α (ng/ml)						
Pre-treatment	2.014±0.58	3.18±0.63	3.26±0.68	<0.0001	<0.0001	0.866
Post-treatment		3.084±0.6	2.98±0.6	<0.0001	<0.0001	0.763
P1 value		0.463	0.042			
% of change		3.83±2.62	8.05±7.12			0.0003
IL-1β (ng/ml)						
Pre-treatment	12.7±2.3	28.5±7.6	27.3±6.2	<0.0001	<0.0001	0.724
Post-treatment		27.4±7.4	25.1±4.4	<0.0001	<0.0001	0.217
P1 value		0.487	0.0488			
% of change		3.9±2.2	7.2±5.6			0.0004

MDA (nmol/ml)						
Pre-treatment	0.492±0.07	1.62±0.28	1.642±0.28	<0.0001	<0.0001	0.709
Post-treatment		1.5±0.26	1.328±0.25	<0.0001	<0.0001	0.0019
P1 value		0.0315	<0.0001			
% of change		7.51±2.83	18.9±7.25			<0.0001
SOD (IU/ml)						
Pre-treatment	1.9±0.12	1.6±0.16	1.56±0.17	<0.0001	<0.0001	0.578
Post-treatment		1.73±0.17	1.838±0.18	0.0004	0.309	0.0376
P1 value		0.0003	<0.0001			
% of change		8.36±2.2	18.25±8.5			<0.0001

Data are shown as mean, standard deviation; C: control group; M: Metformin group; M/O: Metformin/Omega 3 group; TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; MDA: Malondialdehyde; SOD: Superoxide dismutase; P-value indicates the statistical significance of the difference between the three groups; P1 value indicates the statistical significance of the difference between pre-and post-treatment values; P<0.05 indicates a significant difference; P>0.05 indicates a non-significant difference

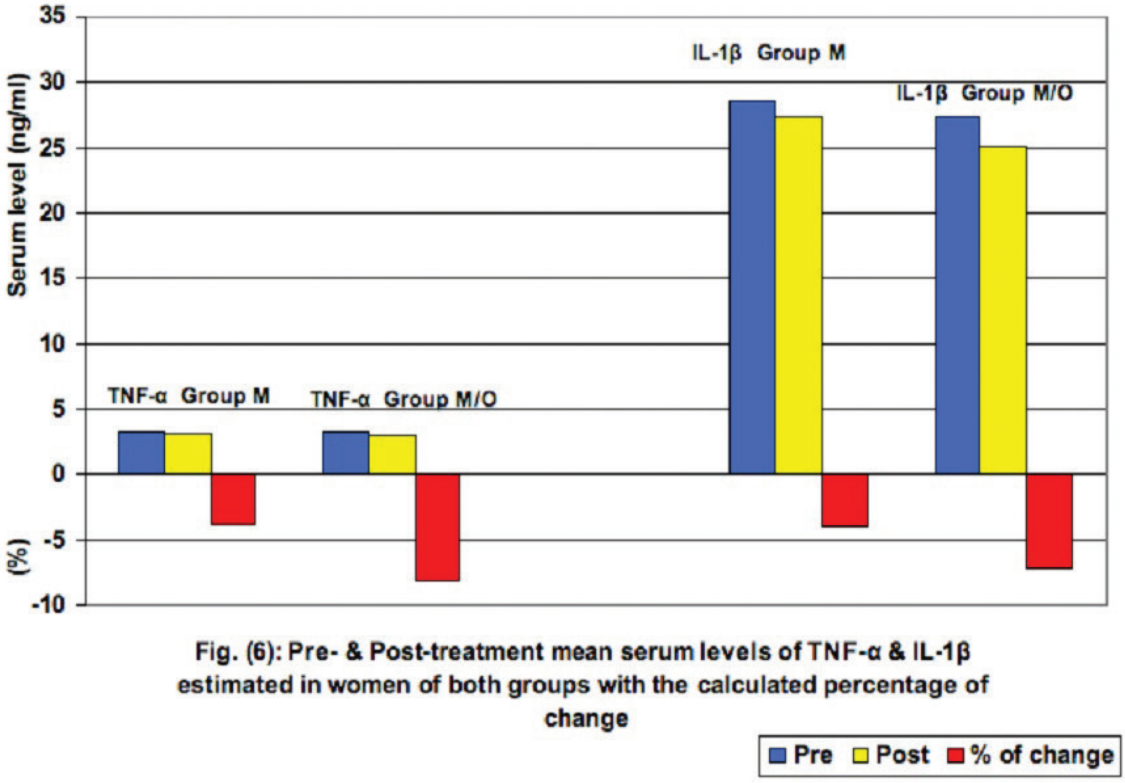

Fig. (6): Pre- & Post-treatment mean serum levels of TNF-α & IL-1β estimated in women of both groups with the calculated percentage of change

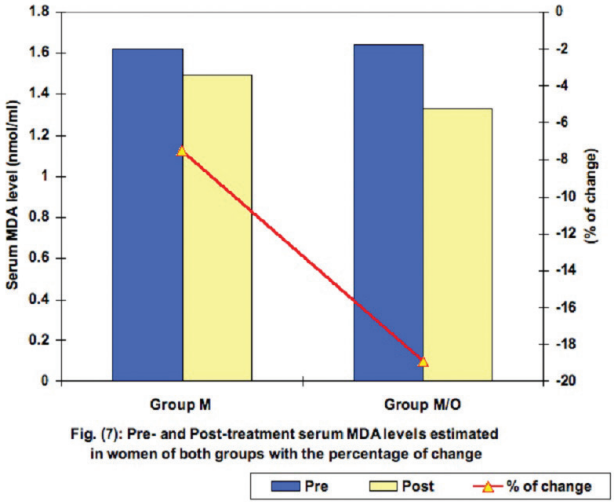

Fig. (7): Pre- and Post-treatment serum MDA levels estimated in women of both groups with the percentage of change

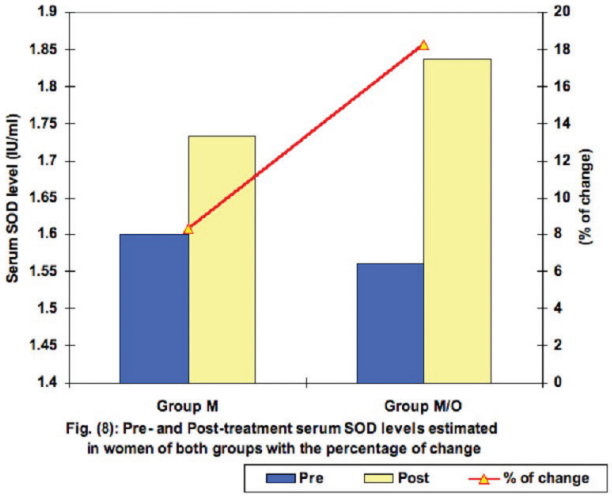

Fig. (8): Pre- and Post-treatment serum SOD levels estimated in women of both groups with the percentage of change

Discussion

The current study included 90 PCOS women with a mean age of 35.7 (±2.7), however, the probable risk for the cardiac event among these women as evaluated using the atherogenic index of plasma (AIP), defined 15.6% and 58.9% of these women had high or intermediate cardiac risk (CR). These findings point to a possible role of PCOS per se, its underlying pathogenic mechanisms, or its associated morbidities for accelerating and accentuating the risk for cardiac events. In support of this assumption, among the studied sample of PCOS women, 59 women were obese and 16 women were morbidly obese, and 52 women were insulin resistant. Moreover, mean serum levels of inflammatory cytokines and lipid peroxidation product malondialdehyde (MDA) were significantly higher in comparison to control fertile women of cross-matched BMI. These findings indicated the fact that PCOS is associated with the disturbed immune milieu in direction of inflammatory arm and redox status in direction of oxidative stress as evidenced by the significantly lower serum levels of SOD in PCOS women than in non-PCOS control women. These disturbances in addition to obesity and IR, both increase the probability of cardiac events, may underlie the reported level of probable CR.

In line with these data, **Ollila et al.** [21] found women at age of 31 years with self-reported PCOS that was manifested as oligo/amenorrhea and hirsutism were found to have higher blood pressure measures than controls and independently of BMI, they will have a higher incidence of cardiovascular morbidity in the premenopausal period. Also, **Mirdamadi et al.** [22] reported that high FBG levels and lipid profiles in obese patients with PCOS are a risk factor for coronary artery disease in PCOS women, but obesity is the more important risk factor and is recommended to assess and monitor CR factors in these women. Moreover, **Duică et al.** [1] documented that PCOS-associated cardiovascular comorbidities gradually lead to endothelial dysfunction and coronary artery calcification, thus posing an increased risk for adverse cardiac events. The applied 3-m therapeutic trial of metformin in combination with omega3 allowed significant improvement of insulin sensitivity with decreased HOMA-IR score, inflammatory status with significant reduction of serum levels of TNF-α and IL-1β, and redox status with a significant decrease of MDA levels with significant elevation of serum SOD. Similarly, **Tosatti et al.** [23] detected a reduction of the inflammatory state in women with PCOS with omega3 supplementation mostly through decreased serum levels of C-reactive protein. In line with obtained results, a meta-analysis to evaluate the effect of omega-3 supplemental therapy on CR

factors in patients with PCOS demonstrated a statistical reduction in serum levels of insulin, total cholesterol, triglyceride, low and very-low-density lipoprotein, and C-reactive protein with the improvement of HOMA-IR score and increased serum levels of high-density lipoprotein [24].

Regarding the probable CR, the metformin/omega 3 combination significantly reduced the AIP in comparison to pre-treatment AIP and to that of women who received metformin alone. Moreover, the effect of adding omega3 to metformin was evident as no woman still had high CR after the end of the therapy, and the frequency of women who had low CR was increased by about 107%. This marvelous effect of omega-3 could be attributed to the reported significant reduction of TNF-α, IL-1β, and MDA levels in comparison to pre-treatment levels and to post-treatment levels in women who received metformin alone.

In support of these findings, a systematic review and meta-analysis revealed that omega-3 and vitamin E co-supplementation have beneficial effects on lipid profile with significantly reduced serum levels of TG and LDL in overweight patients [25]. Another review of the literature reported moderate-certainty evidence suggesting that increasing long-chain omega-3 reduces the risk of coronary heart disease mortality and events with reduction of serum TG and increasing α-linolenic acid slightly reduces the risk of cardiovascular events and arrhythmia [26].

Clinically, **Musazadeh et al.** [27] found omega-3 significantly improvement of serum concentrations of insulin, high-sensitivity C-reactive protein, lipopolysaccharide, total antioxidant capacity, superoxide dismutase activity, MDA, and 8-iso-prostaglandin F2α in patients with non-alcoholic fatty liver in comparison to placebo. Also, **Fazelian et al.** [28] found omega-3 supplementation in chronic kidney disease patients significantly decreased total cholesterol, TG, and MDA levels with a concomitant significant increase of SOD and glutathione peroxidase activities

In line with the effect of omega3 on inflammatory and redox statuses, multiple recent experimental studies detected significant decreases in levels of inflammatory cytokines, improved redox state, and resolution or amelioration of the induced pathologies with the use of omega-3 supplemental therapy [29-31]. Moreover, a recent study had specified this effect to omega-3, not omega-6 where mice fed a diet rich in omega3 showed consistent reductions in serum TNF-α after exposure to 56Fe with no increase in the percentage of osteocytes positive for TNF-α, while this was consistent with the use of omega-6 [32] and another recent study assured a dose-related effect of omega3 on inflammatory cytokines [33].

These effects of omega3 could be attributed to its ability to down-regulate gene expression and mRNA transcription and translation of TNF-α leading to reduction of its serum levels [34]. The positive effect of n-3 PUFAs on lipopolysaccharide-induced inflammatory response was possibly mediated by the nuclear factor-kappa beta (NF-κB) signaling pathway [29]. Moreover, the anti-inflammatory and antioxidant effects of n-3 PUFA could be attributed to resolvin D1 which is the downstream metabolite of docosahexaenoic acid; resolvin D significantly induced higher levels of Bcl-2, SOD, and glutathione, nuclear levels of the nuclear factor erythroid 2-related factor 2 with a significant reduction in reactive oxygen species, MDA, TNF-α, IL-6, NF-κB and expression of cleaved caspase-3 [35].

Conclusion

PCOS is a multifaceted condition possibly induced by or induces obesity, insulin resistance, and systemic pro-inflammatory state and disturbed redox status leading to increased risk of probable cardiac events in these young-aged women. Insulin sensitizers could improve PCOS-associated disturbances. However, omega-3 supplementation as

an adjuvant to insulin sensitizer significantly augmented its effects, minimized the cardiac risk factors, and decreased the risk of probable cardiac events.

Limitations

Evaluation of combined metformin/omega-3 therapy on PCOS-associated hormonal milieu, ovulatory dysfunction, and fertility was also mandatory.

