Issn 1110 -6352

THE EGYPTIAN JOURNAL OF FERTILITY AND STERILITY

Volume 24 Number 3 September 2020

EFSS

EDITOR : ABOUBAKR EL NASHAR

The Egyptian Journal Of Fertility And Sterility

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

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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,

Very important subjects are included in this issue. There was significant increase in oxidant stress biomarkers nitrite and reduced glutathione with significant reduction in superoxide dismutase in patients with repeated abortions. Posterior colpotomy is a promising approach for surgical treatment of tubal ectopic pregnancy especially if laparoscopy is not available. Transcerebellar diameter is the highest statistically significant measurement which could be used in the third trimester for assessing the gestational age compared with femur length and biparietal diameter. Three dimension power Doppler is a useful noninvasive predictor for IVF outcome.

Best regards.

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Pilot Study of Biomarkers of Oxidative Stress and Chlamydia trachomatis in Female Patients with Spontaneous Abortions

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Abstract

The aims of the present study were i. asses the biomarkers of oxidative stress including superoxide anion radical, Nitrite measurement and reduced glutathione in female patients with spontaneous abortions ii. Asses the prevalence of C. trachomatis antibodies IgA and IgG in those patients, iii. Correlate the levels of the oxidative biomarkers to the presence of C. trachomatis antibodies.

The study included one hundred female patients with 2 or more spontaneous abortions in addition to one hundred healthy control females with normal gravidity and parity history. Blood samples were obtained from each subject and subjected to laboratory determination of superoxide anion radical, Nitrite measurement and reduced glutathione by biochemical methods. Determination of specific immunoglobulin A and G (IgA, IgG) was determined by enzyme linked immunosorbent assay (ELISA).

The prevalence of IgA and IgG for C. trachomatis in patients was 4% and 8% respectively and, in the control, subjects the prevalence of IgA and IgG were 1% and 2% respectively. The concentration of oxidative stress products was significantly higher in the patients group compared to the control group (P=0.0001). The concentrations of nitrite and reduced glutathione were 27.9± 4.7, 31.4± 1.9 nmol/ml respectively in patients and the concentrations of nitrite and reduced glutathione were 18.9± 2.1 and 27.9± 3.9 respectively in the control group. The concentration of SOD was significantly reduced in the patients group compared to the control group (P=0.0001). The concentration of SOD was 55.1 6.9 in the patients and in the control the concentration was 64.5± 7.7 nmol/ml In the study of oxidative stress markers in the patients with positive serology for C. trachomatis, there was significant increase in nitrite concentrations compared to patients negative for C. trachomatis serological markers (P=0.005). While reduced glutathione concentration had insignificant increase in patients positive for C. trachomatis with reduced SOD compared to patients with negative serology to C. trachomatis, P=0.6, P=0.07, respectively.

The present study highlights that there was significant increase in oxidant stress biomarkers nitrite and reduced glutathione with significant reduction in superoxide dismutase in patients with repeated abortions. The prevalence of IgG to C. trachomatis was significantly prevalent in patients with recurrent abortion compared to control subjects. The nitrite was significantly correlated with positive serology to C. trachomatis.

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INTRODUCTION

Infertility is a health problem that affects from 8-12% of the worldwide couples. The main cause in about 70% is associated with female infertility due to ovulation problems or male infertility associated with reduced semen quality. However, up to 30% of infertility has no known etiology (1-3). There are causes of infertility attributed to impaired oocyte growth, maturation and implementation (4).

Among pathophysiological process associated with defective oocyte development is oxidative stress with increase in the reactive oxygen species (ROS), high lipid peroxidation (LPO), and decreased total antioxidant capacity (TAC) in follicular fluid (FF) correlate with poor embryo quality and fertilization rates (5, 6), no consensus has yet been achieved in this matter.

Among pathogenic effects of certain microorganisms that lead to infertility is Chlamydia trachomatis (C. trachomatis) infections in genital tract. Chlamydia trachomatis infection affects upper genital tract in females leading to pelvic inflammatory disease and affect the fallopian tubes with fibrosis and tubal factor infertility (6). There is association between C. trachomatis infection in females and increase production of the products of oxidative stress from activated neutrophils and macrophages. The increase products of the oxidativestress affect DNA with oxidative damage leading to aberrant gene expression and inhibition of protein synthesis (7). In vitro experimental study infection of the cell lines with C. trachomatis leads to release of reactive oxygen species with peroxidation of the lipid (8). The effect of peroxidation assists in the spread of the elementary bodies of C. trachomatis by lysis of the infected cells, thus exaggerating the inflammation which results from infection(9). In addition to these effects,there is oxidative damage of the fallopian tubes which leads to increase of 8-hydroxy-2- deoxyguanosineand results in the damage of DNA (10). Moreover, the biomarker of endogenous oxidative DNA damage, 8-hydroxy-2- deoxyguanosine has been reported to be associated with reduced rate of fertilization and low quality oocytes (11, 12).

From the previous study, there is a suggestion for the interaction between the oxidative stress and the persistence of chlamydial infection in the etiology of female infertility. The mechanisms of this interaction can be a clue for providing a new treatment for those patients and in assisting the prognosis (13).

Therefore the aims of the present study were i. asses the biomarkers of oxidative stress including superoxide anion radical, Nitrite measurement and reduced glutathione in female patients with spontaneous abortions ii. Asses the prevalence of C. trachomatis antibodies IgA and IgG in those patients, iii. Correlate the levels of the oxidative biomarkers to the presence of C. trachomatis antibodies.

Material and Method

Subjects

The study was a case- control study. One hundred female patients with 2 or more spontaneous abortions were recruited from Mansoura University Hospital from January 2019 till January 2020. Moreover, one hundred healthy control females with normal gravidity and parity history were included. The patients were above 18 years old with normal hormone profile and no any associated diseases such as liver disorders, renal disorders or autoimmunity. The study was approved by Mansoura ethical committee and approval consent was obtained from each participant in the study. Each female was subjected to full medical history and clinical examination.

Laboratory Investigation

Ten milliliter of heparinized blood was withdrawn from each female under standard precautions. Sera were separated from each blood sample and subjected to laboratory determination of superoxide anion radical, Nitrite measurement and reduced glutathione by biochemical methods. Then aliquots of the serum was kept frozen at -20C for further determination of C. trachomatis antibodies IgA (RIDASCREEN® R-BiopharmAGAn der neuenBergstraße 17 64297 Darmstadt, Germany Chlamydia trachomatis IgA and IgG (Biovision- 155 S. Milpitas Blvd., Milpitas, CA 95035 USA |) by enzyme linked immunosorbant assay (ELISA).

Determination of Superoxide dismutase reductase (SOD)

The biochemical method used for determination of the concentration of superoxide anion depended upon the reduction of nitroblue- tetrazoliumand the formation ofnitroblue-formazan with the measurement of the intensity of the color by spectrophotometry (14). Ten microliter of nitroblue- tetrazolium was added to 100 microliter of plasma with incubation at 37C for 45 minutes. Then 10 microliter of Dimethyl sulfoxide was added and the intensity of the color was measured by spectrophotometer at 550 nm on microplate reader (). The concentration of superoxide dismutase reductase was reported in nmol/ml.

Nitrite measurement

The concentration of nitrite was determined as an indicator to the level of nitric oxide bytheGriessmethod (15). Serial dilution was performed for standard nitrite solution with 100 mM concentration from 100 to 1.6 mM in triplicate microtiter plate and 100 microliters of Griess reagent composed of equal volumes of 0.1% N-(1-naphthyl) ethylenediamine- and 1% sulfanilic acid was added with 50 microliter of plasma samples. After incubation for 5 minute and 10 minute. Measurement was performed by the use of enzyme linked microplate reader at wave length 550 nm (RT-2100C, Rayto, Shenzhen, P.R. China). The results were expressed in nmol/ ml from a standard curve established in each test, constituted of known molar concentrations of nitrites.

Determination of reduced glutathione

The concentration measurement was based upon the oxidation of the reduced form of glutathione with sulphide reagent 5-50-dithiobis-2-nitro-benzoic acid, forming a yellow product of 50-thio-2- nitrobenzoic acid (TNB) (16). The reaction was performed by adding equal volumes of 100 microliter of the samples and sulphosalicylic acid (2.5%) and incubation for 10 minutes. Color reaction was measured by spectrophotometer on microplate reader at 405 nm (). The results were expressed in nmol/ml from a standard curve established in each test, constituted of known molar GSH concentrations.

C.trachomatis Specific IgA

The kit used specific antigen from C. trachomatisouter-membrane-Then substrate will be added for this enzyme which will be converted to blue color. The reaction will be stopped by adding sulphuric acid. The detection of the presence of specific IgA antibodies will be carried out by microplate reader with wave length 450 nm.

Determination of C. trachomatis IgG

The kit used purified Chlamydia trachomatis antigen that was coated on the wells of microplate. In the presence of specific IgG for C. trachomatis in the plasma samples, binding reaction will be formed. Second antibody labeled with enzyme conjugate will be added to bind to the formed complex. Then an enzyme substrate will be added and the reaction will be stopped after incubation. The amount of IgG will be proportional to the intensity of the developed color which will be measured by microplate reader at wave length 450 nm.

Statistical analysis

The data of the results will be analyzed by the use of SPSS24. Quantitative data will be expressed as mean and standard deviation and the comparison will be performed by T test and a nova test. Qualitative data will be expressed as number and percentage and the comparison will be performed by Chi-square. P will be considered significant if >0.05.

Results

The study included 100 patients with spontaneous abortions from 2 up to 4 times with normal hormone profiles with mean age SD 29.9± 5.7. There were also, 100 females with normal parity and gravidity history with mean age SD 29.8± 5.9 years. The prevalence of C. trachomatis IgG in patients was significantly higher in patients (P=0.05) compared to the control subjects. The prevalence of IgA and IgG for C. trachomatis in patients was 4% and 8% respectively and, in the control, subjects the prevalence of IgA and IgG were 1% and 2% respectively. The concentration of oxidative stress products was significantly higher in the patients group compared to the control group (P=0.0001). The concentrations of nitrite and reduced glutathione were 27.9± 4.7, 31.4± 1.9 nmol/ml respectively in patients and the concentrations of nitrite and reduced glutathione were 18.9± 2.1 and 27.9± 3.9 respectively in the control group. The concentration of SOD was significantly reduced in

the patients group compared to the control group (P=0.0001). The concentration of SOD was 55.1 6.9 in the patients and in the control the concentration was 64.5± 7.7 nmol/ml, table 1.

In the patients with spontaneous abortions, the positive serology for C. trachomatis either IgA and/ or IgG was 10%, figure 1.

In the study of oxidative stress markers in the patients with positive serology for C. trachomatis, there was significant increase in nitrite concentrations compared to patients negative for C. trachomatis serological markers (P=0.005). While reduced glutathione concentration had insignificant increase in patients positive for C. trachomatis with reduced SOD compared to patients with negative serology to C. trachomatis, P=0.6, P=0.07, respectively, table 2.

Table 1: Comparison of age and laboratory findings between patients and control.

Parameter	Patients with recurrent spontaneous abortions (n=100)	Control Females (n=100)	P
Age	29.9± 5.7	29.8± 5.9	P=0.9
C. trachomatis IgA	4	1	P=0.2
C. trachomatis IgG	8	2	P=0.005
SOD nmol/ml	55.1± 6.9	64.5± 7.7	P=0.0001
nitrite nmol/ml	27.9± 4.7	18.9± 2.1	P=0.0001
Reduced glutathione nmol/ml	31.4± 1.9	27.9± 3.9	P=0.0001

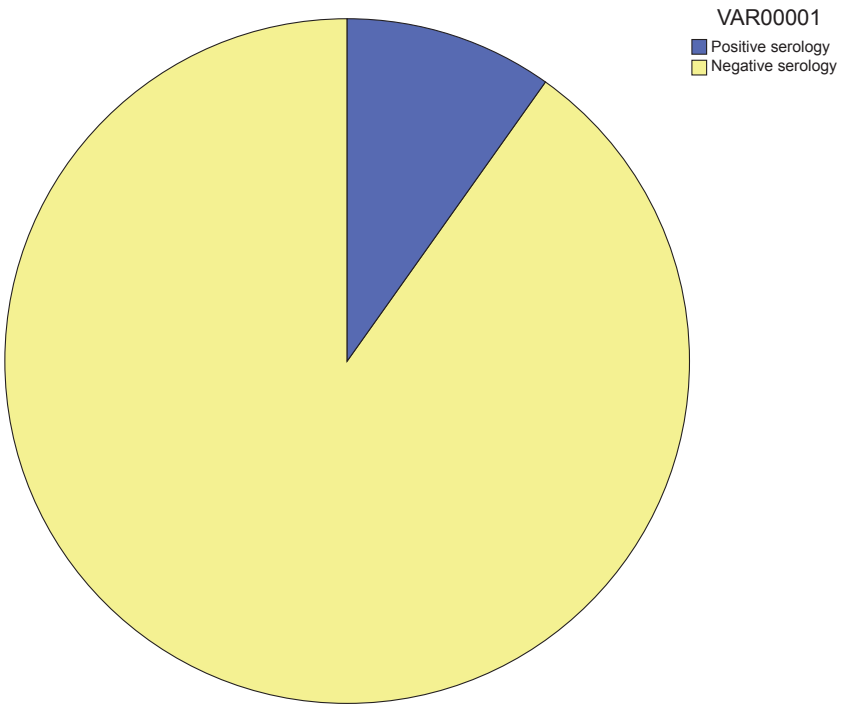

Figure (1): total seroprevalence to C. trachomatis among patients.

Table 2: Comparison between patients with positive antibodies IgA and/or IgG for C. trachomatis and patients with negative antibodies.

	Patients with positive IgG or/and IgA for C. trachomatis (n=79)	**Patients Negative for C. trachomatis IgG and IgA (n=21)**	**P**
Age	28.0± 5.1	30.1± 5.8	P=0.3
SODnmol/ml	53.7± 6.9	57.9± 6.4	P=0.07
nitritenmol/ml	31.6± 3.9	27.3± 4.6	P=0.005
glutathionenmol/ml	28.5 3.9	27.8± 3.9	P=0.6

Discussion

There are various etiologies of recurrent spontaneous abortions which may be attributed to alteration of the chromosomes, hormonal abnormalities, immunologic diseases and infections(17). The imbalance between oxidant/antioxidant may be implicated for such condition. The increase in the products of oxidative stress may lead to recurrent abortions via breaks in the double-strand DNA in the sperm and oocyte. (18, 19).