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Purse string suturing of the lower uterine segment in placenta accreta spectra syndrome for control of bleeding: a prospective case series study

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Abstract

Background: The placenta accreta spectrum (PAS) is a worldwide health problem due to increasing cesarean section rates. A severe maternal complication can occur due to bleeding and invasion of nearby organs. Although many techniques were described to help in intraoperative management, no standard agreement on the best one, and unfortunately, in many conditions, cesarean hysterectomy can only save the mother's life. There's a must to find out new techniques that could help in preserving the uterus.

Objective: This prospective study was taken on 26 patients at Mansoura University Hospital diagnosed with PAS. There were 13 cases PAS grade 1, 7 cases grade 2, 6 cases grade 3A. All the cases were electively delivered at 37 w. Purse string suturing was done to plicate the lower uterine segment and provide natural tamponade to decrease blood loss from the placental bed and preserve the uterus.

Results: the success rate of the procedure in preserving the uterus was 96%. The average blood loss was 1557.69 ± 318.96. Massive blood loss (2500 ml) occurred in 1 case, intraoperative after the procedure. The procedure is simple, not time-consuming, and no special skills are required. A large number of patients and long term follow up are needed to ensure the efficacy of the procedure and the state of future fertility.

Conclusion: management of patients with PAS should be individualized according to PAS grade and patient wishes. The purse-string suure technique can help in reducing blood loss and decrease the cesarean hysterectomy rate.

Keywords: Placenta accrete spectra syndrome, cesarean hysterectomy, lower uterine segment, a purse-string suture.

Introduction

The placenta accreta spectrum (PAS) is a severe obstetric complication of placental invasion into the uterine wall (1). It is associated with serious maternal morbidities globally, and its prevalence is likely to increase due to increasing rates of cesarean sections (C.S) (2)(3)(4).

However, accurate determination of its prevalence and outcome is problematic because most reports lack histopathologic examination (5) (6).

Maternal outcomes depend mainly on diagnosing the condition before(6) or during delivery (7). Cesarean hysterectomy is still the standard of management of PAS to avoid catastrophic haemorrhage (8). However, it is associated with significant maternal morbidities from bleeding, visceral injuries, sepsis, coagulopathy, and the potential risk of mortality(9).

Successful conservative management of PAS has a good impact on a woman's social status and self-esteem (10) and preserving the women's fertility. (11).

Jauniaux et al. defined conservative management as cutting the umbilical cord short and leaving the placenta in situ with close follow-up until resorption of the placenta(12). Some surgical techniques have been described in the literature to help in the conservative management of PAS with variable degrees of success(13).

Obstetricians face a real challenge in delivering such cases without a clear consensus on the best surgical technique(s) to manage PAS. Patients who opt for uterine conservation should be counselled; they may ultimately require an urgent cesarean hysterectomy to save their lives. (14–20).

Innovative techniques to tackle this problem are desperately needed. In this study, we report on a novel purse-string suture technique of the lower uterine segment that helps with the conservative management of PAS, reducing blood transfusion, shortening the operative time, and decreasing overall morbidity associated with cesarean hysterectomy.

Patients and methods

This prospective study was carried out in Mansoura University Hospital, Mansoura, Egypt, on 26 patients, between February 2020 and February 2021, after approval from the institutional research committee (IRB: R.20.02.736.R1), faculty of medicine, Mansoura University.

The patients included in this trial were pregnant in their late third trimester, with a history of one or more previous C.S., diagnosed with PAS by ultrasound, and confirmed intraoperatively as PAS grade 1, 2, 3(A) according to FIGO classification (20). Patients with PAS grade 3(B, C) and extensive adhesions make the lower uterine segment inaccessible; patients with medical disorders or patients with coagulopathy at the time of C.S were excluded.

The primary outcome was the number of patients with successful conservation of the uterus. The secondary outcomes were blood loss (measured directly by weight of soaked towels plus the amount in suction apparatus worked only after delivery of the baby, mean difference between pre-and postoperative haemoglobin levels), number of transfused packed RBCs, and visceral injuries.

Steps of the procedure

1. General anaesthesia was given to all patients, 1 gm cefotaxime before anaesthesia was given, and a wide lower transverse abdominal incision with complete haemostasis was done. The baby was delivered through a transverse incision of the uterus above the suspected margin of the placenta(12), followed by Cord clamping after 30 sec, exteriorization of the uterus, haemostasis of the uterine incision edges was done using many curved artery clamps as shown in figure (1).

2. The following ecbolics were given to all patients: Ergometrine 0.25 MGM + syntocinon 10 I.U., I.V, Carbeprost i.v injection to enhance uterine contraction and separation of non-adherent placental parts.
3. Sharp and/or blunt dissection of the urinary bladder from the lower uterine segment was done to the level of the internal cervical os.
4. Gentle trial of placental separation using controlled cord traction, the adherent parts will be removed by blunt and or sharp excision (8).
5. Purse string suture, using Vicryl 1(Ethicon, Johnson & Johnson international), was inserted with a large 1/2c rounded needle (48 mm) starting from the lowest accessible part on the lower uterine segment (above the level of the cervix), taking the entire thickness of the uterine wall including the decidua/placental tissues, moving in the anti-clock direction, taking bites from the most left lateral border of the anterior lower uterine segment, then 2 cm below lower uterine C.S incision edge, toward the right lateral border of the lower uterine segment, reaching near the starting point, then gentle traction on the threads to plicate the lower uterine segment and compress the whole anterior wall of the lower uterine segment including placental remnants as shown in figure (2).

All patients were followed up by telephone weekly to 6 weeks postpartum and were asked to contact us or return immediately if there is bleeding, offensive discharge, fever, or excessive lower abdominal pain.

Statistical Analysis

Statistical analysis was performed using the SPSS 19 system (SPSS Inc., Chicago, IL, United States). Continuous data were expressed as the mean ± S.D., and categorical variables expressed as a percentage. Means were compared using the unpaired student's t-test, while proportions were compared using the chi-squared test. A P-value of less than 0.05 was considered statistically significant.

Results

This study was carried out on 26 patients using those mentioned above purse-string suturing techniques of the lower uterine segment. Patients' demography is illustrated in table(1). The mean age of patients was 30.54 ± 5.34; the mean C.S number was 2.0 (1.0-5.0). According to the FIGO grading of PAS (2), there were 13 cases grade 1, 7 cases grade 2, 6 cases grade 3A using. All the cases were delivered at 37 w, in elective conditions. The estimated time to do this procedure (the suture) was 2-3 minutes.

In our study, the success rate in preserving the uterus is 96%. Massive blood loss (2500 ml) occurred in 1 case, intraoperative after the procedure, due to more placental tissue in place and over thinning of the uterine wall, urgent hysterectomy was done due to hemodynamic instability.

The average blood loss was 1557.69 ± 318.96; 5 patients (19.2%) reported 2000 ml or more blood loss; the mean number of packed red blood cells transfused was 2.5(1.0-4.0). The mean preoperative haemoglobin was 10.93±0.67 while the postoperative one was 10.45±0.91(t=2.01, p=0.06)

Uterine artery ligation on multiple levels was needed as an extra procedure in 1 case, as the patient continues to bleed after purse-string sutures due to more placental invasion into lateral uterine walls.

The average postoperative hospital stay is 6.0 days (3.0-8.0). All patients were discharged from the hospital in good condition, and no reoperations or readmissions were required. Fortunately, the puerperia of the patients were uneventful, and patients recover well.

Discussion

Varying grades of invasion may be found in the same placenta, creating technical surgical challenges, especially if there is extrautrine invasion (13).

The purse-string suturing technique is effortless, doesn't require special surgical skills as in internal iliac artery ligation, is not time-consuming, and allows the preservation of the lower uterine segment instead of resection. Cervical lips are not a part of the procedure, so, no cervical distortion. There is no risk of obliterating the uterine cavity or hematomata, as the technique involves only the anterior wall.

Purse string suturing mostly control PPH by compressing bleeding areas of the endometrium and myometrium, as the sutures involve the whole wall thickness. Compared to other techniques, it is a blood-saving procedure; the average blood loss is 1557.69±318.96, nearly the same as the cervical inversion technique (1572.5 mL) published by Elgelany et al. (19). Both multifaceted sutures proposed by Meng et al. (18) and multi-positional spiral sutures posted by Liu et al. (21), the average blood losses were 1327.3±1244.1 ml. , (1696± 1397) ml respectively.

The systematic review by Jauniaux et al. (22) reported an 89.7% (208/232) elective or emergent cesarean hysterectomy rate. In our study, the success rate in preserving the uterus is 96%. Meng et al. (18), using multifaceted sutures, reported no cesarean hysterectomy case, while using multi-positional spiral suture achieve uterine preservation in 94% (36/38) of patients (21), nearly the same as in the case of cervical inversion technique (95%)(19).

The mean time needed to perform cervical inversion(19) was 5.4 ± 0.6 min, nearly double the time in our procedure which helps in reducing blood loss and further blood transfusion.

The drawbacks of this study are the limited number of patients, lack of long-term follow-up, and state of future fertility. The placental remnants embedded in the uterine wall could predispose to infection, pyometra, and postpartum haemorrhage.

Uterine wall necrosis can occur due to compression of the feeding vessels and the massive dissection of the bladder from the lower uterine segment to place the suture. Finally, the technique cannot be applied to FIGO grade 3 B, C. Studies with a large number of patients are required to guarantee the technique's safety to incorporate it in the algorithm of management of PAS.

Conflict of interest

None

Aknowledegemt

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Table (1) patient demography

	n=26	%
Age/years Mean±SD (range)	30.54±5.34 (21.0-39.0)	
<30 30-35 >35	16 4 6	61.5 15.4 23.1
Gravidity Median (Range)	4.0(2.0-6.0)	
Parity Median (Range)	2.0(1.0-5.0)	
C.S. number Median (Range)	2.0(1.0-5.0)	

Figure (1)

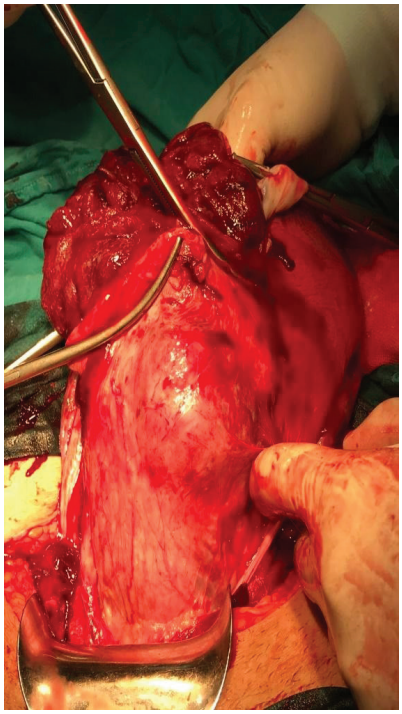
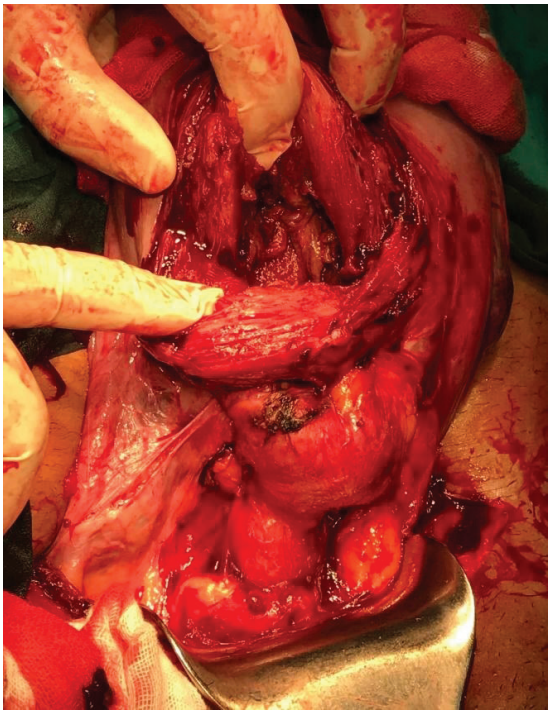


Figure (2)



Laparoscopic Ovarian Drilling in Polycystic Ovarian disease patients: is it hazardous?

Taher Mostafa

Abstract

Objective: to evaluate the outcomes of endoscopic ovarian drilling (LOD) on the Anti Mullerian hormone (AMH) plasma levels, potential changes of the stromal ovarian blood flow, and antral follicular count by three-dimensional power Doppler ultrasonography in an-ovulatory polycystic ovarian disease (PCOD) and if this can explain the ovarian drilling mechanism of action.

Methods: The prospective, comparative (case control) research was completed on fifty patients from Bab El-Shaarea University Hospital (El Sayed Galal). They were selected from infertility and family planning clinics. Women were assigned into one of two equal groups (each 25 patients). The first included infertile women, resistant to clomiphene citrate and scheduled for drilling. The second included healthy women from the family planning clinic, who were seeking for contraception. The main outcomes were AMH levels, Doppler indices and Antral follicular counts before and after drilling. The secondary outcome in the study group included occurrence of ovulation, chemical and clinical pregnancy rate within 6 months after drilling.

Results: The mean initial AMH, doppler indices and antral follicular counts were singingly higher among study than control group. AMH and AFC significantly reduced in cases after than before drilling [2.8 to 3.9], [13.9 to 15.8]. Ovarian FI, VI, and VFI significantly decreased after than before drilling. In the study group, 20 (80%) had ovulation, and 13 (52%) got pregnant within 6 months of the operation. Regression analysis showed that initial AMH, initial AFC, initial VFI, post-LOD AMH, post-LOD AFC, post-LOD VI, post-LOD FI and post-LOD VFI were significant indicators of successful ovulation and successful pregnancy within 6 months after drilling.

Conclusion: Endoscopic ovarian drilling had a significant decreasing effect on serum AMH, AFC, and Ovarian Doppler indices in PCOD patient not responding to clomiphene citrate. The initial values of AMH, AFC and VFI; besides post-drilling values of AMH, AFC, VI, FI, and VFI were significant indicators of ovulation after drilling.

Keywords: Laparoscopic; Ovarian Drilling; Polycystic Ovarian Disease; Anti Mullerian Hormone.

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Introduction

Polycystic ovarian disease (PCOD) is a disorder that hits 5–10% of females of reproductive age group (1). Twelve or more follicles with a dimeter of 2–9 mm or increased the volume of ovaries (>10 cm3) must be present to diagnose the condition by trans-vaginal scanning (2).

Definition of PCOD requires the existence of at least 2 of the next 3 diagnostic criteria: oligo-ovulation and/or anovulation, clinical and/or laboratory features of hyper-androgenism, and ultrasonic polycystic ovarian features (3).

Rising blood flow of ovarian stroma is emerging as an indicator to help in the diagnosis. The average time of maximum ovarian velocity and stromal peak systolic blood flow velocity were significantly higher in PCOD than in normal females (4).

Three-dimensional power Doppler showed significantly higher blood flow of the ovarian stroma in PCOD than in normal ovaries (5).

The pattern assessment of blood flow by 2D ultra-sonography, can be achieved by objective measurement of velocity and the resistance to flow by pulsed-wave Doppler with subsequent analysis of the waveforms derived from a single vessel or by applying color or 2D power Doppler to a single plane (6).

Color Doppler is less sensitive than Power Doppler for detection of low flow velocity, and thus it overcomes the angle dependence of standard color Doppler and provides improved visualization of small vessels (7).

Anti-Mullerian hormone (AMH) belong to the family of transforming growth factor-β (TGF- β). AMH is mainly secreted by the granulosa cells (GCs) of early ovarian developing follicles (8). Its expression is limited to GCs of primary, pre antral, and small antral follicles, suggesting that, it plays a crucial role in folliculogenesis (9).

It was demonstrated that AMH reduces the follicles sensitivity to circulating FSH, an effect that is significant for normal folliculogenesis, also it was shown that AMH is two to threefold rise-up in serum of PCOD than females with intact ovaries (10).

Controversial information is now available regarding whether an intrinsic AMH production by the GCs played a role or cause follicular arrest in PCOD or the AMH excess in PCOD is secondary to the rise in pre-antral follicles number (11).

Increased AMH concentrations may be related to other factors in PCOD, the most obvious being hyper-androgenism (12) and increased resistance to insulin (13).

Increased flow of stromal ovarian blood in PCOD may result from increased production of vascular endothelial growth factor (VEGF), which may modulate the increased insulin-like growth factor I (IGF-I), that enhances gonadotropin stimulated steroid formation in GCs and theca cells, resulting in increased ovarian production of androgens and subsequently increased AMH production (13) and the vascular permeability of theca cells (14).

Knowing factors that determine the response of females with PCOD to drilling will help in selecting cases that may benefit from this treatment modality (15). Factors affecting the success of LOD was reported in a previous study (16).

AMH had been discovered to correlate with ovarian responsiveness to clomiphene citrate (CC) (17) and to FSH controlled ovarian hyper-stimulation for IVF cycles (18). AMH serum level consistency along the menstrual cycle, with very little inter cycle variability, makes it an attractive indictor of response to treatment (19). LOD mechanism is not yet unexplained. Particularly, it is not accurately known whether drilling exerts its effect by a systemic endocrine mechanisms or through a direct action on the ovary (20).

Patients and Methods

The current work was performed at Al-Sayed Galal University Hospital between Marsh 2015 and December 2016. It was a prospective case control study that was held on fifty patients divided into 2 groups. Case group included 25 infertile patients of PCOD not responding to clomiphene citrate and exposed to endoscopic ovarian drilling, and Control group includes 25 healthy patients from family planning clinic seeking for contraception.

Inclusion Criteria:

1. Age between 18-35 years old.
2. Diagnostic features for PCOD included at least 2 of the next 3 features according to Rotterdam ESHRE 2004 :

a)Amenorrhea or oligomenorrhea with chronic anovulation.

b)Clinical and/or labortory evidence of hyperandrogenism.

c)Ultra-sonographic criteria of PCOS.

3. Ovulation failure after administration of CC up to a 150 mg, daily dose from cycle days 2–6 for three consecutive cycles.
4. Ovaries were classified as normal or polycystic by ultrasonic examination, the follicle number of 0.2–0.9 cm in diameter will be counted in both ovaries. The threshold will be established at 12, thus diagnosing polycystic ovaries by the existence of 12 or more follicles. If the average follicle number is less than 12, the ovaries will be considered as normal (21).

Exclusion Criteria:

1. Age <18 or >35 years.
2. Infertility due to male factor.
3. Tubal Factor investigated with hystero-salpingography.
4. Previous or current use of metformin.
5. Diseases potentially affecting the ovarian environment and/or function (including leiomyomas and endometriosis) or any organic pelvic diseases.
6. Women with single ovary.
7. Previous ovarian cystectomy.
8. Hyper-prolactinemia.
9. Thyroid disease.
10. Diabetes mellitus.

Procedure

> For all participants, the age and body mass index (BMI) were recorded. Drilling was performed in the follicular phase of the menstrual cycle. Cauterization of each ovary at 4 points, for 4 seconds at each point, using 40W of power with a high-frequency monopolar current microneedle, 10 mm long regardless of the size of the ovary.

> On the morning of the 3rd day of the same cycle in which the operation would to be performed, a sample of blood was drawn from each patient before drilling for AMH assay.

> Another blood sample was drawn on the third day of the first post-LOD cycle. Blood samples were drawn also from the control women on the third day of the menstrual cycle.

> AFC was obtained in the follicular phase before LOD and repeated in the follicular phase of the first post-LOD cycle. However, the AFC was examined also in the follicular phase of any cycle in the control group.

> A conventional 3D power Doppler ultrasound system having the VOCAL program (Virtual Organ Computer Aided Analysis) was used. Evaluation was performed for both ovaries excluding the ovarian vessels. The site of interest included the whole ovary except for the ovarian vessels.

> Examination was performed in the follicular phase prior to LOD and was repeated in the follicular phase of the first cycle

after LOD. Examination was done in the follicular phase of any cycle in the control group. Three indices were calculated: flow index (FI); vascularization index (VI); and vascularization flow index (VFI).

> The measured items between the 2 groups were compared using the Student t test. Comparisons of values before and after drilling in the PCOD group were done by the paired t test. P value <0.05 will be considered to be statistically significant.

> Follow up of the case group patients was done by doing folliculometry monthly for 6 successive months within them the patients receive clomiphene citrate of a daily dose of 150 mg from the day 3rd to the 7th day.

> The 1ry outcomes were:

> AMH level before and after drilling in case group and its relation with it in the control group. The change in the measures of the 3D power Doppler indices of the stromal ovarian blood flow before and after the operation in case group and its relation to them in the control group.

> The change in antral follicle count before and after drilling in case group and its relation with it in the control group.

> The 2ry outcomes were:

> Ovulation occurrence in the case group patients within 6 months of the operation and with ovulation induction using clomiphene citrate 150 mg daily from 3rd to day 7th day.

> Pregnancy occurrence in the case group patients within 6 months of the operation and with ovulation induction using clomiphene citrate of a daily dose of 150 mg from the 3rd to the 7th day of the cycle.

Statistical Analysis

Statistical analysis was done using SPSS for Windows version 15.0. Data were presented as range, mean and standard deviation (for parametric variables), number and proportion (for categorical variables). Difference between both groups was analyzed using independent student's t-test (for parametric variables), Fischer's exact or chi-squared (for categorical data). Association between variables was estimated using Pearson's correlation coefficient (for parametric variables) and Spearman's correlation coefficient (for non-parametric variables). Significance level was set at 0.05.

Statistical Analysis

Table-1 Difference between Groups regarding Initial Characteristics

	Group I [PCOS Group] (n=25)	Group II [Control Group] (n=25)	MD/OR (95% CI)	P
Age (years)				
Range	19 – 33	18 – 34	-1.6	0.901 [1]
Mean ± SD	26.2 ± 4.27	26.36 ± 4.75	(-2.7 to 2.4)	NS
Parity				
0	20 (80%)	0 (0%)		<0.001 [2]
≥ 1	5 (20%)	25 (100%)	NE	HS
No. of Miscarriages				
0	16 (64%)	21 (84%)	0.34	0.107 [2]
1 – 2	9 (36%(	4 (16%)	(0.09 to 1.3)	NS

Weight (kg) **Range** **Mean ± SD**	 58 – 90 70.6 ± 9.49	 58 – 88 71.2 ± 9.6	 -0.6 (-6.1 to 4.8)	 0.814 [1] NS
BMI (kg/m2) **Range** **Mean ± SD**	 23.34 – 29.74 26.58 1.85	 22.66 – 29.07 25.73 ± 2.01	 0.9 (-0.2 to 1.95)	 0.123 [1] NS

SD standard deviation
IQR interquartile range
MD (95% CI) mean difference and its 95% confidence interval
OR (95% CI) odds ratio and its 95% confidence interval
1 Analysis using independent student's t-test
2 Analysis using chi-squared test
NS non-significant – HS highly significant
NE not estimable

Table-2 Difference between Groups regarding Initial Serum AMH and AFC

	Group I [PCOS Group] (n=25)	**Group II [Control Group] (n=25)**	**MD (95% CI)**	**P**
Initial Serum AMH (ng/ml) **Range** **Mean ± SD**	 3.2 – 11.6 7.19 ± 2.45	 1.2 – 3.8 2.23 ± 0.69	 4.96 (3.9 to 5.9)	 <0.001 [1] HS
Initial AFC **Range** **Median (IQR)**	 24 – 36 29 (27 – 30)	 8 – 18 12 (10 – 14)	 16.9 (15.3 to 18.5)	 <0.001 [2] HS

SD standard deviation
IQR interquartile range
MD (95% CI) mean difference and its 95% confidence interval
1 Analysis using independent student's t-test
2 Analysis using Mann-Whitney's U-test
HS highly significant

Table-3 Difference between Groups regarding Initial 3D Power Doppler Indices

	Group I [PCOS Group] (n=25)	**Group II [Control Group] (n=25)**	**MD (95% CI)**	**P**
Initial VI **Range** **Mean ± SD**	 3.2 – 6.5 4.84 ± 1.08	 1.2 – 2.5 1.84 ± 0.36	 3.01 (2.5 to 3.5)	 <0.001 [1] HS
Initial FI **Range** **Mean ± SD**	 47.1 – 57.1 52.45 ± 3.46	 37.2 – 48.3 41.81 ± 3.21	 10.63 (8.7 to 12.5)	 <0.001 [1] HS
Initial VFI **Range** **Mean ± SD**	 2.2 – 3.5 2.89 ± 0.41	 0.5 – 1.3 0.92 ± 0.25	 1.98 (1.8 to 2.2)	 <0.001 [1] HS

SD standard deviation
VI vascularization index

FI flow index
VFI vascularization flow index
MD (95% CI) mean difference and its 95% confidence interval
1 Analysis using independent student's t-test
HS highly significant

Table-4 Difference between Pre- and Post-LOD Serum AMH and AFC

Group I [PCOS Group]	Pre-LOD	Post-LOD	MPD (95% CI)	P
Serum AMH (ng/ml)				
Range	3.2 – 11.6	1.8 – 6.9	3.34	<0.001 [1]
Mean ± SD	7.19 ± 2.45	3.84 ± 1.51	(2.8 to 3.9)	HS
AFC				
Range	24 – 36	10 – 19	14.9	<0.001 [2]
Median (IQR)	29 (27 – 30)	14 (12 – 16)	(13.9 to 15.8)	HS

SD standard deviation
IQR interquartile range
MPD (95% CI) mean paired difference and its 95% confidence interval
1 Analysis using paired student's t-test
2 Analysis using Wilcoxon signed rank test
HS highly significant