The concentration of oxidative stress products was significantly higher in the patients group compared to the control group (P=0.0001). The concentration of SOD was significantly reduced in the patients group compared to the control group (P=0.0001). Several studies have examined the role of OS in the incidence of pregnancy complications such intrauterine growth restriction or IUGR (20), preterm birth (21), preeclampsia (22, 23), and gestational diabetes (24, 25),**Similar results were reported with increase in different oxidative stress biomarkers and reduced in the antioxidant capacity in patients with repeated spontaneous abortions (13, 19).**

Among infections associated with female infertility is C. trachomatis. C. trachomatis infections are associated with wide varieties of pathological conditions such as salpingitis, pelvic inflammatory disease, ectopic pregnancyand femaleinfertility. C. trachomatis is a recognized agent of preterm labor and premature rupture of membranes (26, 27). Nevertheless, there are limited data about its association with miscarriage (26, 27).

In the present study,the prevalence of IgA and IgG for C. trachomatis in patients was 4% and 8% respectively and, in the control, subjects the prevalence of IgA and IgG were 1% and 2%. There was significant increase in seroprevalence of IgG for C. trachomatis in patients compared to control subjects. There were controversy in the association of C. trachomatis with spontaneous abortions as some studies reported no association between C. trachomatis active infection and spontaneous abortions (28, 29) while other studies reported significantly higher seroprevalence to C. trachomatis in patients with spontaneous abortions compared to females with normal pregnancy outcomes prevalence ranging between 11%-69% in miscarriages compared to 2-7% in healthy pregnant controls (30-32).

Persistant infection of C. trachomatis in female genital tract may lead to abortion via transfer to fetal tissue or endometrium. This effect may be associated with persistent infection and not during acute infection diagnosed by the presence of specific IgA (33).

The pathogenesis of C. trachomatis includes complex interaction between immune response and oxidative stress with the side effects of these reactions that lead to chronic endometritis, salpingitis, pelvic inflammatory disease and distal fallopian tube obstruction due to fibrosis (34, 35). In the present study there was significant increase in nitrite concentrations compared to patients negative for C. trachomatis serological markers (P=0.005). While reduced glutathione concentration had insignificant increase in patients positive for C. trachomatis with reduced SOD compared to patients with negative serology to C. trachomatis, P=0.6,

P=0.07, respectively. Previous studies reported the presence of imbalance in oxidant/antioxidant different groups of infertile in plasma, serum, follicular and peritoneal fluid of infertile women (36, 37). Also, a study reported the presence of this imbalance in patients with tubal infertility and spontaneous miscarriage associated with Chlamydia trachomatis infection (13).

The present study highlights that there was significant increase in oxidant stress biomarkers nitrite and reduced glutathione with significant reduction in superoxide dismutase in patients with repeated abortions. The prevalence of IgG to C. trachomatis was significantly prevalent in patients with recurrent abortion compared to control subjects. The nitrite was significantly correlated with positive serology to C. trachomatis.

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Pregnancy-induced low serum Ficolin levels may underlie the development of Pre-eclampsia and predict it

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Abstract

Objectives: Estimation of serum ficolin-2 and -3 for normotensive pregnant women to find a relation between ficolin levels and development of PE.

Patients & Methods: Primigravida attending the clinic prior to the 12th gestational week underwent blood pressure (BP) measurements and gave blood sample for estimation of fasting blood glucose (FBG) and ELISA estimation of serum ficolin. Enrolled women were asked to attend 4-weekly for BP measurement; 59 PE women (Group PE), 59 normotensive pregnant women (Group NT) and 59 non-pregnant women (Group NP) were enrolled.

Results: At time of PE diagnosis, all pregnant women had higher BP than their baseline measures, with significantly higher measures in PE women. Serum ficolin-2 levels were significantly lower in pregnant than NP women and in PE than NT women, while serum ficolin-3 levels were significantly lower in PE than NT women. Development of PE was positively associated with higher BMI, FBG and BP, while was negatively correlated with ficolin levels. Regression analysis defined low serum ficolin levels as negative predictors for PE development and serum ficolin-2 level <4.793 ng/ml can predict women liable to develop PE with 100% sensitivity and exclude PE with 100% negative predictive value.

Conclusion: Pregnancy has deleterious effect on complement pathway manifested by lower serum ficolin. Low serum ficolin-2 early in pregnancy is a sensitive screening test for pregnant women and can exclude PE development with 100% negative predictive value at level <4.793 ng/ml.

Keywords: Pre-eclampsia, Ficolins, Prediction of frequency & Severity, Fasting blood glucose .

INTRODUCTION

Preeclampsia (PE) is one of the most frequent and difficult illnesses in pregnancy, which jeopardizes both mother and fetus [(1)]. Clinically, PE is characterized by new onset maternal hypertension and proteinuria about the 20th gestational week (GW) in a pregnant woman who was normotensive prior to or at early pregnancy [(2)]. The incidence of PE remains high and because its etiology and pathophysiology are still poorly understood, its management has not been established yet [(3)]. An intact complement system optimizes placental development and function and is essential to maintain host defense and fetal survival [(4)]. Also, significant and intricate immune adapta-

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tions are essential for the establishment and maintenance of normal pregnancy [5].

Altered immune response may play part in PE pathogenesis [6], dysregulation of complement Bb activation between 10th and 20th GW was reported in women who later on develop PE [7], also genetic variations in complement genes C6 and Mannan-binding lectin-associated serine proteases 1 (MASP1) were found to be associated risk of PE and this risk varied by PE subtypes [6].

Ficolins; L-, M-, and H-ficolin [Ficolin-1, Ficolin-2 and Ficolin-3, respectively) are soluble oligomeric defense proteins with lectin-like activity [8] and are structurally similar to the human collections, MBL and surfactant protein A and D [9]. Ficolins are present in human serum and differ in carbohydrate-binding specificity, but in common have the ability to recognize the acetyl group [10]. Ficolins can activate the lectin pathway of the complement system which provides innate immune protection against pathogens, marks host cellular debris for clearance, and promotes inflammation [11]. Ficolins are innate pattern recognition receptors and play integral roles within the innate immune response within organs and throughout the circulation to numerous pathogens [12].

Hypothesis

The current study hypothesized a relation between disturbed serum ficolin-2 and -3 levels and development of PE and so could be used as predictors for PE development and/or its severity.

Objectives

Estimation of serum ficolin-2 and -3 early in pregnancy in women who are normotensives and follow-up for development of PE in trial to find a relation between early levels of ficolin and incidence and severity of PE

Design

Prospective comparative multi-center clinical trial

Setting

University Hospitals, Benha & Tanta, Egypt

Patients & Methods

All primigravida attending the Antenatal Care Unit (ACU) of Benha University Hospital since May 2017 for assurance of being pregnant were evaluated for eligibility for inclusion in the study. All women underwent clinical evaluation and those with infectious diseases, inflammatory states, manifest diabetes, endocrinopathy, essential hypertension, renal, hepatic or cardiac diseases were excluded. Also, women with family history of essential hypertension, metabolic syndrome, or gestational hypertension were excluded from the study. During clinical examination body weight and height were determined and body mass index (BMI) was calculated as weight divided by squared eight and only women with BMI<35 kg/m2 were included in the study. All enrolled women were asked to sign written fully informed consent to attend the ACU 4-weekly till delivery for follow-up. Women presenting after the 12thgestational week (GW), refused to sign the consent, or lost during follow-up were excluded.

The study protocols

At time of presenting to ACU and after assurance of pregnancy, gestational age was determined and all women underwent full history taking and complete clinical examination including blood pressure (BP) measurements; systolic (SBP) and diastolic (DBP) while woman was in supine position. Enrolled women were asked to attend the ACU fasting for at least 8-hr on the start of the 12th GW to give fasting blood samples for routine and study investigations. Then, included women were asked to attend the clinic 4-weekly for measurement of SBP and DBP and evaluation of extent of proteinuria. This protocol was previously approved by the Local Ethical Committee.

Diagnosis and categorization of pre-eclampsia (PE)

Preeclampsia (PE) was defined as development of gestational hypertension in a previously normotensive pregnant woman and is associated with proteinuria quantified as 1+ on dipstick [13]. PE was categorized as mild and severe according to BP measures obtained during follow-up visits, mild PE was diagnosed if SBP and DBP were <160 and <110 mmHg, respectively with proteinuria of <2+ and absence of systemic manifestations. Severe PE was diagnosed 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 [14]. Concerning timing of development of PE in relation to GW, PE was considered of early-onset if diagnosed prior to 34 GW and late if diagnosed after the 34th GW [15, 16].

Groups

1. Group PE included pregnant women who developed PE during pregnancy.
2. Group NT included number of pregnant women equal to that of PE women and were chosen from those who completed their pregnancy free of hypertensive manifestations.
3. Group NP included an equal number of non-pregnant women who were age-matched to women included in other groups and free of infectious or inflammatory disease as control group.

Laboratory investigations

Blood sampling

At the start of the 12th GW, all study participants gave 5 ml blood sample that 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 Eppendorf tube and stores at -80oC till be assayed. Blood samples were collected and numbered by an assistant who was blinded about diagnosis.

Investigations

Serum levels of ficolin-2 and ficolin-3 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 ficolin-2 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab213778, abcam, Cambridge, England) by quantitative sandwich enzyme immunoassay technique[17].

Human ficolin-3 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab213779, abcam, Cambridge, England) by quantitative sandwich enzyme immunoassay technique[18].

Statistical analysis

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using One-way Anova for analysis of variance between groups, paired t-test for analysis within each group and Chi-square test (X2 test) for analysis of non-numeric data. Sensitivity & specificity of studied parameters as predictors were evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05. Regression analysis (Stepwise method) was used for stratification of studied parameters as specific predictors. Statistical analysis was conducted using the SPSS (Version 15, 2006) for Windows statistical package. P value <0.05 was considered statistically significant.

Results

During the study period 491 primigravida attended the ACU for assurance of being pregnant; 73 women were excluded for not fulfilling the inclusion criteria and 418 women were enrolled in the study. During the pregnancy course 67 women developed PE and the remaining women completed their pregnancy free of PE manifestations. Unfortunately, 82 women were missed during follow-up; 8 PE and 73 normotensive women. Thus, 59 PE women had completed the observation period as Group PE and a similar number of normotensive women with cross-matched age and BMI were included as Group NT. A similar number of age and BMI cross-matched women were chosen from those who attended Family Planning Unit seeking for appropriate contraceptive method were chosen as Group NP (Fig. 1).

Revision of enrolment data of women of the three groups showed non-significant variance regarding age and height, while showed significant variance concerning patients' weight, BMI and fasting blood glucose. Interestingly, women who developed PE had significantly higher body weight, BMI and FBG in comparison to non-pregnant, while the differences were non-significant in comparison to normotensive pregnant women (Table 1).

Blood pressure measures estimated at time of enrolment showed non-significant variance between women of studied groups, while at time of diagnosis of PE showed significant variance between women of studied groups. At time of diagnosis of PE, all pregnant women showed elevated BP mea-

sures in comparison to their baseline measures, but the difference was non-significant in women of group NT, while was significant in PE women. Moreover, BP measures estimated at time of development of PE was significantly higher in PE women compared to corresponding measures of women of NT group and to women of NP group with non-significantly higher BP measures in women of group NT versus group NP (Table 2).

Eighteen women developed early PE; 6 had severe and 12 had mild PE, while 41 women developed late PE; 13 had severe and 28 had mild PE. Women developed early PE had SBP and DBP significantly higher compared to those had late PE and women developed severe PE had significantly higher BP measures than those had mild PE (Table 3).

Mean serum levels of studied parameters showed significant variance between women of studied groups. Mean serum levels of ficolin-2 were significantly lower in pregnant compared to non-pregnant women and were significantly lower PE than NT pregnant women. On contrary, serum ficolin-3 were significantly lower in PE women compared to NT and NP women of other groups, while were non-significantly higher in NT women than NP women (Table 4).

Presence of pregnancy was found to be positively associated with at enrolment increased body weight, BMI and higher serum FBG. Moreover, development of PE was found to be positively associated with higher at enrolment BMI, FBG and SBP. Estimated serum levels of ficolins were negatively correlated with presence of pregnancy and such correlation was significant with ficolin-2 (Rho=-0.392, p=0.0007), but was non-significant with ficolin-3 (Rho=-0.051, p=0.498). However, for development of PE, serum levels of both ficlon-2 (Rho=-0.353, p=0.0003) and ficlon-3 (Rho=-0.287, p=0.002) were negatively and significantly correlated with development of PE (Table 5).

Regression analysis of factors correlated with development of PE defined high at enrolment FBG (β=0.181, p=0.020) and SBP (β=0.267, p=0.001) as positive predictors, while low ficolin-2 (β=-0.440, p=0.0005) and ficolin-3 (β=-0.282, p=0.0009) as negative predictors for the possibility of PE development. ROC curve analysis defined high, at enrolment, FBG (AUC=0.617, p=0.028) and SBP (AUC=0.664, p=0.002) as specific predictors for oncoming PE, while low serum levels of ficolin-2 (AUC=0.297, p=0.0008) and ficolin-3 (AUC=0.334, p=0.002) as sensitive predictors for oncoming PE (Fig. 2).

Kaplan-Meier regression analysis defined a mean value for serum ficolin-2 at 4.793±0.326 ng/ml (95%CI: 4.153-5.432) as a cutoff point below which the hazard for PE development increases progressively, but was stationary at values above this cutoff point (Fig. 3). Evaluation of test validity character for this cutoff point, it showed 100% sensitivity for defining women liable to develop PE, 100% negative predictive value, 64.1% positive predictive value, 44.1% specificity rate and 72% accuracy rate.

Estimated serum level of ficolin-2 was negatively correlated with severity and timing of development of PE, but this correlation was non-significant (Rho: -0.195, p=0.138) with timing of PE development and significant (Rho: -0.573, p=0.0003) with severity of PE. ROC curve analysis showed that low serum ficolin-2 was specific predictor (AUC=0.752) for severe PE (Fig. 4).

Discussion

The obtained results showed a positive association between pregnancy and high body weight and mass index (BMI) with high serum fasting blood glucose (FBG) early in pregnancy. These findings indicated that pregnancy itself is obesity inducing and glucogenic state and this could be attributed to the fact that pregnancy induced vicious circle of getting obese with subsequent induction of insulin resistance (IR) that mostly induce a picture of pre-diabetes even if the woman was not diabetic prior to pregnancy nor developed gestational diabetes.

Multiple recent experimental studies assured this assumption and tried to explain these changes, wherein **Chen et al**. [19] found that in normally pregnant animals apo-retinol-binding protein 4 activates the stimulated by retinoic acid 6 signaling cascade, inducing IR through decreased phosphorylation of insulin receptor and insulin receptor substrate 1, and attenuated GLUT4 translocation and

glucose uptake. Also, **Petry et al**. [20] detected a link between pregnancy-associated plasma protein A concentrations in early pregnancy and subsequent glucose concentrations and blood pressures and attributed this to changes in insulin sensitivity and secretion. Thereafter, **Cardenas-Perez et al**. [21] found maternal programming by high-fat diet causes failure in glucose, leptin and insulin sensitivity and fat accumulation and **Olaniyi & Olatunji** [22] reported that obesity and hepatic lipid accumulation during pregnancy is accompanied by increased pyruvate dehydrogenase kinase-4 This study detected a positive significant correlation between high BMI, SBP and FBG at the 12th GW with the development of PE later during the pregnancy course. These findings spot light on the impact of pre-conception obesity and increased blood glucose level on blood pressure measures and subsequent PE development. In line of these data regression and ROC curve analyses defined elevated BMI and SBP early during pregnancy as significant predictors for later on development of PE.

These findings go in hand with **Falcone et al**. [23] who reported that bariatric surgery for obesity could ameliorate pregnancy-induced hypertension (PIH) and with **Kalafat et al**. [24] who found metformin therapy for gestational diabetes significantly reduced the risk of PIH. Moreover, **Davenport et al**. [25], (2018) documented that exercise interventions during pregnancy effectively lowered the possibilities for gestational diabetes, gestational hypertension and PE development. Recently, **Zhuang et al**. [26] reported that BMI was positively correlated with the occurrence of PIH and reduction of BMI may reduce the prevalence of this complication. Also, **Siddiqui et al**. [27] found severe antepartum hypertensive disorders were most strongly associated with obesity.

There was a negative significant correlation between development of PE and serum levels of ficolins. Moreover, statistical analyses reported that low serum level of ficolin-2 is a significant predictor of PE with 100% sensitivity and could exclude its future development with negative predictive value of 100%, especially if its serum level was >4.793 ng/ml which is a diagnostic cutoff point with high specificity as judged by area under ROC.

These findings supported earlier studies reported that mannose-binding lectin pathway activity is involved in pathogenesis of PE [28, 29, 30]. Also, **Halmos et al**. [31] detected significantly lower serum ficolin-2 and ficolin-3 levels in PE women than healthy pregnant women and significantly correlated with PIH-inducing angiogenic factors, plasma VWF: antigen, fibronectin and cell-free fetal DNA concentrations. Recently, **Larsen et al.** [32] found H-ficolin, M-ficolin and MASP-3 serum levels of PE women were lower than in normotensive pregnant women, in decreasing order of significance and MASP-3 levels were increased after delivery in PE and normotensive women, while serum H-ficolin levels were significantly increased after delivery in PE women.

Interestingly, the current study reported significantly lower serum ficolin-2 and non-significantly lower serum ficolin-3 in pregnant women than in non-pregnant women, a finding indicated an impact of pregnancy itself on complement system. This finding assured that previously reported by **Halmos et al**. [31] who detected significantly lower plasma levels of ficolin-2 in healthy pregnant than in healthy non-pregnant women, while ficolin-3 levels did not differ significantly between the two groups.

Conclusion

Pregnancy has deleterious effect on complement pathway manifested by lower serum ficolins. Disturbed complement pathway has a role in pathogenesis of PE. Lower serum ficolin-2 early in pregnancy is a sensitive screening test for pregnant women and can exclude the development of PE with 100% negative predictive value at cutoff point of 4.793 ng/ml. However, wide scale multicenter study is mandatory to establish the value of this cutoff point.

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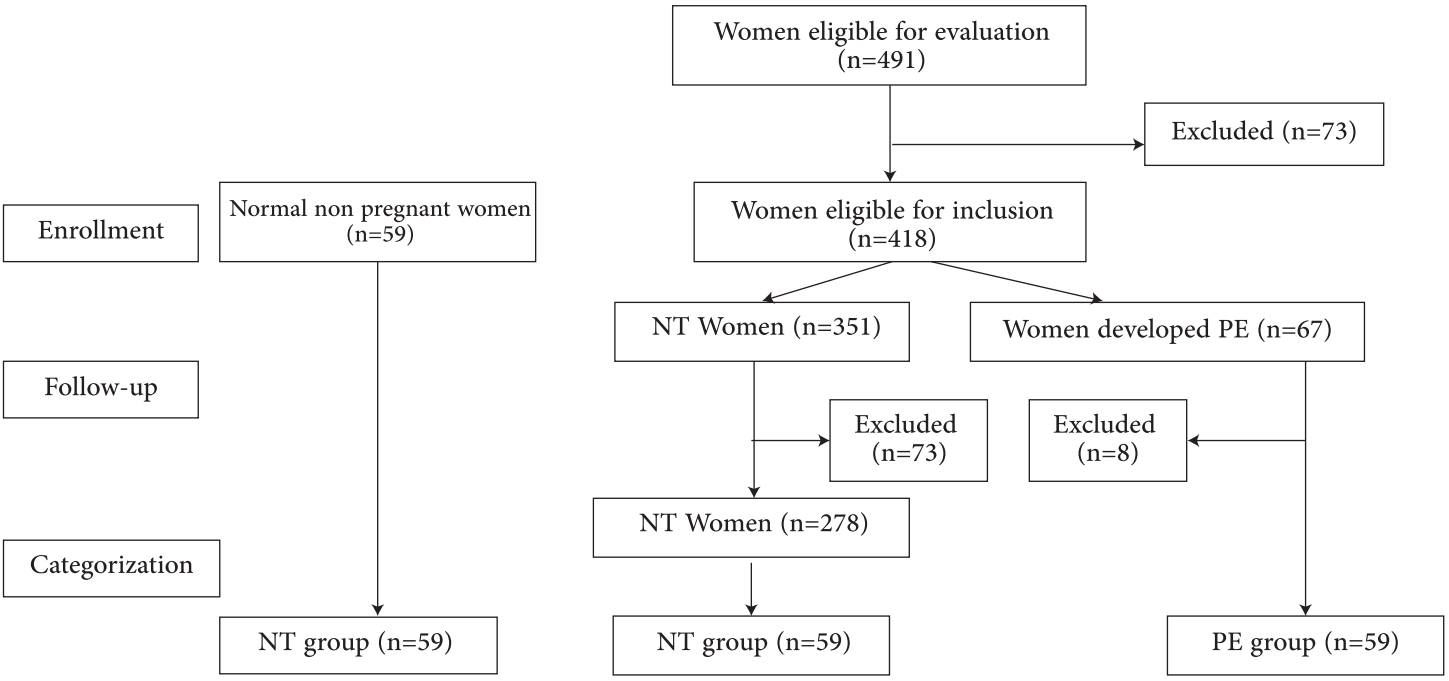

Fig. (1): Flow chart of the study

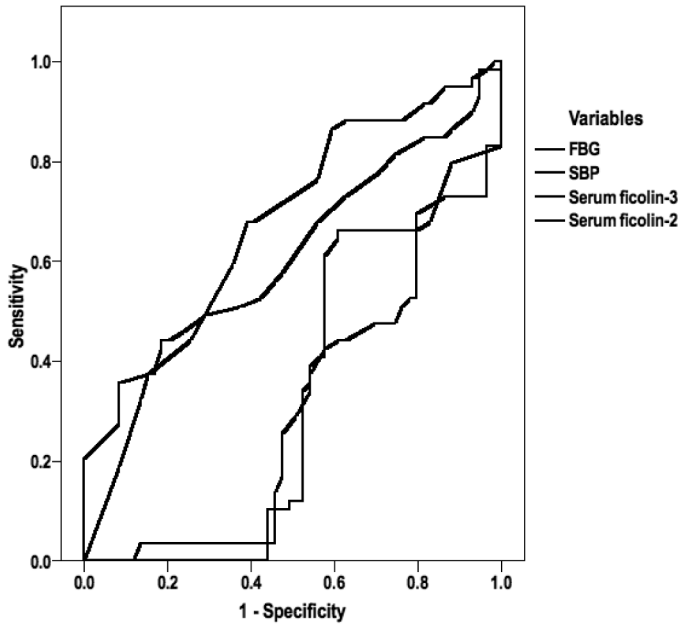

Fig. (2): showing ROC curve for studied variables as predictors for development of PE

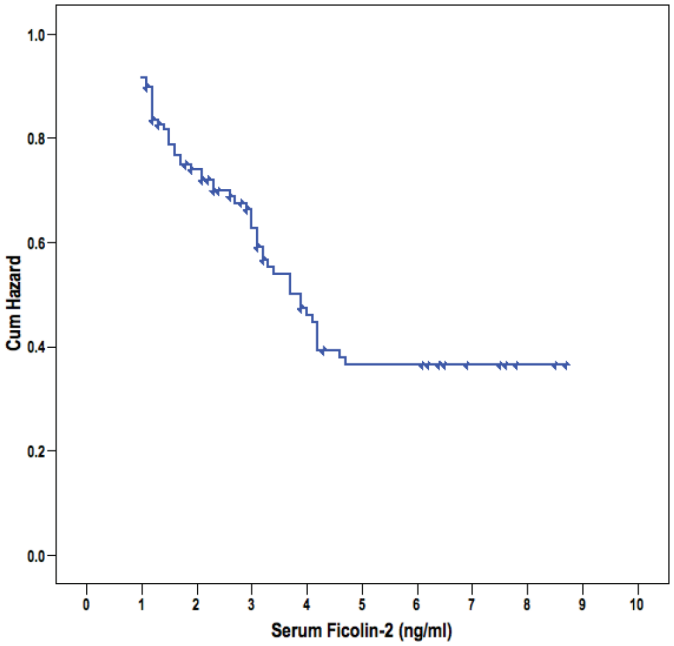

Fig. (3): showing cumulative hazard for developing PE at cutoff point for serum ficolin-2 of 4.793 ng/ml

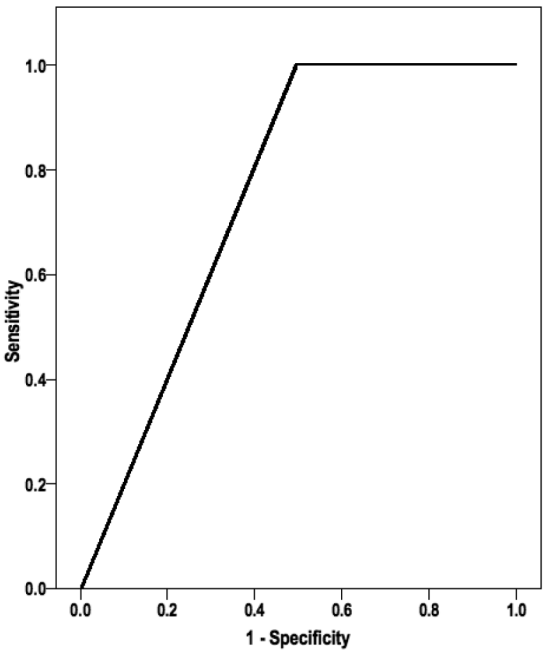

Fig. (4): showing AUC for serum ficolin-2 level as predictor for severe PE

Table (1): Enrolment data of studied patients.

Data		Group NP (n=59)	Group NT (n=59)	Group PE (n=59)	P
Age (years)		28±6.1	26.8±5.6	26.8±7	0.481
BMI	Weight (kg)	74.6±7.5	76±9	79.5±10.2*	0.012
	Height (cm)	169.8±2.1	170±2.4	169.6±2.6	0.653
	BMI (kg/m2)	25.9±2.9	26.3±3	27.6±3.4*	0.008
Fasting blood glucose (mg/dl)		87.2±6	90±9.2	92.3±11.8*	0.013

Data are presented as mean±SD; P value indicates significance of variance between groups; $p>0.05$ indicates non-significant difference; $p<0.05$ indicates significant difference; * indicates significant difference versus NP women.

Table (2): Blood pressure measures estimated at time of enrolment and development of PE manifestations compared corresponding levels in women of other groups.

Time of estimation		Group NP (n=59)	Group NT (n=59)	Group PE (n=59)	P
At enrolment	SBP (mmHg)	117.9±4.5	118±4.4	118.3±5	0.237
	DBP (mmHg)	82.2±4.9	82.9±3.6	83.4±3.5	0.298
At development of PE	SBP (mmHg)	118.2±4.5	119.5±8.8	154±11.4	<0.0001
	P1=		0.092	<0.0001	
	P2=		0.303	<0.0001	
	P3=			<0.0001	
	DBP (mmHg)	82.2±4.9	84.7±3.2	100.1±8.8	<0.0001
	P1=		0.361	<0.0001	
	P2=		0.077	<0.0001	
	P3=			<0.0001	

Data are presented as mean±SD; NP: Non-pregnant; NT: Normotensive; PE: Pre-eclampsia; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; P value indicates significance of variance of measures of the three study groups; P1 indicates significance of difference in comparison to respective at enrolment measures; P2 indicates significance of difference versus respective measures of Group NP; P3 indicates significance of difference of versus measures of Group NT; $p<0.05$ indicates significant difference; $p>0.05$ indicates non-significant difference.

Table (3): Mean blood pressure measures of women developed PE, categorized according to timing of development of PE and its severity

Variable	Category	Early PE (n=18)	Late PE (n=41)	Total (n=59)
SBP (mmHg)	Mild PE (n=40)	149±3.9	145.6±3.1*	146.6±3.7
	Severe PE (n=19)	172±3.9	168.4±2.8*	169.5±3.5**
	Total (n=59)	156.7±11.8	152.8±11.2*	154±11.4
DBP (mmHg)	Mild PE (n=40)	95.3±1.7	93.9±1.7*	94.3±1.8
	Severe PE (n=19)	115±2.6	111±3.7*	112.3±3.8**
	Total (n=59)	101.9±9.7	99.3±8.4*	100±8.8

Data are presented as mean±SD; PE: Pre-eclampsia; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; * indicates significance of difference versus Early PE; **: indicates significance of difference of versus Mild PE.

Table (4): Mean levels of estimated parameters in blood samples obtained at time of enrolment.

Parameter		Group NP (n=59)	Group NT (n=59)	Group PE (n=59)	P
Ficolin-2	Level	5.66±2.6	4.46±2.8	2.34±1	<0.0001
	P1=		0.016	<0.0001	
	P2=			0.0006	
Ficolin-3	Level	24.69±8.5	26.8±14.6	20.1±10.7	0.0059
	P1=		0.339	0.0102	
	P2=			0.005	

Data are presented as mean±SD; NP: Non-pregnant; NT: Normotensive; PE: Pre-eclampsia; PlGF: Placental growth factor; sFlt-1:; P value indicates significance of variance of levels estimated in women of the three study groups; P1 indicates significance of difference versus respective measures of Group NP; P2 indicates significance of difference of versus measures of Group NT; $p<0.05$ indicates significant difference; $p>0.05$ indicates non-significant difference.