Table-5 Difference between Pre- and Post-LOD 3D Power Doppler Indices

Group I [PCOS Group]	Pre-LOD	Post-LOD	MPD (95% CI)	P
Initial VI				
Range	3.2 – 6.5	1.4 – 3.5	2.56	<0.001 [1]
Mean ± SD	4.84 ± 1.08	2.28 ± 0.62	(2.3 to 2.8)	HS
Initial FI				
Range	47.1 – 57.1	40.5 – 47.5	9.03	<0.001 [1]
Mean ± SD	52.45 ± 3.46	43.42 ± 2.22	(8.2 to 9.9)	HS
Initial VFI				
Range	2.2 – 3.5	0.5 – 1.95	1.79	<0.001 [1]
Mean ± SD	2.89 ± 0.41	1.1 ± 0.54	(1.7 to 1.9)	HS

SD standard deviation
VI vascularization index
FI flow index
MPD (95% CI) mean paired difference and its 95% confidence interval
1 Analysis using paired student's t-test
HS highly significant

Table-6 Incidence of Ovulation and Pregnancy within 6 months among Women of Group I [PCOD Group]

Ovulation within 6 months	**20 (80%)**
Pregnancy within 6 months	**13 (52%)**

Data presented as number (percentage)

Table-7 Binary Logistic Regression Analysis for Measured Variables as indicator of pregnancy and Ovulation within 6 months after LOD

	Ovulation within 6 months after LOD		Pregnancy within 6 months after LOD	
	OR (95% CI)	P	OR (95% CI)	P
Age	0.97 (0.8 to 1.2)	0.811	0.91 (0.74 to 1.1)	0.317
Weight	0.92 (0.8 to 1.03)	0.140	0.94 (0.86 to 1.03)	0.197
BMI	0.78 (0.4 to 1.4)	0.387	0.78 (0.49 to 1.23)	0.289
Initial AMH	0.17 (0.03 to 0.96)	0.045	0.54 (0.33 to 0.89)	0.016
Initial AFC	0.26 (0.07 to 0.89)	0.032	0.62 (0.39 to 0.98)	0.040
Initial VI	0.001 (0.00 to 2.56)	0.086	0.46 (0.2 to 1.06)	0.068
Initial FI	0.10 (0.008 to 1.46)	0.084	0.79 (0.6 to 1.03)	0.078
Initial VFI	0.001 (0.00 to 0.74)	0.043	0.09 (0.008 to 0.99)	0.049
Post-LOD AMH	0.28 (0.09 to 0.86)	0.027	0.44 (0.21 to 0.89)	0.023
Post-LOD AFC	0.39 (0.17 to 0.92)	0.032	0.60 (0.39 to 0.92)	0.019
Post-LOD VI	0.01 (0.00 to 0.65)	0.030	0.1 (0.02 to 0.65)	0.016
Post-LOD FI	0.32 (0.12 to 0.86)	0.024	0.5 (0.29 to 0.87)	0.013
Post-LOD VFI	0.006 (0.00 to 0.74)	0.038	0.09 (0.01 to 0.62)	0.014

OR (95% CI) odds ratio and its 95% confidence interval
Analysis using binary logistic regression analysis

Discussion

Polycystic ovarian disease (PCOD) (22) is considered one of the most common endocrine problems among women. PCOD has a diverse range of causes that are not entirely understood (23). PCOD was diagnosed when 2 of the next 3 parameters were present: chronic anovulation, clinical or laboratory hyper-androgenism and clearly defined polycystic ovaries on ultrasound. The criteria are more flexible and permit the diagnosis in patients who had previously excluded by the 1990 NIH criteria, such as anovulatory normo-androgenic or ovulatory hyper androgenic females with polycystic ovaries on ultrasound scan (4). Trans-vaginal ultrasound has become the preferred diagnostic technique for the identification of polycystic ovarian disease. 12 follicles or more measuring 2–9mm in diameter, or increased ovarian volume (10 cm3) should be present on scanning to diagnose polycystic ovarian disease(2). 3D ultrasound has the power to improve the specificity and sensitivity of ultrasound in the diagnosis of PCOD (24). Laparoscopic drilling and CC have been established as the treatment of choice in CC-resistance (25).

The study objective was to determine the effect of endoscopic ovarian drilling (LOD) on Anti Mullerian hormone plasma level, stromal ovarian blood flow changes, by using 3D power Doppler and antral follicle count in females with Poly-Cystic Ovarian disease (PCOD) as primary outcomes and ovulation and pregnancy occurrence within 6 months after drilling with ovulation induction using clomiphene citrate of a daily dose of 150mg from the 3rd to 7th day as secondary outcomes, and whether this can explain the mode of action of drilling.

The study results were compared to previous studies that were held before to measure the results of endoscopic ovarian drilling in PCOD patients not responding to clomiphene citrate mainly with previous studies (15, 20, 26, and 27).

This study did not show statistically significant difference between the 2 groups as regards general demographic data including the age, no. of previous miscarriage, weight and BMI. It was in agreement with those of (15, 20, 26, and 27).

The study conducted that the median parity was obviously significantly higher in females of group II (control group) which was in agreement with the studies of (15, 28).

In the current research, the mean initial AMH and the median initial AFC were higher among cases than control group. The mean initial AMH was 7.19 ± 2.45 for study group, and 2.23 ± 0.69 for group control and the median initial AFC was 29 for group I and 12 for group II when P value was <0.001.

This was in agreement with the study of (15) concerning the initial serum AMH which was 7.4 ± 4.6 for cases group pre LOD and 1.9 ± 0.3 for control group and for the initial AFC which was 29 ± 2.4 for cases group pre LOD and 13 ± 1.9 for control group. It also was in agreement with the studies of (20, 27).

In the current study, the mean ovarian FI, VFI and VI were higher between women of study when compared to control group. This was in agreement with the studies of (4, 15, 27, and 29).

This study showed significant reduction in AMH serum level after LOD between women of cases group which became 3.84 ± 1.51 ng/ml with [MPD 3.34 ng/ml, 95% CI (2.8 to 3.9), p<0.001].

This was in agreement with the study of (15) which was 4.2 ± 2.5 ng/ml and also it was in agreement with the study of (20, 27).

This study showed significant reduction in AFC after LOD among women of cases group which became 14 with [MPD 14.9, 95% CI (13.9 to 15.8), p<0.001].

This was in agreement with the study of (15) which was 15 ± 2.2. And also it was in agreement with the studies of (20, 27).

In this study, there were significant reductions in FI, VI and VFI after than before LOD in study group. This was in agreement with the study of (15, 20, and 27). But this was in disagreement with that of (26) who revealed a significant increase in ovarian FI, VI, and VFI after LOD in women with PCOD.

The cause that this study results were different from the previously mentioned study may be due to the technique of how endoscopic ovarian drilling was performed (number, depth of punctures, type of electrocautary needle, time of application of needle to the ovary and the power of electrocautary.

In this study, bilateral LOD was done by using monopolar electrocautary needle which was applied perpendicular to the ovary aided by a short duration of cutting current 40 W for 4-6 seconds in 4 puncture. In a study done by (26) bilateral LOD was done by using monopolar electrocautary needle which was applied perpendicular to the ovarian surface aided by a short duration of cutting current 30 W for 2-4 seconds in 4 puncture.

Another issue that may explain why our study results (regarding ovarian indices) were different from previous studies is the timing of performing the 3D power Doppler.

In the current study, the 3D power Doppler indices (FI, VI and VFI) were measured in 2nd or the 3rd day of the menstruation before and after drilling.

In the study of Aliaa (26) the indices of 3D power Doppler (FI, VI and VFI) were measured before or after drilling in the day 11 or 12 of the cycle.

The controversy between our and Aliaa (26) results may be explained by different sample size which was smaller our study. The increasing number of included females provide better representation of the general populations.

In our study, of the included 25 women of group I, 20 (80%) had ovulation, and 13

(52%) got pregnant within 6 months after drilling and by clomiphene citrate.

This was in agreement with (15) which had 73.9% ovulation rate and 26.1% spontaneous pregnancy rate.

Conclusion

This study showed that LOD had a significant decreasing effect on AMH serum level, AFC, and Ovarian Doppler indices of FI, VI and VFI in PCOD patients not responding to clomiphene citrate. It also showed that initial AMH, initial AFC, initial VFI, post-LOD AMH, post-LOD AFC, post-LOD VI, post-LOD FI and post-LOD VFI were significant indicators of successful ovulation and successful pregnancy within 6 months after drilling.

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Evaluation of the diagnostic accuracy of designed ultrasound- based scoring system for prenatal diagnosis and differentiation of Morbidly Adherent Placenta (MAP).

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Abstract

Background: Accurate diagnosis of morbidly adherent placenta (MAP) and differentiation between its variants accreta, increta and percreta, allows preparation of life saving measures, and enables planning for alternative surgical procedures saving the future fertility according to the severity of the case.

Objective: Evaluate the accuracy of 2D, color Doppler, 3D and 3D power Doppler in the assessment of Morbidly Adherent Placenta (MAP) and the introduction of designed ultrasound-history based scoring system for prenatal diagnosis of (MAP), and differentiation between its variants in patients with previous cesarean section (CS) scar/s in correlation with the intraoperative findings.

Materials and Methods: Ninety five pregnant women with persistent placenta previa (after 28 weeks' gestation) and pervious history of uterine CS scar/s with mean 2 (1-6) cesarean deliveries were enrolled into the study, with mean age 30.08 ± 7.01 years, gravidity 3.9 ± 1.1 and parity 2.4 ± 1.5. All patients were evaluated with 2D, then color Doppler, Finally 3D and 3D power Doppler ,each US finding suggestive for Morbidly Adherent Placenta (MAP) take a score; the cumulative score compared with the intraoperative final diagnosis. The diagnostic accuracy was evaluated by receiver operating characteristic (ROC) analysis.

Results: 2D had sensitivity (87.4%), specificity (92%), PPV (97.6%) and NPV (65.7%) for diagnosis of Morbidly Adherent Placenta (MAP), while color Doppler had sensitivity (94.7%), specificity (92%), PPV (97.8%) and NPV (82.1%) , 3D power Doppler had sensitivity (93.7%) ,specificity (100%), PPV (100%) and NPV (80.6%) , finally 3D section of the bladder interface had sensitivity (62.1%), specificity (100%), PPV (100%) and NPV (41%). The diagnostic accuracy of the scoring system by using degree of agreement (ROC curve) was (0.049) for 3D power Doppler, (0.049) for 2D US and (0.053) for color Doppler in diagnosis of MAP. The area under the ROC curve was significantly equivalent to all tools of diagnosis (for 3D power Doppler 1.000, 2D US 0.975, color Doppler 0.967 and for overall scoring 0.979).

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Conclusion: 3D power Doppler as an additional tool for diagnosis of MAP had the best sensitivity(93.7%), specificity (100%), PPV (100%) and NPV (80.6%) followed by color Doppler with sensitivity (94.7%), specificity (92%), PPV (97.8%) and NPV (82.1%) and lastly 2D US with sensitivity (87.4%), specificity (92%), PPV (97.6%) and NPV (65.7%), and the introduction of the scoring system facilitates the diagnosis and differentiation between MAP variants with scores (8-12 for accreta, 13-18 for increta,19-25 for focal percreta and > 25 for diffuse percreta), so life saving and fertility preservation procedures could be probably planned

Key words: placenta ,ultrasound ,scoring , morbidly.

INTRODUCTION

Morbidly adherent placenta (MAP) is a term used to describe the clinical condition when part or the entire placenta invades and becomes inseparable from the uterine wall (1).

In normal pregnancy; the Decidua prevents the placenta from invading the uterine wall, while in MAP, the Decidua (Nitabuch's layer) is thin or deficient due to scar defect from prior CS scar/s (2).Therefore in MAP, placenta acts like an invading tumor and how deeply the placenta attaches determines the severity of the case , and so MAP may develop and worsen as pregnancy progresses (3).

Placenta previa with previous cesarean section/s are the main risk factors for MAP, and what is more important, factors related to the closure technique, development of the lower uterine segment, location of the incision and the wound healing which may have an impact in the incidence of placenta accreta (4-6). If no prior cesarean scar with placenta previa the risk of MAP is 5%, while with one prior cesarean scar the risk increases to 24%, with two prior cesarean scars the risk 40% and with four prior cesarean scars the risk reaches 67%, and if the caesarean rates continue to rise as they have in recent years, there will be an additional increase in MAP rates (6).So accurate diagnosis of MAP and differentiation between its variants allows preparations for a possible obstetric emergencies (7) or the use of alternative surgical procedures saving the future fertility according to the severity of the case (8).