Table (5): Spearman's correlation between clinical and laboratory data determined at time of enrolment and presence of pregnancy and development of PE

	Pregnancy		PE	
	Rho	p	Rho	P
Age	0.152	0.053	0.066	0.481
Body weight	0.261	<0.001	0.176	0.057
BMI	0.281	<0.001	0.206	0.025
FBG	0.195	0.009	0.204	0.027
SBP	0.045	0.554	0.286	0.002
DBP	0.015	0.839	0.131	0.159
Ficolin-2	-0.392	0.0007	-0.353	0.0003
Ficolin-3	-0.051	0.498	-0.287	0.002

BMI: Body mass index; FBG: Fasting blood glucose; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

Improving ovarian cancer outcome by studying the clinicopathological characteristics at a tertiary care hospital

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Abstract

Objectives: to optimize the outcome of malignant ovarian tumour via evaluation of the management procedures and protocols in Mansoura university hospitals and how far they are from the international standards.

Method: This descriptive, observational analytical study was conducted at Mansoura University Hospital, oncology unit of the department of Obstetrics & Gynaecology from January 2016 to December 2017 and included 94 patients. The participants were chosen from those attending the gynaecology outpatient clinic and diagnosed clinically and proven by investigations as having ovarian cancer. History, clinical examination and data obtained by abdominal and vaginal ultrasound as well as reports received from MRI and CT scanning we rereserved. Tumour markers were estimated by the same laboratory and technicians and treatment interventions provided with one year follow up results were collected.

Results: demographic patients' data recorded the mean age estimated for all patients is 45.28 ± 15.5 years, 42.5% of which are more than 50 years, and 6.4% younger than 18 years. Patients with low gravidity and parity included near half of the cohort (48.9 % and 44.7 respectively).Family history of ovarian, breast and colon cancers were positive in 9 patients only. Premenopausal ones recorded the highest number. From all of the cohort 6 cases gave a history of infertility. The main complaint was abdominal discomfort (40 cases), followed by abdominal swelling (24). Epithelial ovarian cancers were the most common (74.5%), with serous cyst adenocarcinoma constituting the majority (68.6 %) followed by granulosa cell tumour (10.6%) then border line and germ cell tumours(6.4%) for each group. The least reported subclass was immature teratoma (1%). Two cases were found to be Krukenbergmetastasis from colonic cancer. CA125 mean was+SD 510.41 ± 131.42 IU/ml. AFP and HCG were elevated in germ cell tumour and sex cord tumour. Most of the patients (74.5%) presented with advanced stage disease III and IV, whereas 25.5% of patients presented with stage I and II. Eighty-eight patients did primary debulking surgery. Two patients received neoadjuvant chemotherapy followed by secondary debulking surgery. The majority of patients (68%) had a combined surgery and chemotherapy.25.5% of the patients had the chance of fertility preserving surgery as they underwent unilateral oophorectomy. Estimated cancer mortality in our cohort proved 18 cases died (19.1%) within a year after treatment, 76 patients (80.9%) survived beyond a year after the initial treatment. The stage of the disease at presenta-

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tion was strongly correlated to survival beyond a year after treatment ($p < 0.001$).

Conclusion: mproving the health care system and promoting effective clinical management of ovarian cancer is an important issue to eliminate the survival disparities in our locality that requires improvement in guidelines adherent care.

Keywords: ovarian cancer, tertiary care.

INTRODUCTION

Ovarian cancer is a highly aggressive neoplastic disease in women with a high mortality rates [1]. Being diagnosed usually in advanced stage, the treatment is usually less valuable[2]. Symptoms reported for ovarian cancers include abdominal discomfort, bloating, nausea, and urinary urgency are vague and often mistaken with other minor diseases. Currently, the best available way for screening and detecting early stages ovarian cancer are transvaginal sonography (TVS) and elevated CA-125 [3].Histologically, over 90% of ovarian neoplasm arise from the surface epithelium, the rest from germ cells or stromal cells. The epithelial neoplasms are subsequently classified as serous(30-70%), endometrioid (10-20%), mucinous (5-20%), clear cell (3-10%), and undifferentiated (1%) with a 5-year survival rates recorded are 20-30%, 40-63%, 40-69%, 35-50%, and 11-29%, respectively. The subtypes differ with regard to risk factors, biological behaviour, and treatment response [3].Ovarian malignancies are surgically staged according to International Federation of Gynecology and Obstetrics (FIGO) staging system through staging laparotomy [4]. Treatment of ovarian cancer depends on the extent of metastasis at the time of diagnosis. Therefore, surgery is necessary for diagnosis, staging, and primary treatment and the goals of initial surgery is to provide the therapeutic benefit with cytoreduction. On the other hand, systemic treatment or adjuvant therapy, needs a precise histologic diagnosis and accurate staging [5, 6].The current study was held to optimize the management of malignant ovarian tumours via evaluation of the management procedures/protocols at Mansoura University hospitals and how far they are from the international standards.

PATIENTS AND METHODS

This prospective, descriptive, observational and analytical study was conducted at Mansoura University Hospital, oncology unit of the department of Obstetrics & Gynaecology from January 2016 to December 2017. The study protocol was reviewed and approved by the department of obstetrics and gynaecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt and approved by local ethical committee, institutional research board [IRB number R/16.11.11].The participants number was 94 and were chosen from those attending the gynaecology outpatient clinic and diagnosed clinically and proven by investigations as having ovarian malignancy. All women selected were subjected to verbal and written informed consents after explaining the basics of the study with her own rights to withdraw at any time. Patients who had associated endometrial carcinoma with ovarian malignancy were excluded together with those having incomplete data available verifying diagnosis or refusing participation. Full history taking and examination were recorded for all cases. All patients were subjected for trans abdominal and transvaginal ultrasound (TAS, TVS) by the same machine(Canon / Aplio500) and with the same sonographer. Also; it should be noted that, MRI and CT scanning being a part of malignancy workup pre and postoperatively were evaluated by the same radiologist (MRI: 1.5 T, sequences included conventional T1 and T2-WI, T2 fat sat images and CT:Inginia , Philips, Netherland, slice acquisition 3mm). Expected ovarian tumour markers including CA125, AFP and HCG were estimated by the same laboratory and technicians. These clinical data were collected together with investigations done or treatment interventions given; whatever the type or planned for the patients, then tabulated and subjected for statistical analysis. Follow up of patients was by clinical examination and transabdominal ultrasonography, vaginal ultrasonography and CT.

Statistical analysis

Data were collected, tabulated and statistically analysed by IBM computer using the Statistical Package for the Social Sciences (SPSS version 22). Chi- square test was used to compare the association between categorical variables between

groups and Fisher exact test was used where the cell count is less than 5. Student t-test was used to compare means± SD of quantitative variables in parametric data. P value <0.05 was set significant.

Results

The majority of cases(42.5%) were more than 50 years, 36.2 % from 30-50, 14.9 % from 19-30 years meanwhile about 6.4% of patients are younger than 18 years. The mean age estimated for all patients was 45.28 ± 15.5 years with a range from 9-83 years. Patients with low gravidity and parity included near half of the cohort (48.9 % and 44.7 respectively). About a half of the patients were premenopausal. Family history of ovarian, breast and colon cancers were positive in 9 patients only, who represent 9.5 % of all patients. Premenarchal tumours reported in2 cases (2.1%), premenopausal ones recorded the highest number being 48 (51.1%) while postmenopausal patients were 44 cases (46.8%). The family history for ovarian cancer was found in 9 cases (9.5) whereas 6 cases (6.4) gave a history of infertility.

Patient's clinical data in addition to surgical staging, histopathological types and associated tumour markers were represented in table (2).

Table (1): Distribution of cases according to main clinical presentation, surgical staging and histopathological grading and tumour markers.

Variable			No (%)
Main clinical presentation:			
Pelvic pain			12 ((12.8)
Abdominal discomfort			40 (42.6)
abdominal swelling or mass			24 (25.5)
GIT symptoms			10 (10.6)
Urinary symptoms			2 (2.1)
Histopathology types:			**No %**
1- Granulosa cell tumor			**10 (10.6)**
2- EOC:			**70 (74.5)**
Serouscystadenocarcinoma			48/70 (68.6)
Mucinous cystadenocarcioma			12/70 (17.1)
Undifferentiated adenocarcinoma			8/70 (11.4
clear cell carcinoma			2/70 (2.9)
3- Germ cell tumor			**6 (6.4)**
Immatureteratoma			1/6 16.6
Yolksac tumor			3/6 50.0
Dysgermenoma			2/6 33.4
4- krukenbergtumor			**2 (2.1)**
5- Borderline tumor			**6 (6.4)**
Stage:			
Stage I			10 (10.6)
Stage II			14 (14.9)
Stage III			44 (46.8)
Stage IV			26 (27.7)
Grade:			
GI			14 (14.9)
GII			34 (36.2)
GIII			40 (42.5)
Tumour markers	**Positive**	**Range (IU/ml)**	**Mean ± SD**
CA125	84/94 (89.3%)	11.00 – 7621.00	510.41 ± 131.42
AFP	14/18 (77.7%)	3 – 1811	282.63 ± 576.89
HCG	2/16 (12.5%)	3 – 637	51.13 ± 159.49

Data presented in number (%), EOC = epithelial ovarian cancer.

Analysis of EOC patients alone showed a mean age of 47.5 ± 11.7 years and that all age groups were represented within this group. On the other hand, at the age of 19-50 years Germ cell tumour represent 11.1% and Granulosa cell tumour 18.5%. Simple linear regression showed that age at presentation was strongly correlated to histopathology at presentation ($p < 0.05$), table 2.

Table (2): Correlation between histopathology types and age of the patients,

Variables	19-50y (n=54)	>50y (n=40)	P value
Granulosa cell tumour	10 (18.5)	0 (0%)	□2 (23.486) P < 0.05*
EOC Serous cystadenocarcinoma Mucinous cystadenocarcioma Undifferentiated adenocarcinoma clear cell carcinoma	**32 (59.3)** 22 (40.8) 6 (11.1) 4 (7.4) 0 (0)	**38 (95%)** 26 (65%) 8 (20%) 4 (10%) 2 (5%)	
Germ cell tumour Immature teratoma Yolk sac tumour Dysgermenoma	**6 (11.1)** 1 (3.7) 3 (5.5) 2 (3.7)	0 (0) 0 (0) 0 (0) 0 (0)	
krukenbergtumor	2 (3.7)	0 (0)	
Borderline Tumor	4 (7.4)	2 (5)	

Data presented in number (%), EOC = epithelial ovarian cancer* Significant P < 0.05

Table (3) shows the different modalities of treatments used and their related mortality.

More than 95% of patients had surgery during their treatment course. The majority of the patients (68) had a combination of surgery and chemotherapy,

Out of those who underwent primary debulking surgeries, 6 (6.3 %)had secondary debulking surgery either due to residuals or tumour recurrence (table (3).

Table (3): The different modalities of treatments used and their related mortality.

Variables	No (%)
Type of Surgery as treatment: **Primary Surgery** Unilateral adenexectomy TAH+BSO Debulking (GIT metastasis) **Interval debulking surgery**	 **88 (93.6)** 24/88 (27.3) 55/88 (62.5) 9/88 (10.2) **6 (6.3)**
Chemotherapy: No chemotherapy Postoperative chemotherapy Neoadjuvant Range of cycles needed	26 (27.7) 62 (65.9) 6 (6.4) 1-17 (Mean ±SD 5.66± 2.82)
Response rate of 6 months follow up of chemotherapy: Stage I (8 cases) Stage II (12 cases) Stage III (32 cases) Stage IV (16 cases)	 8 (100%) 12 (100%) 23 (71.8%) 6 (37.5%)

Mortality rate during 1 year follow up in relation to stage:	
Negative mortality: (N = 76)	
Stage I	10 (13.1)
Stage II	12 (15.8)
Stage III	38 (50)
Stage IV	16 (21.1)
Positive mortality: (N = 18)	
Stage I	0 (0)
Stage II	2 11.1
Stage III	6 33.3
Stage IV	12 66.6
	[□2 18.798; P <0.001*]

Data presented as number (%), mean ± SD. P value is set significant when <0.05.

T AH+BSO = total abdominal hysterectomy and bilateral salpingoopherectomy, GIT= gastrointestinal tract.

Discussion

The study cohort included 94 women diagnosed with various types of ovarian cancer. Ovarian cancer is the 7thmost common cancer and 8thmost common cause of cancer death among women [7] and it is predominantly a disease of older postmenopausal women with the majority of cases being diagnosed in women over 50 years [8].In this study the majority of our cohort were below 50 years (57.5%) including about 6.4% of patients younger than 19 years meanwhile those diagnosed above fifties included 42.5 %. The mean age of all patients was 45.28 ± 15.5 years and for EOC alone, being the commonest appeared to be 47.5 ± 12.7 years. Actually, this postulated more than a decade lower in the mean age of our patients than what is seen in Western populations[6, 7]but appearing similar to a study from our locality at Alexandria University by Mostafaa et al., 2012 [9] who proved the age was around 48 years and another one population-based cancer registry study from Gharbia governorate which estimated the mean age at diagnosis was 47.2 years [10].Also, Abdel Aziz et a 2014[11] reported that the mean age of patients in Menoufia study was 53.4 years and this despite slightly higher than our findings but still also recognizing a decade lower than what is seen in Western populations and supporting our findings. Compared to recent studies done outside Egypt [12], the middle east cancer consortium (MECC) evaluated the incidence of ovarian cancer among four countries, namely Turkey, Israel, Cyprus, and Jordan compared to the US SEER database and noted that, the highest age of patients diagnosed with ovarian cancer were in US women followed by Israeli Jews, Cyprus and Izmir (Turkey) as having nearly 10 years more and lowest in Jordanians being almost below the age of 50 years and this comes very close to our result. Therefore, the age incidence of our patients is similar to that of other parts of Egypt and Jordan, while it is nearly 10 years younger than those published from developed counters [11].This might be connected to the average life expectancy in Egypt that is shorter than reported in the developed countries but this needs to be further studied.The majority of the studied patients (51.1%) were premenopausal, despite the notion that ovarian cancer is a disease of post-menopause, and this appeared similar toHong Kong study where 49.5% were premenopausal [13] and a Nigerian study where 60%were premenopausal [14].The high incidence within the premenopausal age in this study may suggest a shift to an earlier age of occurrence in the population which is a worrisome development and in contrast with international studies done in the United States, United Kingdom and Australia which showed higher incidence in the postmenopausal age [15].

A large proportion of the women in this study were nulliparous (25.5%) and this is consistent with the incessant ovulation theory; (Fathalla 1971) where autorsugesstednulliparity as a risk factor for ovarian cancer due to repeated cycles of ovulation, resulting in an increased trauma and scar tissue formation on the surface epithelium of the ovary thereby an increasing risk of malignant transformation (16).

In our study, positive family history to cancers was present in 9.5% of case and this comes similar to the percentage in Pakistanian study (Sarwar et al., 2006)(17) and still lower than those with proved higher incidence in other localities especially when the patient had BRCA1 and BRCA2 Mutation Carriers. Really this point is not included in our investigations and considered as a shortcoming in our work [18].Studying clinical presentation of our patients, most of patients (74.5%) presented at late stage disease (III and IV) with vague abdominal bloating or distension. Our results were similar to results presented by Malik, 2002 and Sarwar et al., 2006 [17.19] but lower than that proved by Peas et al 2011 and Fatiregun et al., 2015 [20. 21]. The atypical presentation mentioned should suggests that clinicians must have a high index of suspicion when patients present with vague abdominal symptoms and signs and it is important for women and medical practitioners and health care providers to know the symptoms of ovarian cancer so that early diagnosis could be made as screening in general population is not yet effective. In our results, simple linear regression showed that stage at presentation was strongly correlated to age at presentation ($p < 0.001$) and showed that younger patients are more likely to present early with slight better prognosis.

In our study, EOC predominates constituting 74.5% of cases with higher incidence of serous followed by mucinous subtypes and this come in agreement of some other results [9, 11, 12, 19, 22). Also, in our study the percentages of granulosa cell tumor and germ cell tumors, 10.6% and 6.4 % respectively, appeared similar to results published by Freedman et al., 2001 [22].

CA 125 is generally recommended for clinical use in the diagnosis and follow up treatment of ovarian cancer. In our study, CA-125 was elevated in more than 89% of all cases and this comes similar to Alexandria study where CA-125 was elevated in all epithelial tumors [9], while in the study done by Sarwar et al [17],CA-125 was elevated only in 70% of epithelial ovarian cancer.

The standard care for ovarian cancer is proper surgical staging with optimal cytoreduction and chemotherapy. The aim of surgical effort in ovarian cancer is to reduce the burden of residual tumor to a point at which the chemotherapy will be optimally effective. The recommended surgical procedure is total hysterectomy, bilateral salpingo-oophorectomy and omentectomy aimed at radical cytoreductive treatment. Actually, this was the rule in this study where nearly 93.6 % of patients were managed initially with primary debulkimgsurgery. Many studies proved similar results [23]. On the other hand, a study done by Thrall et al., 2011 [24], reported that surgery was performed initially in 58.8% of the women with advanced ovarian epithelial carcinoma. The gynecology oncology group has defined optimal debulking as residual implants up to 1 cm to give a positive hope for postsurgical survival and such measurements are subjectively determined at the completion of surgery [25, 26].

Of those who underwent surgery, 36.4% of cases had optimum cytoreduction (no residual or residual less than 2 cm) and despite this comes near to figures found by Gerestein et al., 2011 [27] but much lower than figures proved by Brand, 2011[28] being 45 % and 65% respectively. It is obvious from the comparison that in this study, optimal cytoreductive surgery is performed in a much less frequency than done in Western countries and this can be explained by; the very late presentation in most of our cases and non-availability of multidisciplinary team evaluating all the cases before surgery. Therefore, the authors advice here is to increase the efficacy of adjuvant chemotherapy and success of surgery for ovarian cancer is patient's selection and stabilizing a skilled surgical team to achieve a survival benefit.

Different response rates to chemotherapy in ovarian cancers were seen in the literature with different percentages of complete responses. It was found to be 51% for cisplatin by Thigpen et al., 1994 [29] meanwhile for paclitaxel followed by either cisplatin or carboplatin it ranged between 64–74%[29]. In other studyused paclitaxel plus cisplatin versus paclitaxel plus carboplatin the response rate recorded, 46% vs. 53% respectively [30].After comparing our results to those of the large international studies, we found that the response rate including clinical complete response in our patients is closely similar to those of the international studies.

Regarding the type of chemotherapy regimen used, Paclitaxel-carboplatin was the most frequently

used regimen as a first line in 62.3%, followed by carboplatin single agent in 24.1% of cases. The response rate to the first line chemotherapy (including both neo-adjuvant and following surgery) after three cycles was seen in 72 % of the cases. In our study there was a statistically significant correlation between response and stage ($P < 0.001$). FDA recently approved the use of Bevacizumab, a monoclonal antibody, in the management of platinum resistant ovarian cancer[31], but this had no role in our patients because of the limitation of the resources.

The follow up period for our patients was 12 months and the progression free survival after first line chemotherapy was 10.8 months and this comes slightly lower than some international studies as this found to be ranged between 11 and 21 months [32].The possible explanation of that difference could be explained by; first; the number of the patients in our study was relatively small in comparison to those studies, second; no standard chemotherapy protocol was given among all patients in our study, and third; the high frequency of chemotherapy under-dosage and frequent interruption of the treatment were due to limited resources and at sometimes unavailability of the drugs specially paclitaxel. Further research on outcomes of implementing quality improvement programs in ovarian cancer care will improve the ability to implement centralized care and further identify factors improving outcomes in ovarian cancer care. Hence, the authors' advice for further study involving a large number and better to be multicentric for obtaining results near or similar to that listed international.

In conclusion

Accurate and annual studies on patients with ovarian cancer will help improving the health care system and promote effective clinical management to eliminate the survival disparities, and thereby improved the clinical outcome.

Conflict of interest

the authors declare that there is no conflict of interest.

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Posterior colpotomy versus laparoscopy for surgical management of ectopic pregnancy

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Abstract

Background and aim: to assess the the posterior colpotomy approach versus laparoscopic approach in the surgical management of ectopic pregnancy in stable patients.

Methods: A prospective cohort study conducted on 40 women with ectopic pregnancy, divided into two groups; **Group 1** included 20 patients for whom laparoscopic salpingectomy was done and **group 2** included 20 patients for whom salpingectomy was done via posterior colpotomy approach. Operative and postoperative data were collected and analyzed.

Results: Operative time was significantly shorter in group 2; 45 ± 8.1 min versus 56.5 ± 13 min in group 1($P<0.05$). Postoperative pain scoring was significantly lower in group 2 (5.7±1.1 vs 6.8±1.6 in group 2 and group 1 respectively) ($P<0.05$). Urgent laparotomy was performed for one patient in group 1and two patients in group 2 ($P>0.05$). There was no significant difference between the two groups regarding patient characteristics, clinical data, hospital stay and the need for blood transfusion.

Conclusion: Posterior colpotomy is a promising approach for surgical treatment of tubal ectopic pregnancy especially if laparoscopy is not available.

Keywords: posterior colpotomy; ectopic pregnancy; salpingectomy; tubal pregnancy.

INTRODUCTION

Ectopic pregnancy occurs when the fertilized ovum is implanted outside the uterine cavity with tubal ectopic being the commonest type [1].

It is one the most important causes of maternal morbidity and mortality. Up to 6% of pregnancy associated mortality are attributed to ectopic pregnancy [2].

Serial transvaginal ultrasound (TVS) along with serum β-human chorionic gonadotropin (hCG) allowed the possibility to diagnose early ectopic pregnancy. Management options include expectant, pharmacological (methotrexate) and surgical management [3].

Surgical management can be via laparoscopy which is the gold standard or laparotomy if laparoscopy is not possible either due to lack of equipment, surgeon experience or unstable patient [4].

Before the era of laparoscopy, posterior colpotomy approach was used for diagnosis and /or treatment of ectopic pregnancy [5,6,7].

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When laparoscopy is unavailable, posterior colpotomy approach has been suggested as an alternative procedure with shorter operative time hospital stay and reduced blood loss by a relatively recent reports [8,9].

The aim of the study was to assess the the posterior colpotomy approach versus laparoscopic approach in the surgical management of ectopic pregnancy in stable patients.

Materials and methods

A prospective cohort study conducted in Obstetrics and Gynecology department, Gama hospital, Al-khobar, Kingdom of Saudia Arabia in the period between March 2017 and March 2020. All women attending early pregnancy clinic or emergency room and diagnosed with tubal ectopic pregnancy by abnormal doubling of beta-hCG, emopty uterus and ectopic mass by transvaginal ultrasound (TVS) were invited to participate in the study. The institutional review board and the ethical committee in Gama hospital have formally reviewed and approved the study protocol and all women who accepted to participate signed the informed consent form after thorough explanation of the study objectives. Women aged 18-40 years, 6-8 weeks of gestation, vitally stable, minimal or no free fluid in the pouch of Douglas by TVS, diagnosed as ectopic pregnancy, candidates for surgical management and accepted to participate were included in the study. Women who were vitally unstable, disturbed ectopic with massive intra-peritoneal collection, previous laparotomy , previous vaginal surgery and those with chronic medical disorders were excluded from the study.Also, women with documented ovarian pregnancy and those who refused to participate, there data were not included in the analysis.The study included 40 patients divided into two groups; Group 1 included 20 patients for whom laparoscopic salpingectomy was done and group 2 included 20 patients for whom salpingectomy was done via posterior colpotomy approach. Laparoscopy was done using the closed technique, main port in the umbilicus and two ports in the right and left iliac fossae.Posterior colpotomy was done in the lithotomy position after vaginal retraction using a weighted speculum against the posterior vaginal wall, clamping and traction of the posterior lipof the cervix, transverse incision in the transition between the mucosa of the posterior vaginal wall and cervix followed by dissection of the vaginal wall, drainage of free blood,salpingectomy, washing of the pelvic cavity then closure.The first surgeon was the same person in all patients. In some cases, some surgical difficulties were faced; improper field in some was managed by using long- bladed vaginal retractors. Inability to access the tube was another important difficulty seen in few cases and was managed by antero-superior traction on the cervix by volsellum making the uterus retroverted (RVF). If still not accessible, Zumi uterine manipulator injector (disposable one used in laparoscopy in the hospital) was inserted to allow better manipulation; better RVF and side displacement of the uterus and that maneuver facilitated caching the tube and bringing it down by Babcock forceps. As per hospital policy, salpingectomy was the procedure of choice as there is no difference between conservative surgery and salpingectomy in terms of subsequent intrauterine pregnancy from the literature [10].

Outcome measures included mean operative time, average blood loss, pain. Post-operative pain intensity and the need for blood transfusion. Pain intensity was evaluated using the Visual Analogue Scale (VAS) with range from zero to ten directly with points of 0 = no pain at all and 10 = the most distressing pain. Close monitoring was done to report any postoperative complications then at discharge, they were given appointment in the outpatient clinic after seven days for evaluation of persistent symptoms or complications.

Statistical analysis: data were statistically analyzed by SPSS version 20 (SPSS Inc, Chicago, IL). Normally distributed numerical data were presented as a mean and standard deviation and were compared with unpaired Student's t-test. Qualitative data were presented as the number and percentage. Qualitative data were compared using chi-square test.For all tests, the statistical significance was considered when $p<.05$.

Results

There was no significant difference between the two groups regarding patient characteristics in terms of age, parity, body mass index, gestational age, beta-hCG level and ectopic mass size by TVS as revealed in table 1.

Operative time was significantly shorter in group 2 (posterior colpotomy group), on the other hand, there was no significant difference between the two groups regarding the need for emergency laparotomy and no intraoperative blood transfusion as depicted in table 2.

Pain intensity was significantly lower in group2 and there was no significant difference between the two groups regarding length of hospital stay and postoperative blood transfusion (table 3).

Discussion

The study included 20 patients in each group who were candidates for surgical management. Salpingectomy was done successfully in 19 patients of group 1 (laparoscopy group) and one patient needed urgent laparotomy because of significant intraoperative bleeding. . Salpingectomy was done successfully in 18 patients in posterior colpotomy group (group 2),urgent laparotomy was done for two patients, one due to significant intraoperative bleeding and another due to difficulty to access the fallopian tube. Mean operative time and postoperative pain were significantly lower in the posterior colpotomy group. Hospital stay as well as the need for blood transfusion was comparable between the two groups.

Women with successful procedure expressed their satisfaction specially due to absence of abdominal scar.

Surgical treatment of ectopic pregnancy through laparotomy was the standard procedure until Shapiro and Adler described the laparoscopic approach in the early 1970s [11].

With great development of diagnostic modalities and early diagnosis, expectant and pharmacological treatments are successful in many patients. But surgical treatment, being the most definitive, is still indicated in some cases [12].

With advancement of laparoscopic equipment and skills, operative laparoscopy is considered the gold standard for surgical management of ectopic pregnancy patients who are hemodynamically stable.

In 2008, there was report of two cases of tubal pregnancy treated successfully via posterior colpotomyapproach. The first patient refused laparoscopy as she did not wish to have incision scars in her abdomen. The second patient had an early ectopic pregnancy, candidate for medical treatment however, was not offered because of concerns regarding compliance with the treatment protocol [13].

Posterior colpotomy was a feasible approach for myomectomy through vaginal route where it was successful in 40 out 45 patients indicated for myomectomy [14].

The procedure is reported also for tubal ligation for sterilization with Advantages including absence of abdominal scar, minimal morbidity, no need for special equipment and shorter hospital stay, albeit there were some complications including urinary retention, urinary tract infection and occasional rectal injury [15].

The authors acknowledge that this is the first trial comparing posterior colpotomy approach versus laparoscopy for surgical treatment of ectopic pregnancy.

The small cohort in addition to a considerable number of women refused to participate represented unintended limitation of the study.

Future research is to conduct a randomized controlled study and to measure cost effectiveness of the posterior colpotomy approach against the standard laparoscopy for surgical treatment of ectopic pregnancy in stable patients.

Conclusion

Posterior colpotomy is a promising approach for surgical treatment of tubal ectopic pregnancy especially if laparoscopy is not available.

Acknowledgments

The authors would like to acknowledge the cooperation of the nursing staff in the outpatient clinic and operation room of Gama hospital.

Conflicts of interest

The authors declare that no conflicts of interest in relation to this article exist.