PATIENTS AND METHODS

This retrospective study was carried out in Mansoura University Hospital, Obstetric and Gynecological Department together with women imaging center of Professor / Magda Shady between May 2013 to May 2017. Ninety five pregnant women with persistent placenta previa (in third trimester) and pervious history of uterine CS scar/s with mean 2 (1-6) cesarean deliveries were enrolled into the study, with mean age 30.08 ± 7.01 years, gravidity 3.9 ± 1.1 and parity 2.4 ± 1.5 (table 1). All patients were subjected by the same operator to 2D, color Doppler, then 3D and 3D power Doppler using (Voluson E8 expert), the sonographic data of all patients were reviewed, calculating their sensitivity, specificity and predictive values, while images interpretations were guided by parameters listed in tables (2, 3, 4, 5, 6), then a score was given to each criteria for diagnosis of MAP and the cumulative score was calculated compared with the scoring system listed in table (7). The scoring results were compared with operative findings and the results were statistically analyzed using SPSS software, Chi-square, T-test, Monte Carlo test, Krusskal-walis test and ROC Curve.

RESULTS

Among this study patients were divided in to four groups: accreta, increta, focal percreta, and diffuse percreta.

MAP and its variants were confirmed in 95 patients at the time of Cesarean delivery (intraoperative), in which placenta accreta (n= 13, 14% %), placenta increta (n=24,

25%) percreta focal (n=20, 21%) and diffuse percreta (n=38, 40%).

According to the history (table 1) the number of CS scars had significant difference between groups (P = 0.001), but past history of placenta previa had no significant difference (P =0.3), each historical criteria take a score then the sum of them give score (3-5) for MAP.

In order to test the accuracy of sonographic criteria for the diagnosis of all types MAP (accreta, increta, percreta), 2D sonographic data (table2, 3) revealed that: Type of the placenta had significant difference between groups (P = 0.001), as more severe cases associated with anterior placenta previa complete centeralis.

Myometrial thinning had sensitivity (47.4%), specificity (100%), PPV (100%) and NPV (33.3%), when the myometrium partially lost or completely absent or had placental bulge / exophytic mass it had specificity (100%), PPV (100%) with sensitivity (20, 87.4, 16.8% respectively) and NPV (24.8, 67.6, 23.8% respectively).

As regarded lacunae, suggested cut off value for Lacunar number (5 lacunae) had sensitivity (38.9%) , specificity (92%), PPV (94.9%) and NPV (28.4%), and cut off value of lacunaer size (2 cm) had sensitivity (61.1%) , specificity (72%), PPV (89.2%) and NPV (32.7%), these cut off values help in decreasing bias ,as regarded lacunaer shape, branching ones were found to be associated with severe cases of MAP (P=0.001), and when both lacunae eye of typhoon and lacunae continuous with bladder wall added to the lacunar evaluation specificity and PPV increase to (100%) but with low specificity(5.2,6.3 respectively) .

Disrupted bladder had specificity and PPV (100%), but with lower specificity (11.6%) and NPV (22.9%).

The overall sensitivity of 2D US (87.4%), specificity (92%), PPV (97.6%) and NPV (65.7%) for diagnosis of MAP, each criteria described above take a score then the sum of them give score to each MAP variant (accreta=5-8, increta=9-12, focal percreta=13-18, diffuse percreta= >18).

As regarded color Doppler criteria for diagnosis of MAP (table 4):- Hypervascularity was found to have sensitivity (95.8%), specificity (68%), PPV (91.9%) and NPV (81%),and with its classification to mild , moderate and marked there was significant difference between groups (P=.000) , within the hypervascularity the presence of gap had sensitivity (61.1%), specificity (88%), PPV (95.1%) and NPV (37.3%), and The blood flow PVS more than 15 cm had sensitivity (50.5%), specificity (96%) ,PPV (98%) and NPV (33.8%).

With over all sensitivity of the color Doppler (94.7%), specificity (92%), PPV (97.8%) and NPV (82.1%). For diagnosis of MAP, each criteria described above take a score, the sum of them give score to each MAP variant (accreta=2-4, increta=5- 7, percreta>7).

With 3D power Doppler (table 5), we had to examine two views:-

(A)Basal (sagittal) view: - Numerous coherent vessels including the bladder interphase and the placental base had sensitivity (64.2%), specificity (100%), PPV (100%) and NPV (42.4%). Lacunar aneurysm had sensitivity (26.3%), specificity (100%), PPV (100%) and NPV (26.3%).

(B)Lateral (coronal) view:-The presence of blood vessels passing perpendicular to the uterus had sensitivity (75.8%), specificity (96%), PPV (98.6%) and NPV (51.1%). **Chaotic branching & detour vessels** at the bladder interphase, **had sensitivity** (82.1%), specificity (96%), PPV (98.7%) and NPV (58.8%). The resistance index (RI) was measured at 3 different points on bladder wall then the lowest value was used to be our referral to identify high risk cases, with cut off value less than 0.4, with sensitivity (98.9%),

specificity (64%), PPV (91.3%) and NPV (94.1%).

With over all sensitivity of the 3D power Doppler (93.7%) and specificity (100%), PPV (100%) and NPV (80.6%) for diagnosis of MAP, each criteria described above take a score and the sum of them give score to each MAP variant (≤ 4 =Focal percreta and > 4 =Diffuse percreta).

Finally 3D US section at the bladder interface shows (table 5):- interrupted bladder mucosa which had sensitivity (62.1%), specificity (100%), PPV (100%) and NPV (41%) ,the presence of this criteria take score 3 in case of placenta percreta only ,to be added to the previous scores.

A total score made by the sum of previous scores for diagnosis and differentiation of MAP and its variant (accreta=8-12, increta=13-18, focal percreta=19-25, diffuse percreta= > 25).

Based on the criteria described above (preoperative) when compared with intraoperative findings, 3D power Doppler as an additional tool for diagnosis of MAP had the best sensitivity (93.7%), specificity (100%), PPV (100%) and NPV (80.6%) followed by color Doppler with sensitivity (94.7%), specificity (92%), PPV (97.8%) and NPV (82.1%) and lastly 2D US with sensitivity (87.4%), specificity (92%), PPV (97.6%) and NPV (65.7%) (table 6).

The diagnostic accuracy of the scoring system by using degree of agreement (ROC curve) was (0.049) for 3D power Doppler, (0.049) for 2D US and (0.053) for color Doppler in diagnosis of MAP. The area under the ROC curve was significantly equivalent to all tools of diagnosis (for 3D power Doppler 1.000, 2D US 0.975, color Doppler 0.967 and for overall scoring 0.979) (figure 7).

DISCUSSION

The main core of prenatal diagnosis of MAP and its variants in cases of placenta previa with previous cesarean scar /s is to reduce maternal and fetal morbidity and mortality due to lack of adequate preparation at time of delivery (1).

The introduction of clinical and ultrasound scoring system for the diagnosis of MAP and its variants (accreta, increta, percreta), could be helpful for improvement of the diagnostic performance and management.

In the current study diagnosis of MAP and its variants (accreta, increta and percreta) was confirmed in 95 patients, placenta accreta represented (n= 13, 14%), placenta increta (n=24, 25%) focal percreta (n=20, 21%) and diffuse percreta (n=38, 40 %).

Cases with placenta accreta had less number of previous CS scars and no past history of placenta previa, by sonographic evaluation they associated with anterior placenta centeralis (n=6), anterior placenta marginalis (n=5) and posterior placenta centeralis (n=2), they had thinmyometrium and serrated bladder interface without abnormal vascularity, no lacunae or lacunae ≤ 5 in number, < 2 cm in size with regular shape and no vascularity.

While cases with placenta increta were associated with increasing number of previous CS scars and no past history of placenta previa ,the sonographic evaluation revealed that the majority of the cases had anterior placenta centeralis (n=22), the myometrium may be thin , partially lost or even completely lost and disrupted bladder wall with hypervascularity and blood vessels passing parallel to the bladder wall having low RI < 0.4 , also had no lacunae or lacunaer number ≥ 5 with size ≥ 2 cm with irregular shape and with abnormal blood flow had PSV > 15 cm.

In cases with placenta percreta they had increasing number of previous CS scars with past history of placenta previa, the sonographic evaluation showed that the majority of the cases had anterior placenta centeralis (n=56), and only few cases (n=2) seen with

posterior placenta centeralis, they had complete myometrial loss with placental bulge / exophytic mass and hypervascularity with gap in , that differentiate cases of focal percreta from cases of diffuse percreta which have severe hypervascularity with no gap, and the bladder interface seen disrupted which may be focal or diffuse with blood vessel/s passing perpendicular to the uterus and according to their number we can differentiate between percreta types, by 3D power doppler they had hypervascularity with low RI < 0.4 , forming coherent , chaotic branching and detour vessels, they may have no lacunae or lacunaer number ≥ 5 with size ≥ 2 cm with irregular shape or even branching ones open at bladder wall with abnormal blood flow had PSV > 15 cm , they may form lacunaer aneurysm when their vascularity fuse with the vascularity of the bladder wall in cases of diffuse percreta , lastly by 3D section diagnosis of interrupted bladder mucosa which might be focal or diffuse.

The overall performance of 2D US for diagnosis of MAP had sensitivity (87.4%), specificity (92%), PPV (97.6%) and NPV (65.7%),so by 2D grayscale abdominal ultrasound we have an idea about cases highly suspected to be MAP and even we can localize the site of the myometrium defect which could have more vascularity and need more evaluation by Doppler US , and that was in agreement with FatemehRahimietal (9), who revealed ultrasound sensitivity and specificity reached to 71.4% and 88.5%: consecutively with accuracy of 87%,and the study carried out by Dwyer BK etal (10) who detected 2D US sensitivity 93% and specificity 71%.

Adding color Doppler the accuracy of diagnosis increases to sensitivity (94.7%) ,specificity (92%), PPV (97.8%) and NPV (82.1%), similar to our study, Levine D - et al (11) that showed color Doppler imaging with a sensitivity of (86%) and a specificity of (92%), while Shweel MAG et al (12) results with color doppler was (90%) for sensitivity and (70%) for specificity, with the current study the high false positive(PPV 97.8%) results were observed because the majority of the cases had more than one previous CS with the formation of bladder varices and neovascularized vessels mistaken as abnormal bladder–uterine serosa interface hypervascularity which was assumed to be MAP and that was in agreement with Bonnie KD et al (13) and Cali G et al. (14,15), While the false-negative cases (NPV 82.1%) was mostly in cases evaluated early at 29-32 weeks and delivered later after 35 weeks , because MAP is a progressive condition that increase with increasing the period of pregnancy(16,17), also depending on more than one criteria increases the accuracy of diagnosis and differentiation of MAP and that agree with Shih et al (18).

Adding 3D power Doppler increases the accuracy of diagnosis and decreases the false negative and false positive results, and it was carried in two views(table 5):- Basal view:-the presence of numerous coherent vessels and lacunar aneurysm both had specificity (100%) and PPV (100%), they were found with more severe cases of MAP (diffuse percreta).

lateral view :-the presence of blood vessels passing perpendicular to the uterus, Chaotic branching & detour vessels had specificity (96%) and PPV around (98%), so they could be used not only for differentiation between placenta increta and percreta but also between percreta variants, and that was in agreement with the study carried out by Calì G etal (14,15) and Chou M-M etal (19), and with the addition of RI value as done by the study of Shih et al (18) the sensitivity increase to (98.9%), but also the NPV had increased to (94.1%), because of neovascularity of the previous CS scars (13,14).

3D US section (table 5) at the bladder wall , interrupted mucosa due to abnormal placental invasion could be identified , with specificity (100%), PPV (100%) and low NPV(41%), that criteria was associated with all cases of diffuse percreta and all most all

cases of focal percreta, and that was to lesser extent in agreement with Chou M-M-etal (19), so that criteria could add an additional tool in the differentiation between placenta increta and percreta and also between percreta variants according to the part of mucosa involved (focal or diffuse) ,also 3D US section at the bladder interphase can differentiate between focal percreta and cases of placenta increta with outer varicosity , so decreasing the false positive results.

Each of the previous diagnostic criteria in each tool of diagnosis had taken a score according to its severity and its ability of differentiation between different types of MAP, and the sum of scores was evaluated for each tool in one hand and the total score of all tools in the other hand, and it was found that the 3D power doppler criteria alone was as accurate as the total score in diagnosis and decreasing the false positive results (table 6,figure 7) , this scoring system may help in standardization of diagnosis for MAP and differentiation between its variants, our results was to lesser extent in agreement with that of Nelson T etal (20) ,that introduces placenta accreta index(PAI) , which is a scoring system based on history of previous cesarean delivery (s), placental location, placental lacunae , lower uterine segment thickness, and the presence or absence of bridging vessels , with score of > 4 predicted abnormal placenta invasion in patients with at least 1 prior CD, while Rac MWF etal (21) had published scoring system parameters include loss of retroplacental clear zone, irregularity and width of uterine-bladder interface, smallest myometrial thickness, presence of lacunar spaces, and bridging vessels, together with number of cesarean deliveries and anterior placental location ,each parameter was weighted to create a 9-point scale in which a score of 9 provided a probability of invasion, with sensitivity 61%, specificity 93%, positive predictive value 87%, and negative predictive value 82%,

Close to the current study woodring TC et al (22) study, who developed a mathematical model using antenatal clinical signs to identify patients with PA, suggesting that combining antenatal factors (placenta previa, numbers of previous caesarean deliveries) with ultrasound suspicion of PA had better positive predictive value than ultrasound suspicion of PA alone . From previously discussed, the current study scoring system had the advantage of diagnosis MAP cases and differentiation between its variants while other scoring systems gave an idea of cases more liable to be MAP, and that had an important effect on childbirth timing and management, as the mean gestational age of delivery in the current study was 37 weeks and in many cases it could reach 39 weeks, and that agreed with many studies that had planned CS between the 36th and 38th week, after fetal pulmonary maturity (23), and disagreed with other studies that had scheduled CS at 34- weeks after course of corticosteroids to avoid emergent CS (1,24).

Conclusion

3D power Doppler as an additional tool for diagnosis of MAP had the best sensitivity (93.7%), specificity (100%), PPV (100%) and NPV (80.6%) followed by color Doppler with sensitivity (94.7%), specificity (92%), PPV (97.8%) and NPV (82.1%) and lastly 2D US with sensitivity (87.4%), specificity (92%), PPV (97.6%) and NPV (65.7%), together with the introduction of the scoring system, that had better accuracy in diagnosis of , and differentiation between MAP variants with (1.000) for 3D power Doppler, (0.975) for 2D US and (0.967) for color Doppler , with score (8-12 for accreta, 13-18 for increta,19-25 for focal and > 25 for diffuse percreta) , so life saving and fertility preservation procedures could be probably planned

Case presentation (Diagnostic performance):

Case (1):

27 years old, G3P2 pregnant ±30 weeks, had previous 2 CS scars with no past history of placenta previa in the previous pregnancy, history score=3(MAP).

2D US shows anterior placenta complete centeralis, thinning of the myometrium, lacunae (≤ 5 in number, small ≤ 2 cm in size and irregular), the score =8 (accreta).

Color Doppler shows mild myometrial hypervascularity with PSV >15 cm, the score =3(accreta).

3D power Doppler in the lateral view shows blood vessels passing parallel to the bladder wall with the lowest RI < 0.4,the score = 2 (focal percreta).

Total score sum =16, which correspond to placenta increta according to our scoring system, and was confirmed operatively.

Case (2):

33 years old, G4P2 pregnant ±32 weeks, had previous 3 CS scars, no past history of placenta previa in the previous pregnancy, the score=3 (MAP)

2D US shows anterior placenta complete centeralis, absent myometrium/retroplacental space, lacunae (≤ 5in number, small ≤ 2 cm in size) and disrupted bladder wall, the score =10 (increta).

Color Doppler shows moderate hypervascularity, the score = 2 (accreta).

3D power Doppler in the lateral view shows blood vessels passing perpendicular to the uterus, chaotic branching & detour vessels with the lowest RI ≤ 0.4,the score = 7(diffuse percreta).

Total score sum =22, which correspond to focal percreta according to our scoring system, and was confirmed operatively.

Case3 (3):

35 years old, G4P3 pregnant ±31 weeks, had previous 3 CS scars, no past history of placenta previa in the previous pregnancy, history score = 3 (MAP).

2D US shows anterior placenta complete centeralis, partially lost myometrium/retroplacental space, lacunae (≥ 5 in number, large ≥ 2 cm in size, irregular), and disrupted bladder wall, the score =14(focal percreta).

Color Doppler shows marked hypervascularity with gap, blood flow PSV >15 cm, the score = 8 (percreta).

3D power Doppler in the basal view shows numerous coherent vessels, , the lateral view shows blood vessels passing perpendicular across the bladder wall, chaotic branching & detour vessels with the lowest RI < 0.4,the score = 10 (diffuse percreta).

3D at the bladder interface identify interrupted bladder mucosa, score =3 (percreta).

Total score =38, which correspond to diffuse percreta according to our scoring system, and was confirmed operatively.

Case (4):

40 years old, G3P2 pregnant ±36 weeks, had previous 2 CS scares, no past history of placenta previa in the previous pregnancy, history score = 3 (MAP).

2D US shows anterior placenta complete centeralis, partially lost myometrium/retroplacental space with placental bulge and lacunae (≤ 5 in number, large ≤ 2 cm in size, irregular), and disrupted bladder wall which, the score =15 (focal percreta).

Color Doppler shows marked hypervascularity with gap and blood flow PSV >15 cm, the score = 8 (percreta).

3D power Doppler in the basal view shows numerous coherent vessels, , the lateral view shows blood vessels passing perpendicular

across the bladder wall, chaotic branching & detour vessels with the lowest RI < 0.4,the score = 10 (diffuse percreta).

3D at the bladder interface identify interrupted bladder mucosa, score =3 (percreta).

Total score =39, which correspond to diffuse percreta according to our scoring system, and was confirmed operatively.

Case (5):

31 years old, G5P3A1 pregnant ±35 weeks, had previous 3 CS scares, with past history of placenta previa in the previous pregnancy, history score = 5 (MAP).

2D US shows anterior placenta complete centeralis, absent myometrium /retroplacental space, lacunae (≥ 5 in number, large ≥2 cm in size, irregular/branching and lacunae continuous with bladder wall) , and disrupted bladder wall which, the score =18(focal percreta).

Color Doppler shows marked hypervascularity with gap and blood flow PSV >15 cm, the score = 8 (percreta).

3D power Doppler in the basal view shows numerous coherent vessels, the lateral view shows blood vessels passing perpendicular across the bladder wall, chaotic branching & detour vessels and lacunaer aneurysm with the lowest RI < 0.4,the score = 13 (diffuse percreta).

3D at the bladder interface identify interrupted bladder mucosa, score =3 (percreta).

Total score =47, which correspond to diffuse percreta according to our scoring system, and was confirmed operatively.

Case (6):

28 years old, G4P2A1 pregnant ±34 weeks, had previous 2 CS scars, no past history of placenta previa in the previous pregnancy, the score=3 (MAP).

2D US shows anterior placenta complete centeralis, absent myometrium /retroplacental space and placental bulge/ exophytic mass, lacunae (≥ 5in number, large ≥2 cm in size, irregular/branching) with lacuna continuous with the disrupted bladder wall, the score =21 (diffuse percreta).

Color Doppler shows moderate hypervascularity with gap, PSV >15 cm, the score =7(increta).

3D power Doppler in the basal view shows numerous coherent vessels and in the lateral view shows blood vessels passing perpendicular to the uterus, chaotic branching & detour vessels and lacunar aneurysm with the lowest RI ≤ 0.4,the score = 13(diffuse percreta).

3D at the bladder interface identifies interrupted bladder mucosa, score =3 (diffuse percreta). Total score sum =47, which correspond to diffuse percreta according to our scoring system, and was confirmed operatively.

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Table (1): History risk factors of MAP.

	Accreta n = 13	Increta n =24	percreta focal n =24	percreta diffuse n =34	Significance test
Age in years mean ± SD	31.5 ±3.6	30.08±5.01	32.1± 4.6	30.1±4.5	MCT,P = 0.1
Gravidity mean ± SD	4.5 ± 2.02	3.9 ± 1.1	4.2 ± 0.87	4.1 ± 1.5	F= 0.5,P = 0.8
Parity mean ± SD	2.4± 1.5	2.5±1	2.5±0.8	2.7±1	F = 1.4,P= 0.2
Number of scars	2(1-4)	2(1-5)	2(1-4)	2(1-6)	Krusskal-walis testP = 0.001
History of placenta previa	0 (0)	1 (4.2%)	3 (35%)	3 (8.8%)	MCT P = 0.3

Table (2):-Type of placenta previa.

Type of placenta	Accrete n = 13	Increta n =24	percret a focal n =24	percreta diffuse n =34	Significan t test
	No (%)	No (%)	No (%)	No (%)	
Anterior placenta (centeralis)	6(46.2)	22(91.7)	23(95)	33(97.1)	MCT P ≤ 0.001
Anterior placenta (marginalis)	5(38.5)	1(4.2)	0 (0)	0 (0)	
posterior placenta (centeralis)	2(15.4)	1(4.2)	1(5)	1(2.9)	

MCT= Monte Carlo test P ≤ 0.05 statistically significant

Table (3):- Preoperative diagnosis by 2D US.

2D US	MAP* N=95	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
The myometrium					
Thinning	45	47.4	100	100	33.3
Partially lost	19	20	100	100	24.8
Absent myometrium/retroplacental space	83	87.4	100	100	67.6
Placental bulge/ exophytic mass	15	16.8	100	100	23.8
Lacunae					
Number	37	38.9	92	94.9	28.4
Size	58	61.1	72	89.2	32.7
Lacunae continuous with bladder wall	6	6.3	100	100	21.9
Lacunae eye of Typhon	5	5.2	100	100	20.9
Urinary bladder interface					
Disrupted	95	93.7	100	100	79.3
Over all diagnosis of 2D US	83	87.4	92	97.6	65.7

Table (4):- preoperative diagnosis by color Doppler.

Color Doppler	MAP* n= (95)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hypervascularity	85	89.5	68	91.4	63
+ve gap	58	61.1	88	95.1	37.3
Blood flow with PVS >15 cm	48	50.5	96	98	33.8
Lacunae eye of typhoon	5	5.2	96	80	20.9
Bladder interface hypervascularity	58	95.8	68	91.9	81
Over all diagnosis of color Doppler	90	94.7	92	97.8	82.1

*Or its variants, placenta increta and placenta percreta. NPV, negative predictive value; PPV, positive predictive value

Table (5): Preoperative diagnosis by 3D color power Doppler.

	MAP n=(95)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
(Basal view)					
Numerous coherent vessels	61	64.2	100	100	42.2
Lacunar aneurysm	25	26.3	100	100	26.3
(Lateral view)					
blood vessels passing perpendicular to the bladder wall	73	75.8	96	98.6	51.1
Chaotic branching & detour vessels	79	82.1	96	98.7	58.5
Lowest RI	95	98.9	64	91.3	94.1
3D section					
Interrupted bladder mucosa	60	62.1	100	100	41
Over all evaluation	89	93.7	100	100	80.6

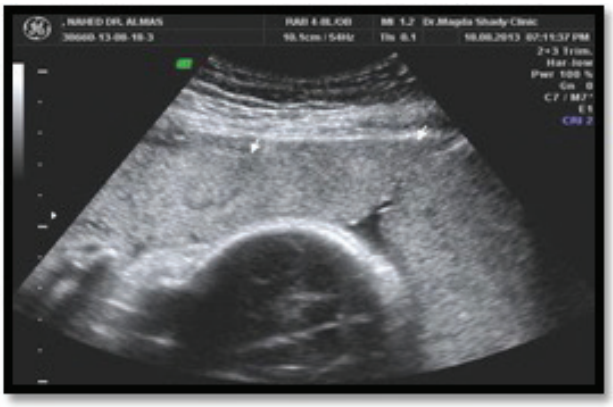
Table (6): Comparison between diagnosis tools.

Over all evaluation	**MAP n= (95)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**
3Dpower Doppler	89	93.7	100	100	80.6
Color Doppler	90	94.7	92	97.8	82.1
2D US	83	87.4	92	97.6	65.7

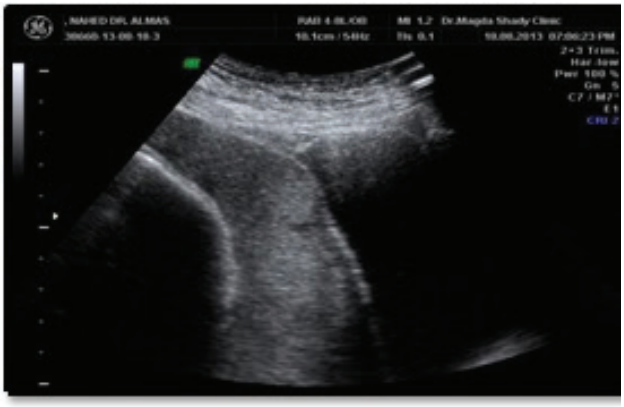
Table (7): Scoring system for diagnosis of MAP.