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Table 1: patient characteristics

	Group 1: N=20	Group 2: N=20	Student t-test	P-value
Age (years)	29.7±3.1	29.9±3.2	0.2	0.84
Parity	3.1±1.7	3.3±1.9	0.35	0.7
Body mass index (Kg/m²)	25.7±4.2	26.1±3.9	0.3	0.76
Gestational age (weeks)	7.3±2.4	7.8±2.8	0.61	0.55
Serum β-hCG levels (mIU/mL)	7846±5122	7782±4982	0.04	0.97
Mass diameter by TVS (mm)	42±23	45±21	0.43	0.67
Fetal cardiac activity N (%)	4 (20%)	3(15%)	0*	1

*: Chi-square

Table 2: operative data for both groups

	Group 1: N=20	Group 2: N=20	Student t-test	P-value
Mean operative time (min)	56.5 ± 13	45 ± 8.1	3.36	**0.0018**
Average blood loss (cc)	428±116	411±121	0.45	0.65
Intraoperative blood transfusion	0	0	-	-
Emergency laparotomy	1(5%)	2(10%)	1.08*	0.3

*: Chi-square

Table 3: post-operative outcomes for both groups

	Group 1: N=20	Group 2: N=20	Student t-test	P-value
Pain intensity(VAS)	6.8±1.6	5.7±1.1	2.5	0.02
post-operativeHospital stay (hs)	25±4.5	23±4.3	1.44	0.16
Post-operative blood transfusion	2(10%)	3(15%)	0*	1

*: Chi-square

Estimation of Gestational Age in Third Trimester of Pregnancy by Fetal Transcerebellar Diameter and its Accuracy
Article type: original article.

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Abstract

Objective: estimation of accurate gestational age correctly remains a crucial step in the antenatal care provided to all pregnant women. Our aim was to find out if transcerebellar diameter (TCD) could aid precisely in detecting accurate gestational age using ultrasound.

Patients and methods: One hundred and three healthy women with singleton pregnancy were included. Pregnancies complicated with fetal anomalies, intrauterine growth restriction or death were excluded. Eligible participants were examined extensively by ultrasound to confirm the gestational age using TCD, biparietal diameter (BPD) and femur length (FL). Then, data were collected and statistically analyzed comparing the three measurements.

Results: TCD was accurate within in 88.3% of the cases, while FL was accurate in 65% of the cases and BPD was accurate 51.5%. Bland-Altman comparison of actual GA and GA estimated by TCD, FL and BPD showed narrow limits of agreement indicated low bias and better test with TCD, thus GA estimated by TCD was the best .There was good correlation between actual GA and GA estimated by TCD ($r=0.989^{**}$, $p< 0.001$).

Conclusion: TCD is the highest statistically significant measurement which could be used in the third trimester for assessing the gestational age compared with FL and BPD without any effect of parity, gestational age or fetal presentation on its accuracy.

INTRODUCTION

The pregnancy date estimation is mandatory for the pregnant ladies to have the expected time of delivery in which various tests will be taken to achieve the estimated time. There are methods used to determine the gestational age including the date of the first day of the last menstrual cycle, clinical assessment, and ultrasonography [1]. The four basic measurements, including biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), femur length (FL), can be performed using standard American institute of ultrasound in Medicine guidelines[2].

In third trimester, various ultrasound parameters including FL are used for gestational age assessment, yet it shows a margin of error of two to three weeks from the actual gestational age [3]. Also, the BPD shows a margin of error of three or four weeks from actual gestation because of the large biological variations in fetal skull shape and size [4].

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In third trimester when there is excessive molding of the head, BPD and HC become unreliable, in this case transcerebellar diameter (TCD) becomes a reliable marker for estimation of gestation since the cerebellum is not liable to change in its form and size as it is protected very well inside the posterior fossa surrounded by dense petrous and occipital bones [5].

TCD can better predict gestational age especially in cases where there is variation of fetal head shape, such as dolichocephaly and brachycephaly [6,7]. TCD applied in cases where it is difficult or impossible to calculate biparietal diameter, or cases where it is unsuitable because of the expressed molding of head [8]. In this study we compare between the TCD the BPD and the FL in accuracy of assessment of gestational age in the third trimester.

Patients and methods

Prospective cross-sectional study carried out between January 2019 and June 2019 in Obstetrics and Gynecology Department, Kasr-AlAiny medical school, Cairo University after obtaining the approval from the local ethical committee. Pregnant women were recruited from the obstetrics outpatient clinic while attending for routine antenatal care between 31 to 36 weeks a total 103 women were examined for this study.

The sample size calculated according to equation [9]:

$N = 4\sigma 2\ (Zcrit + Zpowr)2/D2$

N= Total sample size which is the sum of the sizes of both comparison groups

σ = The standard deviation of each groups, assumed to be equal in both groups=1

Zcrit= The desired significance criterion. =0.05

Zpowr = Desired statistical power. =80%

D = The minimum expected difference.

The calculation showed that we should include at least 100 women to achieve study power of 80%. Women in the age range from 18 to 40 years old with healthy singleton pregnancy at 31-36 weeks' gestation (calculated by the first day of last menstrual periods and confirmed by 1st trimester ultrasound scan) were included. Exclusion criteria were intrauterine growth restriction, intrauterine fetal death, and congenital anomalies of fetus.

Women included in this study were subjected to verbal consent that was obtained from all candidates. Then, full history was taken and included personal history, menstrual history, obstetric history, medical history, drug allergy, operative history and any obstetric or operative complications. Women were examined by general examination, abdominal examination and full ultrasound examination done for gestational age determination, placental position, fetal anatomy, amniotic fluid volume, confirming the fetal presentation and position and measure the fetal TCD, BPD and FL.

Technique of ultrasound

- Trans-abdominal ultrasound using (TOSHIBA Xario 100) was performed on all cases while women were in a tilted position with the head of the bed raised 30 degrees and with a small pillow under the right loin [10].
- BPD measurement was taken in the transthalamic view with a rugby-football-shaped skull, rounded at the occiput and more pointed at the sinciput. Along midline halfway between the proximal and distal scale echoes. The cavum septum pellucidum divided the midline one-third of the distance from the sinciput to the occiput. The two anterior horns of the lateral ventricles proportionally located about the midline. All or part of the posterior horns of the lateral ventricles equally placed near the midline. The BPD includes the thickness of only the upper parietal bone (outer to inner measurement) [10].
- FL measurement was obtained with both ends of the ossified metaphysis are clear. The longest distance of the ossified diaphysis was measured regarding the angle between the femur and the insolating ultrasound beams that is typically between 45◦ and 90◦. Each caliper was placed at the ends of the ossified diaphysis without containing the distal femoral epiphysis if it is evident. This dimension should eliminate triangular spur artifacts that can incorrectly encompass the diaphysis length [10].
- Measurement of the TCD was obtained by getting the transthalamic view of BPD then rotating the probe slightly downwards, allowing the posterior horns of the lateral ventricles to

disappear from the view being replaced by the cerebellum. The TCD measured at 90 degree to the long axis of the cerebellum across its widest point, using the outer to outer method. All collected data were tabulated and subjected to proper statistical analysis.

Statistical Analysis

Collected data entered using Microsoft excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. Qualitative data was represented as number and percentage. Quantitative data represented by mean ± SD. Differences between quantitative variables by independent by T test. Correlation between groups was tested by Pearson's correlation and the agreement by Kappa agreement. P value was set at <0.05 for significant results.

Results

One hundred and three pregnant women with healthy singleton pregnancy were included for statistical analysis. The age of pregnant women included in the study was in the range of 18 to 39 years old, the gestational age of pregnancy ranges from 31 to 36 weeks. As regards to data found in the Table (1) the mean age was 28.87, the mean gestational age was 32.93, PG were (39.8%) and the of multi gravida were (60.2%).

As regards to data found in the Table (2) the mean GA by TCD was 32.87 weeks, mean GA by BPD was 33.19 weeks and GA by FL was 32.98 weeks among studied population. When we compared TCD measurements between primipara and multipara at different gestational ages, there was no statistically significant difference between them as shown in table (3). Therefore, it seems that conditions related to multiparity as obesity or pendulous abdomen to affect TCD readings. Moreover, comparing TCD measurements according to fetal presentation, as presented in table (4), did not show any statistically significant difference. They were comparable in cephalic, breech and transverse presentation.

There was significant positive correlation between GA ultrasound parameters and their estimated GA. The highest was with TCD followed by FL at the last BPD as found in Table (5). There was no significant difference between actual GA and GA estimated by TCD or FL measurements, while BPD cannot be used alone for estimation of GA as seen in Table (6). The frequency and percentage of correct assessment by TCD was highest followed by FL and at the last is BPD as revealed in Table (7). The narrow limits of agreement indicated low bias and better testing results accordingly GA estimated using TCD was the best as in Table (8). Finally, Table (9) showed that TCD was significant predictor for GA when compared with others.

Discussion

Accurate gestational age estimation is the corner stone in any obstetric management. Fetal development monitoring is now possible with the introduction of ultrasound. Several biometric parameters were in use for the detection of fetal gestational [11]. Among the various clinical criteria, using the first day of the last menstrual period is the most used, but it is not useful when the woman is unsure of her dates[12].

The cerebellum represents the earliest system to begin to develop in neural system and the last one to complete after birth and it is representing the most complex structure in the embryo [13]. TCD may be a more reliable predictor than BPD since the external pressure does not affect the posterior fossa, for example in fetal malposition as breech presentation or with oligohydramnios, which may induce distortion of the fetal head [11].

Because TCD seems unaffected by intrauterine growth restriction measuring TCD is especially advantageous when it is suspected or when GA is uncertain or with macrosomia [14].

The aim of this study is to compare the accuracy of TCD measurement in estimation of the gestational age in the third trimester with the current fetal biometric measurements (FL and BPD) and gestational age estimated by last menstrual period. The mean age of studied group was 28.87 years the mean gestational age was 32.93 weeks at time of measurement. All the studied group had singleton uncomplicated pregnancy, with known LMP.

In the present study the mean GA estimated by TCD was 32.87 weeks, mean age estimated by

BPD was 33.19 weeks, and that estimated by FL was 32.98 weeks. The three sonographic measurements were compared to the actual gestational age estimated by LMP, and measurement of the mean gestational age was 32.932 weeks, the means of gestational age estimated by TCD near to the actual GA.

A study in 2013 studied demonstrated that TCD was a reliable method of gestational age determination in third trimester than BPD [4]. A study in 2014stated that TCD gave correct assessment corresponding to the gestational age by LMP in 80.1% of patients, and as FL in 70.9% of patients [3].

A study in 2014, studied pregnant women in their third trimester to determine the accuracy of the TCD in assessment of gestational age. The TCD considered a dependable method for assessing gestation in third trimester of pregnancy [15].

A study in 2004, concluded that TCD measurement gave a gestational age within 3 days in 59% and within 1 week in 90%. While the FL gave a gestational age within 3 days in 46% and within 1 week in 80%. While, the BPD gave a gestational age within 3 days in 29.5% and within 1 week 60%, being the least accurate measurement amongst the 3 ones used [16].

A study in 2016, studied pregnant women in the period of 15 to 40 weeks. They performed a linear correlation between TCD and GA and the progressive changes in cerebellum from grade I to grade III with advancing gestational age [11].

A study in 2015, studied pregnant women between the 15th week of gestation and term. TCD positively correlated with BPD, HC, AC and FL so that TCD can be used as a reliable parameter for determination of gestational age [17].

A study in 2003, studied pregnant women for usefulness of TCD as a sole parameter for calculating gestation in the third trimester. The gestational age by TCD, BPD, FL and AC compared with actual gestation. The gestational age measured by TCD correlated with that measured by FL [18].

A study in 2015, studied pregnant women in the second and third trimesters. The accuracy of TCD in detection of GA was constant throughout the second and third trimesters of pregnancy [19].

Bhimarao et al. conducted a study over one year on 50 clinically suspected IUGR and showed one of the important limitations in the ultrasound study that it depended on the operator for precise measurement, there was also technical limitation due to the dense shadowing in the posterior fossa in the third trimester, which may limit adequate visualization of the cerebellum [20].

Naqvi et al. asked two US Maternal-Fetal trainers and 10 trainees participating in a 3-day hands-on fetal biometry workshop to join his study. To assess agreement, 3 trainees and 1 trainer obtained two measurements of the BPD, HC and TCD from 16 pregnant women in the second and third trimester. Agreement was assessed by Bland Altman plots, TCD measurements obtained by trainees were 0.2 cm less than expert measurements, this represented a 12.7% difference. Self-reported confidence in obtaining TCD measurements increased following the training. That meant sonography trainees can obtain acceptable TCD measurements in late pregnancy after a brief didactic and hands-on training workshop [21].

From the study we can conclude that TCD is an accurate method for assessment of gestational age in third trimester followed by FL, and the least accurate is the BPD. It can also be used for gestational age estimation in cases who are not sure about their LMP. All previous studies concluded that TCD was more accurate than other parameters in estimation of gestational age. Therefore, further studies with larger numbers of subjects and blinded observers are needed to assess the accuracy of TCD measurement in estimation of the gestational age.

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Table (1): Distribution according to age and parity among study group (n=103).

%	Number (n = 103)	Age (In years)
3.88 % 33.00% 2.91% 60.19%	4 34 3 62	< 20 21: 25 - 26 – 30 > 30
%	**Number (n = 103)**	**Parity**
39.8 60.2	41 62	Primigravida Multigravida

Table (2): Mean and standard deviations of all parameters GA among study group (n=103).

Maximum	Minimum	Median	Std.Deviation	Mean	N	Parameters
36.00	31.00	33.00	1.35951	32.932	103	GA
36.00	30.00	33.40	1.45979	32.873S8	103	TCD
36.00	30.00	33.00	1.60868	32.9806	103	FL
36.00	30.00	33.00	1.32885	33.1942	103	BPD

Table (3): TCD according to parity clustered by gestational age among studied group (n=103).

GA	Parity	n.	Mean ± SD	p-value
31 w (n=20)	Primi	5	35.02± 0.52	0.12(NS)
	Multi	15	34.6± 0.43	
32 w (n=18)	Primi	9	36.4± 0.56	0.11(NS)
	Multi	9	36.6± 0.44	
33 w (n=32)	Primi	9	38.4± 0.24	0.074(NS)
	Multi	23	38.07± 0.47	
34 w (n=17)	Primi	12	39.25± 0.27	0.10(NS)
	Multi	5	39.04± 0.19	
35-36w (n=16)	Primi	6	40.6± 0.68	0.26(NS)
	Multi	10	41.0± 0.56	

Table (4): TCD level according to position among studied group (n=103).