Item	Score	Type of placenta
History:		
Number of previous scars ≤ 2		
Number of previous scars ›2	1	≤ 2=no accreta
No history of placenta previa	2	3-5=MAP
History of placenta previa	1	
2D US:	3	
Anterior placenta	2	≤ 4=no accreta
Other types of placenta	1	5-8=accreta
Thinning myometrium	1	10-12=increta
myometrium Partially lost	2	13-18=focal percreta
Absent myometrium.	3	> 18= diffuse percreta
Placental bulge/exophytic mass	3	
Lacunar number ≤ 5	1	
Lacunar number > 5	2	
Lacunar size < 2 cm	1	
Lacunar size ≥ 2 cm	2	
Irregular/ branching lacunae	3	
Lacunae continuous with bladder wall.	3	
Disrupted bladder wall	3	
Color Doppler:		
Blood flow with PSV >15 cm	2	< 2= no accreta
Lacunae eye of Typhon	3	2-4=accreta
Hypervascularity: mild/moderate/severe	1/2/3	4-6=increta
+ve gap	3	> 6=percreta
3D power Doppler :		
The presence of blood vessels passing perpendicular to the uterus	2	≤ 4 =Focal percreta
Lowest RI< 0.4	2	> 4 =Diffuse percreta
Numerous coherent vessels	3	
Chaotic branching & detour vessels	3	
Lacunaer aneurysm	3	
3D section at bladder wall:		
Interrupted bladder mucosa	3	3=percreta
Total score:		
No accreta	≤ 8	
Accreta	8-12	
Increta	13-18	
Focal percreta	19-25	
Diffuse percreta	> 25	

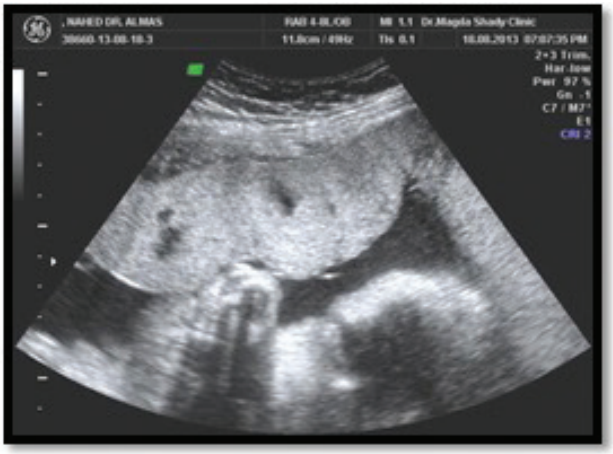
Figure (1):- Case (1) diagnosis:



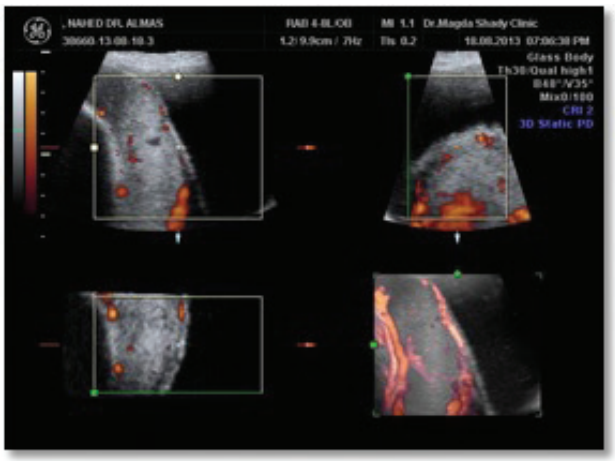
A: Thinning of the myometrium by 2D US.



C: lost retroplacental space and disrupted bladder wall.

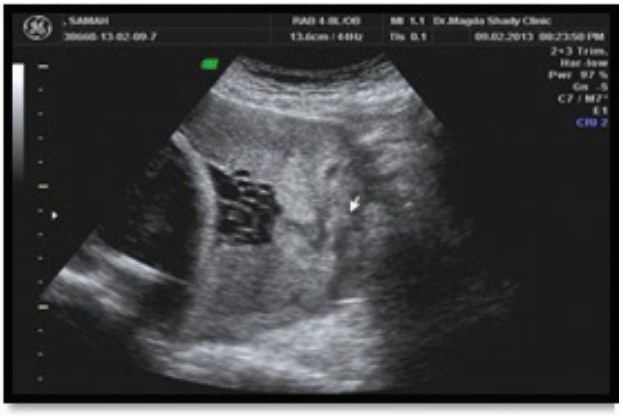


B: lacunae (≤ 5, small ≤ 2 cm in size and irregular)

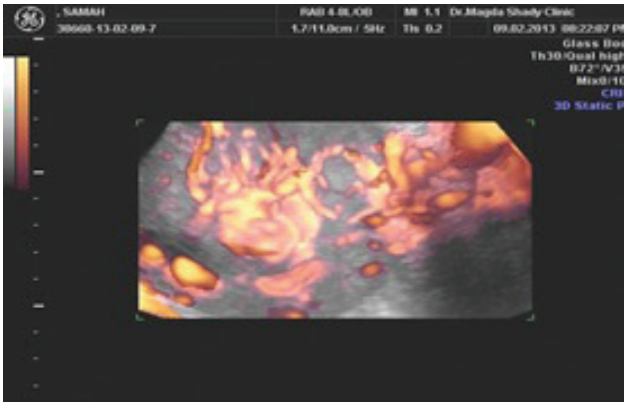


D: blood vessels passing parallel to the bladder wall by 3D power Doppler.

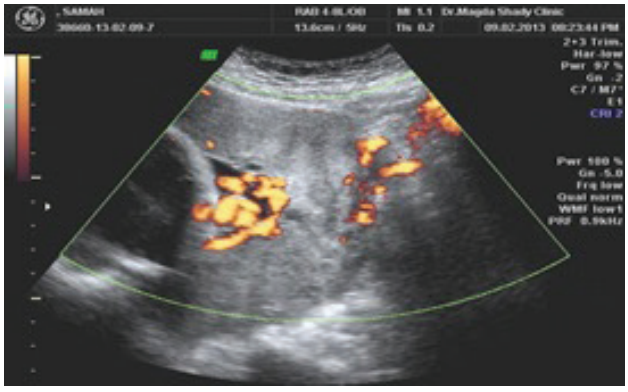
Case (2) diagnosis:



(A):-Absent myometrium/retroplacental space and no significant lacunae by 2D US.

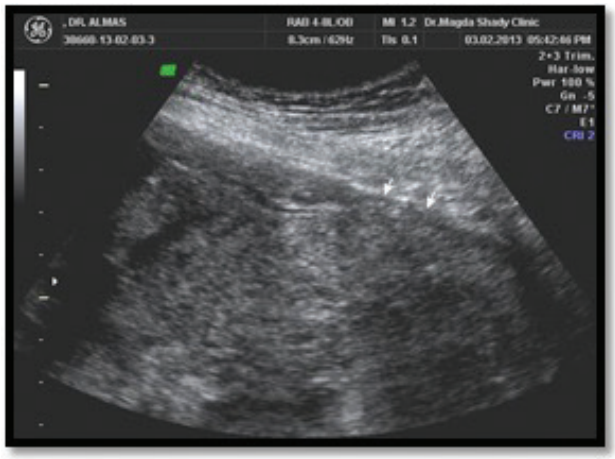


(C):- Chaotic branching & detour vessels by 3D power Doppler.

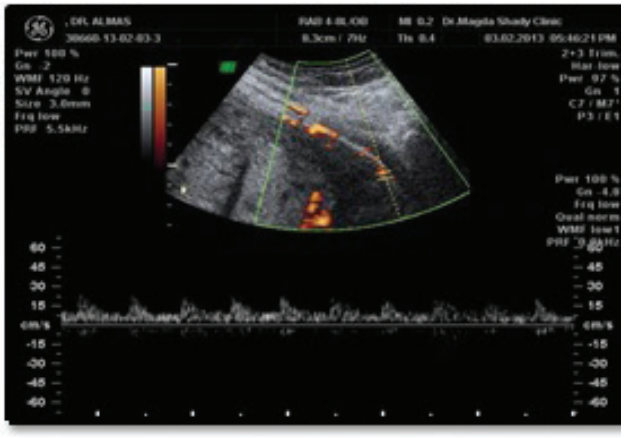


(B):-Moderate hypervascularity at bladder wall by Doppler.

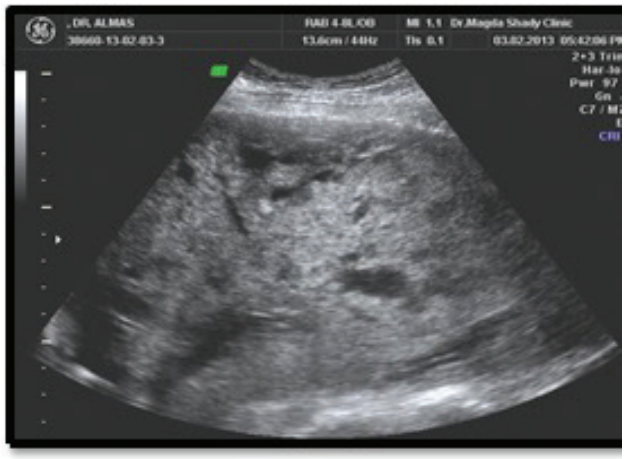
Figure (3):- Case (3) diagnosis:-



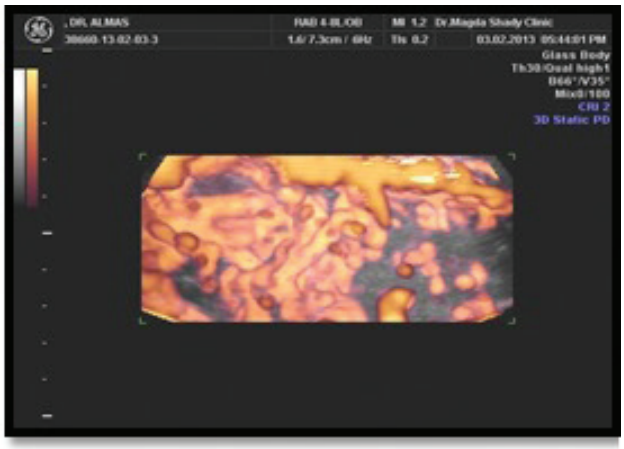
(A):-Partially lost myometrium by 2D US.



(C)-Presence of gap within bladder interface hypervascularity. With PSV >15 cm by Doppler.

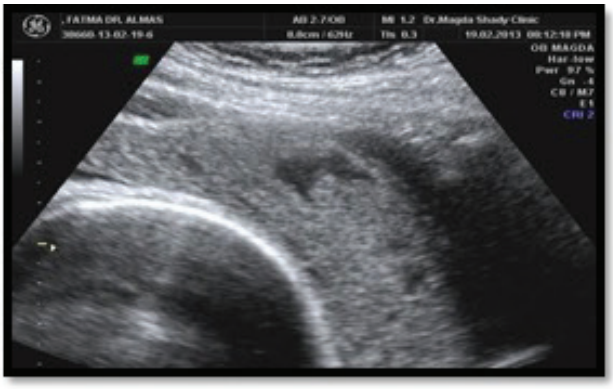


B: Lacunae ≥ 5 in number, ≥ 2 cm in size, and irregular by 2D US.(B):-

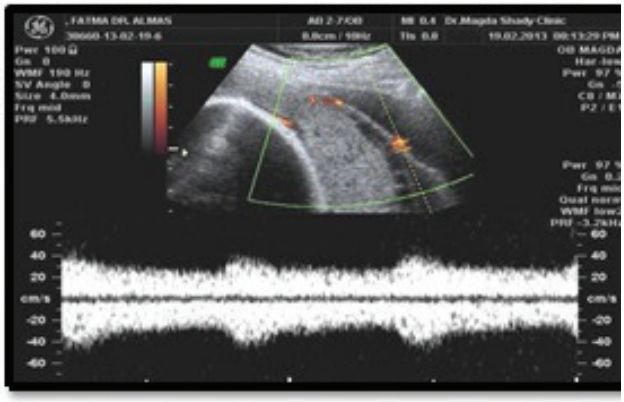


(D):-Coherent, Chaotic branching & detour vessels by 3D power Doppler.

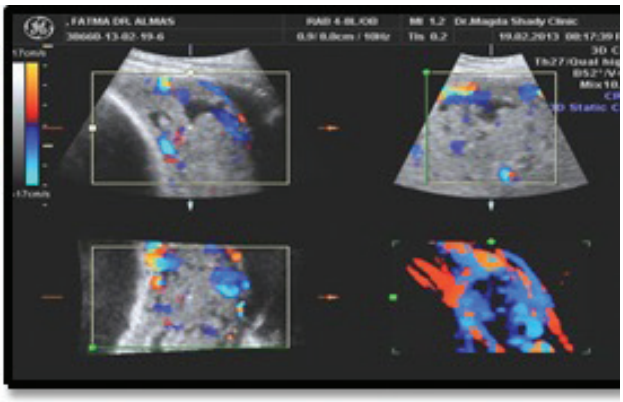
Figure (4):- Case (4) diagnosis:



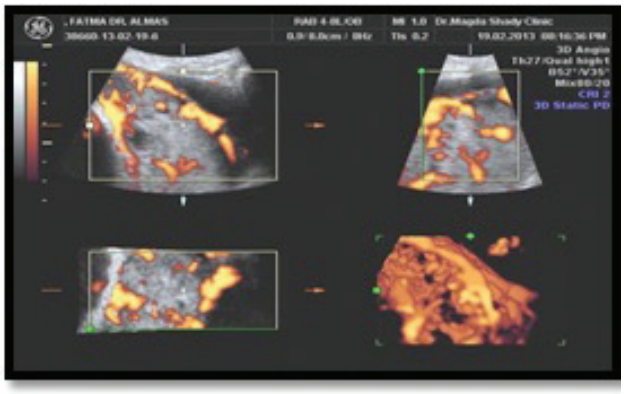
(A):-partially lost myometrium with placental bulge and lacunae (≤ 5 in number, small ≤ 2 cm in size and irregular) by 2D US.