Position	n.	TCD			KW test	P value
		Mean	± SD	Range		
Cephalic	70	37.8	2.04	34.1-41.9	3.06	0.21 (NS)
Breech	21	38.2	1.97	34.6-41.0		
Transverse	12	36.9	2.05	34.1-41.5		

Table (5): Correlations between GA estimated by LMP and GA estimated by sonar parameters among studied group (n=103).

p value	r value	Parameters
<0.001	0.989**	TCD
<0.001	0.824**	FL
<0.001	0.762**	BPD

Table (6): Paired analysis between actual GA and GA estimated by sonar parameters from 31 to 36 weeks among studied group (n=103).

		Mean	N	Std. Deviation	Paired t	P
Pair 1	GA	32.9320	103	1.35951	1.124	0.121
	TCD	32.8738	103	1.45979		
Pair 2	GA	32.9320	103	1.35951	-2.187	0.034*
	BPD	33.1942	103	1.32885		
Pair 3	GA	32.9320	103	1.35951	-0.541	0.593
	FL	32.9806	103	1.60868		

Table (7): Comparison of correct assessment of gestational age by TCD, FL and BPD among studied group (103).

Correct assessment of gestational age by TCD (Frequency and percentages)					Correct assessment of gestational age by FL (Frequency and percentages)					Correct assessment of gestational age by BPD (Frequency and percentages)				
Weeks ofgestation	Total no (n)	yes	no	%	Weeks ofgestation	Total no (n)	yes	no	%	Weeks ofgestation	Total no (n)	yes	no	%
31	20	14	6	70	31	20	12	8	60	31	20	6	12	50
32	18	16	2	88.9	32	18	10	8	55.6	32	18	6	12	33.3
33	32	31	1	96.9	33	32	25	7	78.1	33	32	25	7	78.1
34	17	15	2	88.2	34	17	10	7	58.8	34	17	7	10	41.1
35	14	13	1	92.9	35	14	9	5	64.2	35	14	8	6	57.1
36	2	2	0	100	36	2	1	1	50	36	2	1	1	50
Total	103	91	12	88.3	Total	103	67	36	65	Total	103	53	50	51.5

Table (8): Bland-Altman comparison of GA by LMP and GA by TCD, FL and BPD

GA (TCD) vs. GA (LMP) in weeks	
Limits of agreement	-0.412 to 0.529
Mean difference (95% CI)	0.058 (0.012 to 0.104)
Range	30.5 to 36
Pitman's test of difference in variance	<0.001
GA (BPD) vs. GA (LMP) in weeks	
Limits of agreement	-2.082 to 1.557
Mean difference (95% CI)	-0.262 (-0.444 to -0.081)
Range	30.5 to 36
Pitman's test of difference in variance	0.724
GA (FL) vs. GA (LMP) in weeks	
Limits of agreement	-1.835 to 1.738
Mean difference (95% CI)	-0.048 (-0.227 to 0.130)
Range	30.5 to 36
Pitman's test of difference in variance	0.003

Table (9): Simple linear regression analysis for TCD as a predictor of GA.

Model summary	**R2**	**Adjusted R2**		**SEE**	**F**	**P-value**
	0.942	0.942		0.32	1647	<0.001(HS)
Variable	**Unstandardized Coefficients**		**Standardized Coefficients**	**95% CI of B**	**t**	**P**
	B	**Std. Error**	**Beta**			
(Constant)	8.55	0.6		7.3-9.7	14.2	<0.001 (HS)
TCD	0.65	0.016	0.971	0.61-0.68	40.5	<0.001 (HS)

IS Three D power Doppler of the endometrial and sub endometrial regions effective in predicting endometrial implantation? Prospective cohort study

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Abstract

Objective: This study aimed to evaluate the three dimension power Doppler indices together with uterine artery Doppler indices during the day of embryo transfer in predicting the outcome of ICSI cycle.

Study design: One hundred and three healthy women with singleton pregnancy werprospective cohort study.

Patient and methods: One hundred twenty patients were included in the study during ICSI cycles. This work was done at IVF unite of Dar El Teb hospital, Egypt. All patients included in the work had these criteria; age; 22–35 years, BMI; < 35 kg/m^2, oligo- or oligoasthenospermia. All patients received along agonist protocol of ovarian hyperstimulation and after follicular retrieval; embryos were transferred at the stage of blastocyst. Three D Power Doppler was done at the day of embryo transfer. Quantitative pregnancy tests were done for every patient. The rates of clinical and ongoing pregnancy were estimated. All women were categorized into two categories: with pregnancy and without pregnancy.

Results: Thirty-five percent of patients became pregnant. Our study showed non-significant differences in both groups regarding demographic, clinical and laboratory data except for some vascular parameters (endometrial VI, FI, VFI-subendometrial FI- u PI). Our study revealed a significant increase of some vascular parameters (endometrial VI, FI, VFI-subendometrial FI- u PI).and correlated to pregnancy. The endometrial VFI is the most sensitive vascular parameter correlated to pregnancy.

Conclusion: 3DPD is a useful non invasive predictor for IVF outcome.

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INTRODUCTION

Many factors determine the success in the cycle of IVF/ET; few of them are known to be directly related to the successful outcome. The implantation of good quality embryos remains a rate limiting step in IVF/ET management .In spite of the advances in ovarian stimulation protocols, improvement in culture conditions and the method of assisted fertilization, the implantation rate remains low. The success of embryo implantation depends on a dialogue between the transferred embryo and the receptive endometrium (1).

The receptivity of endometrium was investigated by different strategies like the histological dating of an endometrial biopsy (2), intra-uterine flushing for detection of endometrial cytokines (3), the genomic study of a timed endometrial sample(4). Nevertheless, ERA requires an invasive method, and high cost. Ultrasound can evaluate changes in the endometrium during stimulated cycles by non-invasive technique (5).

The uterine receptivity are controlled by many variables like the endometrial, and the sub-endometrial perfusion (6, 7).Many studies observed a positive correlation between the characteristics of endometrium, and implantation rate after IVF/ ICSI cycles, and the poor uterine receptivity was related to impaired blood flow in endometrial and sub-endometrial regions (8, 9, 10).

Ultrasonography was used as a non invasive tool to measure the endometrial thickness to show the effect of endometrial thickness on embryo implantation and endometrial receptivity but unfortunately conflicting findings were obtained. (11).

Some tried to assess the flow of blood in the uterine arteries by Doppler US and they found that uterine arteries Doppler did not represent the actual blood flow in the endometriam .Others tried to use three-dimensional power Doppler ultrasound for measurements of endometrial and sub-endometrial blood flows (1).The endometrial receptivity was evaluated in the endometrial and sub-endometrial blood supplies, especially in intrauterine insemination and IVF-ET cycles (12). This study aimed to investigate the three dimensional power Doppler indices together with uterine artery Doppler indices at the day of embryo transfer in predicting the outcome of ICSI cycle.

Patient and methods:

This prospective cohort research was done at IVF unit of Dar El Teb, Dokki, Egypt, since January 2015 till September 2019. Before the conduction of the study, the Local Ethical Committee approved the work. All women gave consent to participate in the work. One hundred twenty couples included in the work had these criteria; age; 22–35 years, BMI; < 35 kg/m2, male factor with oligo- or oligoasthenospermia. Exclusion criteria; -gross pathology in the uterus and tube, -Development of OHSS, -inadequate response to super ovulation, -failure of mature ovum to fertilize or inadequate development of the embryos to the stage of blastocyst and - If the couple refused to be included in the work at any stage of the treatment cycle.

All patients received long protocol for controlled ovarian overstimulation as described by Chang et al (13). The LHRH agonist ampoules were commenced in the prior mid-luteal phase (decapeptyl R 0.1mg, Triptorelin-Acetate, Ferring GmbH, Wittland 11, D-24109, and Kiel, Germany). After the pituitary down regulation was confirmed, the r FSH vials were given by 225 IU/day (Gonapure 75 IU, IBSA Institute Biochimique SA, Switzerland). During the follow up period of hyperstimulation, dosages were scheduled regarding the response of every woman.

When at least three dominant follicles (a size 18-20 mm) were reached in every patient, the HCG 10000 IU (Epifassi 5000 IU, Epico, Egypt) was taken. The follicles were retrieved 35 hours following HCG administration. Dydrogesterone 30 mg daily (Duphaston, Dydrogesterone 10 mg, Abbott, Pentapharma, Egypt) was used to support the luteal phase. At the day of embryo transfer (blastocyst stage), every woman underwent 3 D power Doppler US. Serum pregnancy test was done after twelve days later to embryo transfer, and if positive (chemical pregnancy), the TVS was used to detect clinical pregnancy while the ongoing pregnancy was detected at the end of first trimester.

Technique of 3D Power Doppler:

We used dedicated 3D transducers to obtain 3D US image. Firstly, determination of the volume box. Secondly, activation of the 3D probe while it was held stationary. Thirdly, the volume data were presented in multi planner display. By using 3D Power-Doppler ultrasound and the VOCAL program (the rotation angel was 30° in our study), we can evaluate the tissue vascularity. Three vascular parameters were used: the Vascularization Index (VI) represents the number of the blood vessels inside the volume box. Flow Index (FI) represents the intensity of blood flow within the area of interest. The Vascular-Flow Index (VFI) represents the number of the blood vessels and the intensity of blood flow within the area of interest. (14) (Figure 1). The “shell” function was used at different

thickness around the predetermined endometrium (in this study, it is estimated to be 5 mm) to measure the sub endometrial volume and estimate the vascularization in this region". (Figure 2)

Statistical analysis

Calculation of the sample size was done by using Open Epi (version 3, open source calculator-SSProor) depending on the number of patients fulfilling inclusion criteria in 6 months and attending to IVF unit of Dar El Teb hospital was estimated by 185 patients and percentage of ICSI success in a prior research is 32.4%(15), so to obtain a research power 80% and CI 95%, at least 120 patients must be included in the research. The variables were presented as mean ±SD. Independent t-test, Mann -Whitney-test and ROC curve analysis were used for statistical analysis. The SPSS program Version 18 was used. The statistical significance was considered when P value <0.05.

Results

Nine patients were excluded from the work from 129 women participating in the study due to; development of OHSS in 2 patients, 2 patients with inadequate ovarian response, failure of mature ovum to fertilize in 3 patients or inadequate development of the embryos to the stage of blastocyst in 2 patients. Figure 1

All included patients had demographic, clinical and laboratory data as presented in table 1. On assessment of serum pregnancy tests, it was found the chemical pregnancy was 39.2% while clinical pregnancy rate was 35%. Only 11 patients had abortions at 7-12 weeks gestation (ongoing pregnancy rate =25.8%). The included women were subdivided into two groups; group A (with pregnancy) and group B (with non pregnancy). Table 2. The no significant differences in both groups regarding the demographic data were presented in Table 1

Assessment of endometrial-sub endometrial and uterine blood flow at the day of embryo transfer, revealed significant increase of endometrial vascularity(VI,FI,VFI) in the pregnant women, significant increase of sub endometrial blood flow (FI) in the pregnant women. Also significant increase of pulsatility index of uterine artery correlated with pregnancy. The endometrial volume was comparable in both groups. Table 1

At the day of embryo transfer,3 D power Doppler of the endometrial and sub endometrial areas and uterine artery Doppler were used to predict endometrial implantation by The ROC curve analysis .The areas under the curve for E VFI, u PI,E FI ,SE FI and E VI were 0.82, 0.75. 0.66, 0.65, and 0.62 at a cut off ≥ 0.96 & ≤ 1.5, ≥ 26.7, ≥ 19.9, ≥ 19.8, ≥ 4.7 indicating that the endometrial VFI is more sensitive and specific than other vascular parameters. Table 3

In other words, the endometrial VFI is the most sensitive endometrial vascular parameter (figure 3), while the sub endometrial FI is the only sensitive sub endometrial vascular parameter (figure 4)detected by 3D PD US. On evaluation of the colour Doppler of uterine artery, it was found that only the u PI was significantly correlated to pregnancy. (Figure 5)

Discussion:

The 3D PD-US angiography is the most important diagnostic tool to evaluate restricted tissue, by showing and calculating relevant parameters (16, 17, and 18).The power Doppler ultrasound has extreme sensitivity to slight blood flow to detect overlapping vessels (19). The restricted tissue like endometrium is important for uterine receptivity (18). Our study showed that the thickness and volume of endometrium were not correlated with pregnancy.

The endometrial thickness was investigated for several years for detection of its relation to pregnancy and the results were controversial because the endometrial thickness was affected by different factors like mechanical stimulation or by the ovarian stimulation treatment method (18). For some times, the endometrial volume was considered an important index for endometrial receptivity with reporting it should be at least 2.0–2.5 ml for establishing pregnancy (20) while another research showed no pregnancy with volume less than 1ml (21). Our study, similar to several studies (22, 23, and 24), found no relation between the endometrial volume together with the endometrial thickness and pregnancy while others concluded a positive

correlation with pregnancy (17). This controversy can be seen in other view as the impact of endometrial thickness or volume on the pregnancy was favored by endometrial vascularization that should be investigated (25, 26). Because sufficient blood supply is necessary for endometrial receptivity (24), the endometrial neomicrovascularisation increases significantly in the follicular and early luteal phase (27) and is affected by different factors like age, medication, hormones (23). The endometrial neomicrovascularisation can be assessed by power Doppler combined with 3D US(17).Vascular indices like VI, FI, and VFI, can be estimated from the total number of color voxels and intensity of blood flow (28).Our study, similar to Wang et al (29) and Singh et al (1), found a positive correlation with pregnancy regarding endometrial vascular indices and sub endometrial flow index, in spite of the assessment of vascularization was questioned in different reports (30, 31,32). The clinical value of 3D-PD US has been intensively studied. Jinno et al (33) stated that the endometrial blood flow during second half of the cycle could predict the outcome in IVF cycles. Ng et al (23) found a positive correlation between flow of blood in endometrial and sub endometrial regions and pregnancy outcome. Furthermore there was a positive correlation between flow of blood in sub endometrial region and some cytokines like IL 15(34) and IL 18 and IL 18 B P (35). Our study, similar to Kim et al (16), found that the endometrial VFI was the most sensitive vascular parameter in predicting pregnancy (0.8) so it is included, recently, in Ultrasound multimodal score to assess the endometrial receptivity. The total score was 18, the lower the score, the worse the endometrial receptivity, and vice versa (36). Based on these results, it is reasonable to hypothesize that the endometrial vascularity is correlated to embryo implantation.