(D):-PSV > 15 cm by Doppler.

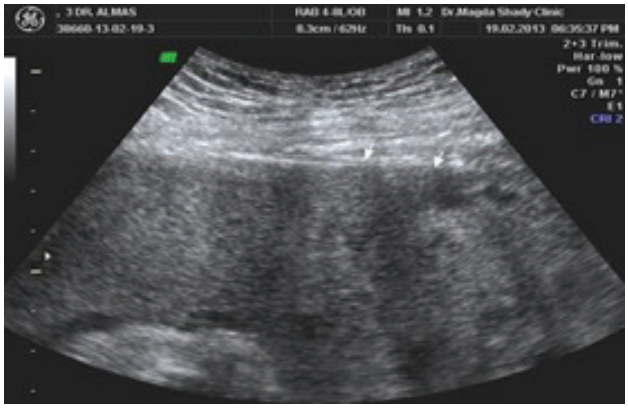


(B):-Severe bladder wall hypervascularity with gap by color Doppler.

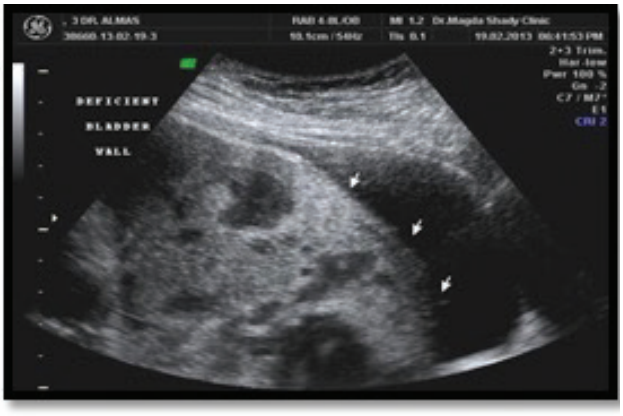


(E):- Numerous coherent vessels by 3D power Doppler.

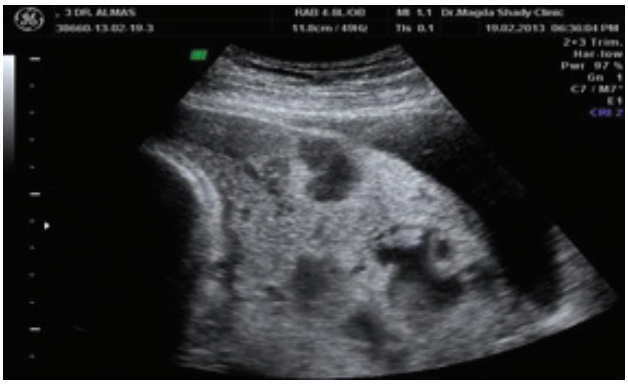
Figure (5):- Case (5) diagnosis:



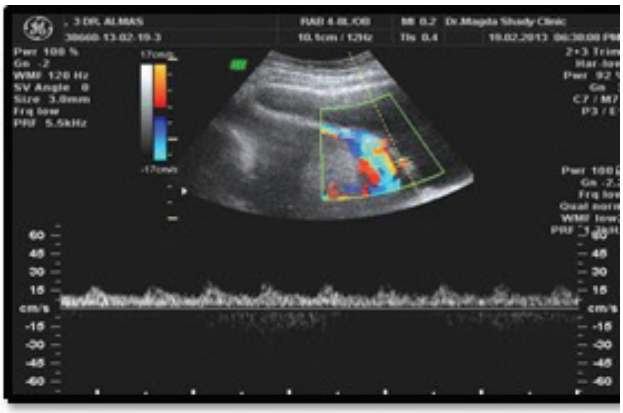
(A):-Absent myometrium/retroplacental space by 2D US.



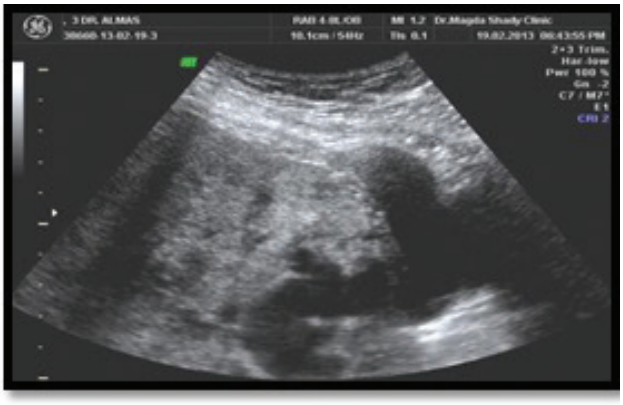
(C):-Disrupted bladder wall with large branching lacunae continuous with it.

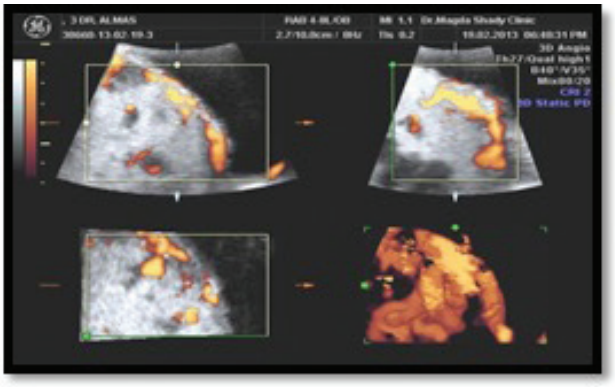


(B):-lacunae (≥ 5 in number, small ≥ 2 cm in size and irregular/branching)



(D):-Marked bladder wall hypervascularity with PSV >15 cm by doppler.

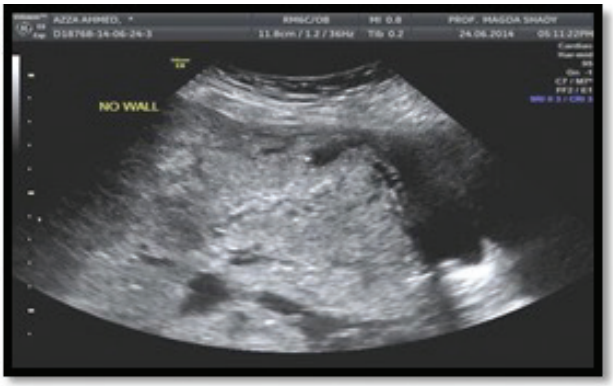




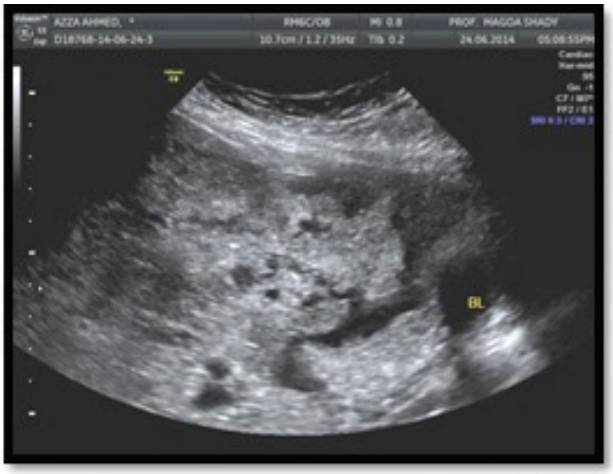
(E):- Chaotic branching & detour vessels by 3D power Doppler.

Fig. (6): ROC curve analysis of serum βhCG and Kiss-1 as predictors for getting EPL

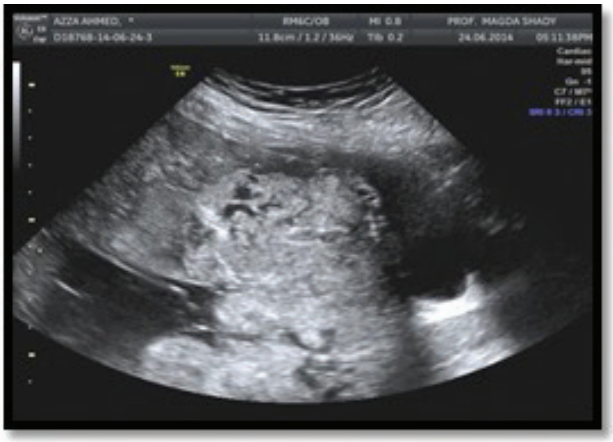
Figure (6):- Case (6) diagnosis: -



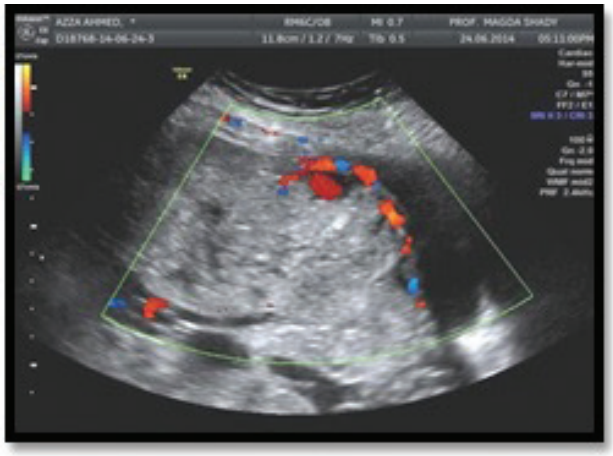
(A):-Absent myometrium/retroplacental space by 2D US.



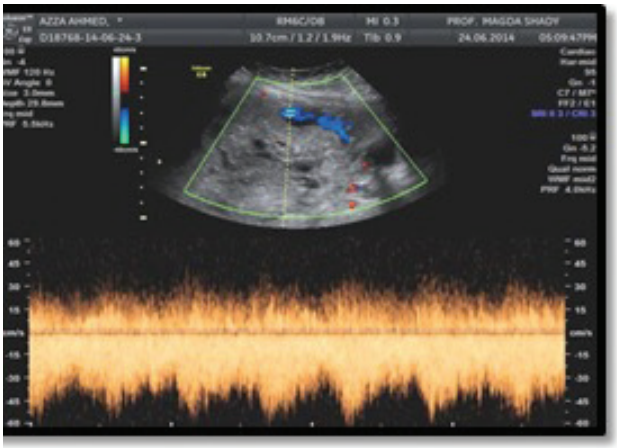
(B):- lacunae (≥ 5 in number, large ≥ 2cm in size and irregular/ branching) with large branching lacunae continuous with the bladder wall by 2D US.



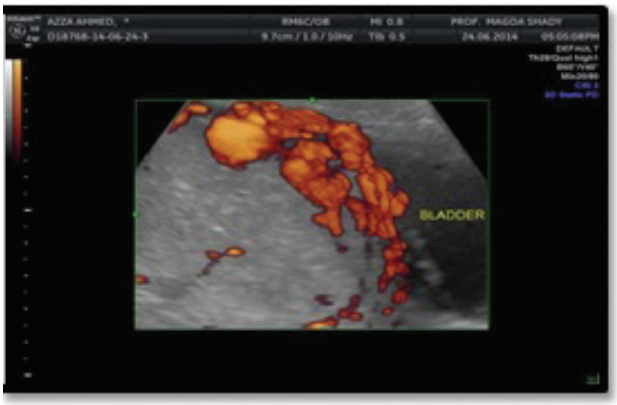
(C):-Disrupted bladder wall with placental bulge/exophytic mass.



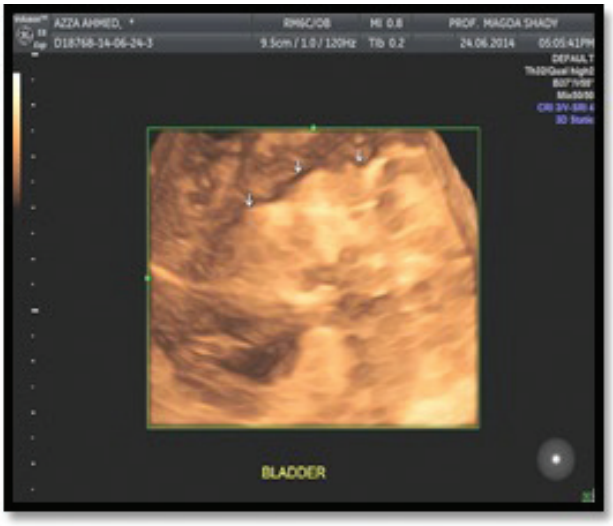
(D):- Moderate bladder wall hypervascularity with gap, by Doppler.



(E):- Blood flow with PSV >15 cm by color doppler.



(F):-Coherent vessels, chaotic branching & detour vessels by 3D power Doppler.



(G):- Interrupted bladder mucosa by 3D section at the bladder wall.

Figure (7):-ROC curve for scoring system accuracy.