Othman et al (37) stated that the blood flow, in the endometrial and sub endometrial tissue, differed significantly according to BMI. It was lower in obese and overweight women. In obese women, the relatively hyperestogenemia may have negative impact on the receptivity of the endometrium (38).Also the relatively hyper insulinemia decreases the glycodelin level that reduces the fertility at the level of endometriam. (39, 40)

Numerous studies have evaluated the value of measurement of vascularity in endometrial and sub endometrial regions in predicting IVF outcome, at the day of embryo transfer and the day of HCG administration but the results were conflicting(19, 24 , 41, 42, 43, 44, 45). These conflicting results and the variations of day timing of measurement explained why still no consensus as to when these measurements should be done (24).

Different thickness of sub endometrial shell was used to assess the sub endometrial vascularity. Some used 2mm (24) and5mm (19) while others used 1mm (22, 23). Our study used 5mm shell. The difference of thickness of subendometrial shell between different studies explained the variation of measurements.

This study was limited as it included only women received long agonist protocol and shell of 5 mm in the sub endometrial region. Our protocol of COS cannot be generalized to other protocols of IVF. Also, this study did not compare other rFSH types.

Conclusion

The use of 3DPD to predict embryo implantation in IVF is of significant value.

Abbreviations

Ovarian hyperstimulation syndrome (OHSS), Estradiol (E2), gonadotropin-releasing hormone agonist (GnRH-a), Luteinizing hormone (LH), in-vitro fertilization (IVF), intra-cytoplasmic sperm injection (ICSI), trans vaginal ultrasound (TVS), human chorionic gonadotropin (hCG), interleukin (IL), embryo transfer (ET), assisted reproductive technology (ART), percent (%),kilogram per square meter Kg/m2 (Kg/m2), recombinant follicle stimulating hormone (rFSH), milli international unit (mIU), milligram (mg), millimeter (mm), number (NO), SD (standard deviation), metaphase 2 (M11) Odd Ratio (OR) confidence interval(CI) , region of interest (ROI) , virtual organ computer aided Analysis(VOCAL) , binding protein (BP) , controlled ovarian stimulation (COS) ,Three dimensions Power Doppler Ultrasound(3 DPD US) ,uterine artery pulsatility index (u PI) , Vascularization Index (VI), Flow Index (FI) Vascular-Flow

Index (VFI),endometrial(E) subendometrial(SE),Endometrial Receptivity Array(ERA).

Declarations

Acknowledgments

Authors would like to thank our nursing staff who provided help for completion of data collection

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Availability of data and materials

The datasets used and/or analyzed during the current study were available from the corresponding author on reasonable request.

Authors' contributions

KMS: Analysis, Manuscript Drafting, Acquisition of data, Critical Discussion, Management and Follow up of cases; IIS: Study Design, Manuscript Drafting, Acquisition and interpretation of data, Management and Follow up of cases;. Both authors read and approved the final manuscript.

Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was approved by the Local Ethical Committee of Benha University Hospital and written informed consent was obtained from each participant before the study.

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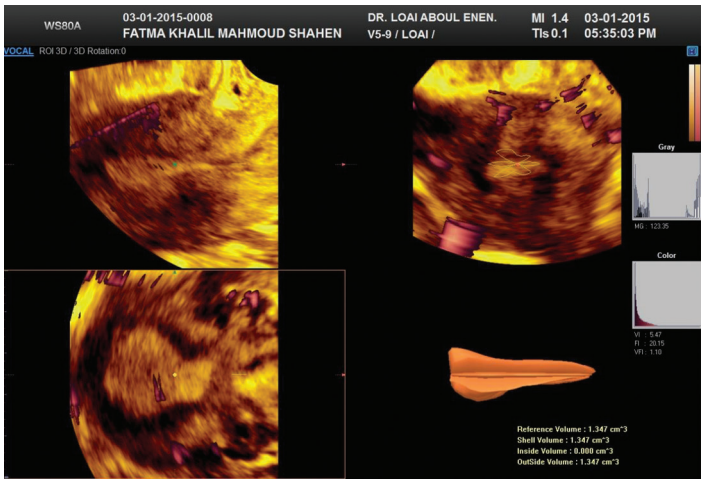

Figure (1): 3 D PD US with VOCAL programme showing endometrial VI, FI, and VFI.

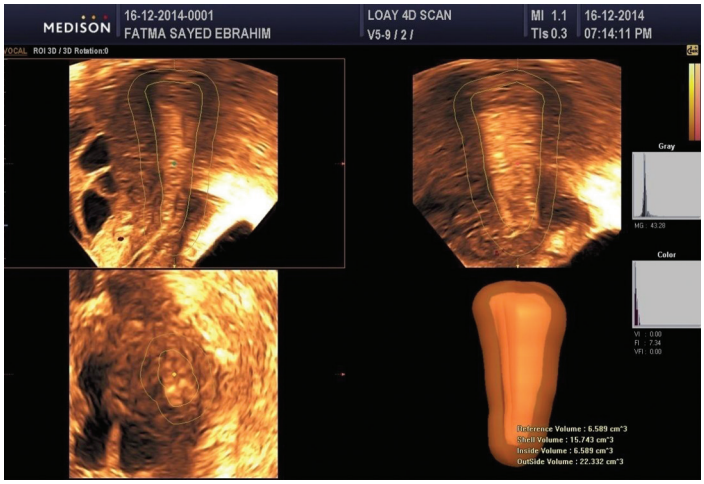

Figure (2):3 D PD US with VOCAL programme showing subendometrial VI, FI, and VFI.

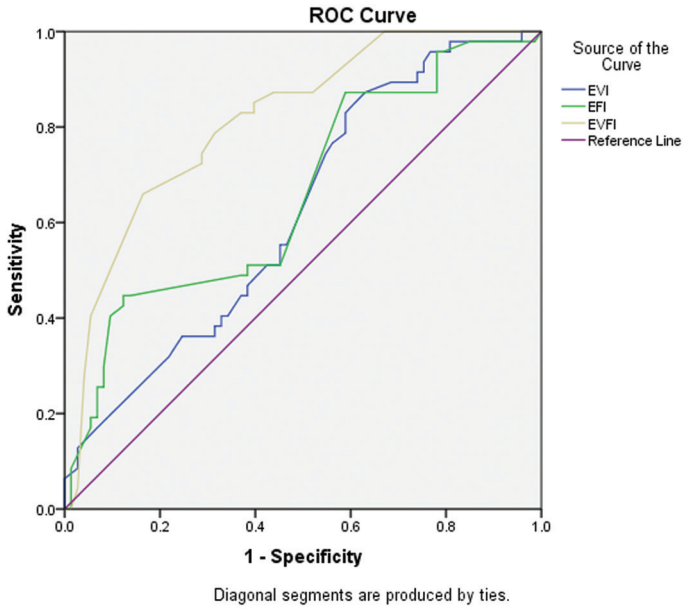

Figure (3):ROC curve of endometrial vascular indices

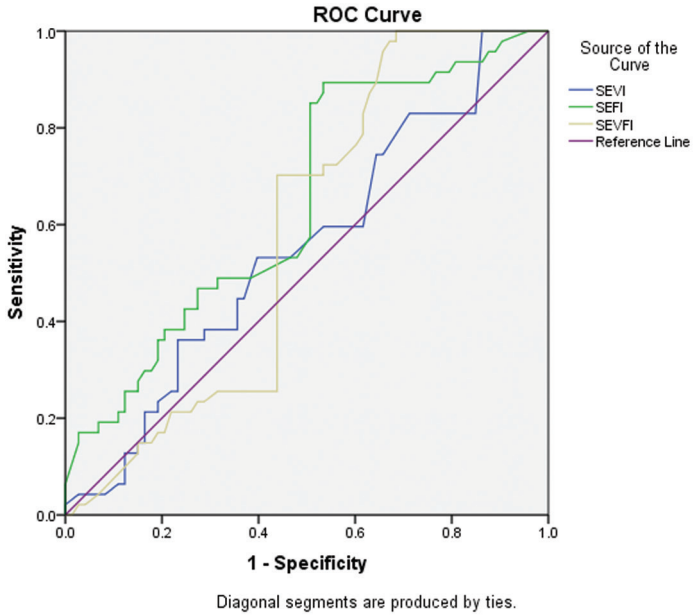

Figure (4): ROC curve of subendometrial vascular indices

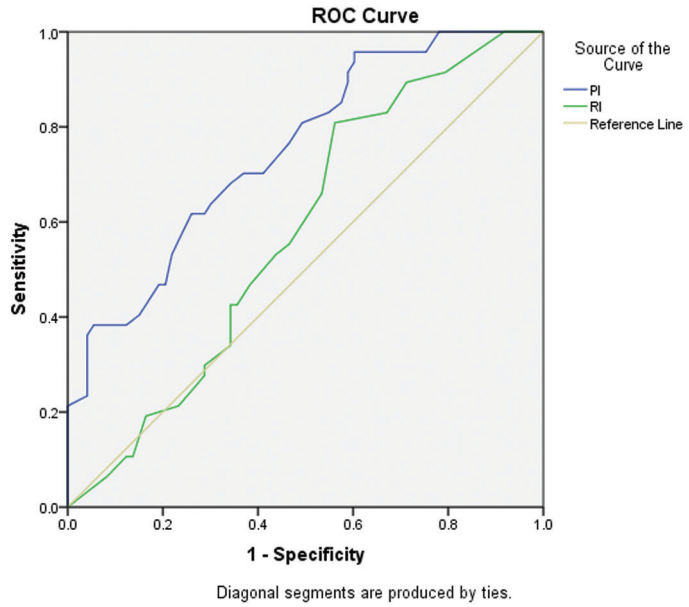

Figure (5):ROC curve of uterine artery Doppler indices

Table 1: shows demographic, clinical and laboratory data and their relations to pregnancy

Variables	Pregnant (n=47)	Non-pregnant (n=73)	Total (n=120)	t	p
Age (years)	29.87±4.2 (20.0-35.0)	30.38±4.28 (23.0-35.0)	31.52±6.48 (20.0-35.0)	0.64	0.52
BMI (kg/m^2)	30.56±3.54 (23.41-38.37)	29.92±2.35 (23.67-39.64)	30.28±3.27 (23.41-39.64)	1.18	0.24
Duration of infertility (year)	5.66 ± 1.56 (3-9)	5.6±2.11 (2-10)	5.79±2.56(2-10)	0.16	0.87
No. of HMG amp	29.94±2.38 (25-37)	30.85±1.84 (26-37)	30.49±2.62 (25-37)	1.89	0.062
Duration of induction : (day)	2.94±1.63 (11-15)	13.32±1.29 (11-15)	13.17±1.44 (11-15)	1.41	0.16
No. of follicles retrieved	15.06±5.08 (9-26)	15.22±4.25 (7-25)	15.16±4.57 (7-26)	0.18	0.86
No. of Metaphase II oocytes:	8.49±2.33 (5-15)	8.7±2.37 (5-14)	8.62±2.35 (5-15)	0.48	0.64
No. of transferred blastocyst:	2.72±0.62 (2-4)	2.68±0.72 (2-4)	2.70±0.68 (2-4)	0.30	0.76
Endometrial VI	4.69±0.89 (0.33-5.52)	4.24±1.28 (0.28-5.51)	4.41±1.16 (0.28-5.52	2.1	0.038*
Endometrial FI	20.21±1.31 (17-22)	19.44±1.22 (17-23)	19.46±1.19 (17-23)	10.72	0.001**
Endometrial VFI	1.1±0.17 (0.86-1.4)	0.90±0.19 (0.06-1.5)	0.98±0.21 (0.06-1.5)	6.26	<0.001**
Endometrial volume	5.62±1.98 (3.572-8.922)	4.94±2.41 (1.26-8.79)	5.35±2.36 (1.26-8.922)	1.6	0.112
Sub VI	2.16±1.71 (0.67-5.93)	1.95±1.78 (0.06-5.86)	2.03±1.75 (0.06-5.93)	0.64	0.53
Sub FI	36.03±20.75 (7.34-65.88)	27.34±20.07 (6.73-65.62)	30.74±20.70 (6.73-65.88)	5.22	0.024*
Sub VFI	1.26±0.67 (0.57-2.48)	1.20±0.98 (0.01-2.53)	0.83±0.78 (0.01-2.53)	0.40	0.69
PI	1.41±0.15 (1.22-1.83)	1.59±0.22 (1.29-1.90)	1.56±0.21 (1.22-1.90)	5.1	<0.001**
RI	0.83±0.06 (0.72-0.92)	0.85±0.07 (0.72-0.95)	0.84±0.07 (0.72-0.95)	1.21	0.23
Endometrial thickness	12.77±0.69 (11.3-13.9)	12.91±0.48 (11.4-14.3)	12.66±0.92 (11.3-14.3)	1.27	0.21
Basal FSH	6.78±0.49 (6.28-7.7)	6.79±0.42 (6.3-7.7)	6.79±0.45 (6.28-7.7)	0.14	0.89
Basal LH	6.07±0.91 (4.9-7.6)	5.92±0.88 (4.8-7.5)	6.09±0.93 (4.8-7.6)	0.87	0.39

Data are presented as mean±SD, and ranges are in parenthesis; *: Significant ($p<0.05$); **: Highly Significant ($p<0.01$)

Variables	(n=120)	
	No.	%
Pregnancy test		
-ve	73	60.8
+ve	47	39.2
Chemical pregnancy rate	47	39.2
Clinical pregnancy rate	42	35.0
Ongoing pregnancy rate	31	25.8
No. of gestational sac (47)		
1	27	57.4
2	15	31.9
3	5	4.2

Table 3: shows validity of some predictors in prediction of success ICSI.

Variable	Cutoff	AUC	CI	Sens.	Spec.	+PV	-PV	Accuracy	p-value
EVI	≥4.66	0.62	0.52-0.72	40.4	67.1	44.2	63.6	56.7	0.027*
EFI	≥19.87	0.66	0.56-0.76	51.1	61.6	46.2	66.2	57.5	0.003**
EVFI	≥0.96	0.82	0.74-0.89	74.5	71.2	62.5	81.2	72.5	<0.001**
SEVI	≥1.35	0.55	0.45-0.66	53.2	60.3	46.3	66.7	57.5	0.34
SEFI	≥19.79	0.65	0.55-0.75	55.3	50.7	41.9	63.8	52.5	0.008**
SEVFI	≥1.01	0.58	0.48-0.68	63.8	56.2	48.4	70.7	59.2	0.14
PI	≤1.47	0.751	0.664-0.838	68.1	65.8	56.1	76.2	66.7	<0.001**
RI	≤0.86	0.578	0.476-0.68	61.7	49.3	43.9	66.7	54.2	0.15

AUC: Area under curve; CI: Confidence interval; +PV: Positive predictive value; -PV: Negative predictive value; Sens.: Sensitivity; Spec.: Specificity; *: Significant (p<0.05); **: Highly Significant (p<0.01)