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Acknowledgments

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

References

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

2- Books:

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

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

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

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Contents :

Letter from the Editor:

Dear colleagues,

Warm greetings
Very interesting subjects are included in this issue. Previous CS was found to be the most important risk factor for Placenta previa and accrete. Decreasing rate of primary CS and optimizing obstetric care are mandatory to prevent maternal and fetal complication that could happen due to having future placenta accrete. Fertility sparing surgery is feasible during surgical management of placenta accrete. Buserelin addition to the luteal phase of antagonist cycles appears to improve pregnancy outcomes with no associated increase in OHSS risk. Estimation of the studied array of cytokines at the 1st antenatal visit has a high diagnostic value for the upcoming pregnancy-induced disorders (single or combination) with higher predictive value than the reliance on clinical data. Cerebro-Placental Doppler Ratio (CPR) can predict adverse perinatal outcome of IUGR. CPR ≤1.1 has 95.5% sensitivity in predicting adverse perinatal outcome.

Best regards.
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Patient Characteristics and feto-maternal Outcomes Among Cases Of Placenta Previa and Accidental Hemorrhage

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Abstract

Introduction: Third trimester bleeding is one of the major obstetric emergencies, which contribute greatly to maternal and fetal morbidity and mortality. It is defined as bleeding from or into the genital tract prior to delivery of the baby anytime from 20 weeks gestation, in some developed countries or 24 weeks gestation, in others or 28 weeks in countries with low resource settings thus lacking adequate neonatal support incubators.

The aim of this study: The study aimed to elucidate the outcomes with the associated morbidities, which will help define the magnitude of the problem posed by antepartum hemorrhage in order to better the management measures available to promptly tackle and alleviate this condition.

Patients & Methods: This study was prospective observational study conducted in Department of Obstetrics and Gynecology, Faculty of Medicine, Fayoum University Hospital and El-Sahel Teaching Hospital. All cases of antepartum hemorrhage admitted to emergency unit at maternity hospital after the age of 28 week of gestation during the period from (1st of August 2019 to end of November 2020) were included in this study, meeting the inclusion and exclusion criterion.

Results: Total number of patients who were admitted to obstetric department with APH during the study period was 120 case of them 25 cases were elective and all of them were placenta previa cases and 95 cases were emergency. 67 cases (55.83%) with placenta previa (25 elective and 42 emergency) & 44 (36.636%) with accidental hemorrhage (all are emergency or urgent cases), 9 (7.5%) due to other causes. Maternal outcome in PP include Increased numbers of CS 67 case (100%), Increased number of units of blood transfusion (1-18) unit with mean 4.31 ± 3.27, Hysterectomy 21 case (31.3 %), Shock 29 case (43.3 %), Urinary injury either bladder or ureteric injury 5 cases (7.5 %) (4cases bladder injury and 1 case Ureteric injury) all of them were placenta percreta, ICU admission 14 case (20.9 %), postpartum hemorrhage occurred in 6 cases and maternal mortality one case (1.5%). While maternal out come in accidental hemorrhage patients was numbers of CS delivery

was 35 cases and 9 cases delivered vaginally, number of units of blood transfusion (1-18) unit with mean 3.57 ± 3.08, Hysterectomy 3 cases (6.8%), Shock 22 case (50%),Conclusion Previous CS was found to be the most important risk factor for Placenta praevia and accreta Pre-eclampsia& previous abruption were the most important risk factors for abruption. Fetal morbidities associated with both placenta previa & abruption were prematurity, low birth weight, low Apgar score, admission to NICU.

Keywords: Placenta Previa , APH, Accidentalhemorrhage, perinatal mortality, maternal mortality.

Introduction

Third trimester bleedingor antepartum hemorrhage is one of the major obstetric emergencies, which contribute greatly to maternal and fetal morbidity and mortality. It is defined as bleeding from or in to the genital tract prior to delivery of the baby anytime from 20 weeks gestation, in some developed countries or 24 weeks gestation, in others or 28 weeks in countries with low resource settings thus lacking adequate neonatal support incubators.(1)

APH as courses can be grouped into obstetric (bloody show, placenta praevia, abruption placenta, vasa praevia, uterine rupture, disseminated intravascular coagulation) and non-obstetric (cervicitis, cervical cancer, cervical polyps, cervical eversion, vaginitis, vaginal laceration). (1) From these, APH is caused majorly by placenta praevia and abruptio placenta and occasionally some local causes however, the incidence of APH is much more than the combined incidence of the above. (1)

It is recognized that the amount of blood lost is often underestimated and that the amount of blood coming from the introitus may not represent the total blood lost (for example in a concealed placental abruption). It is important therefore, when estimating the blood loss, to assess for signs of clinical shock. The presence of fetal compromise or fetal demise is an important indicator of volume depletion (2).

The following definitions have been used (3):

1. Spotting–staining, streaking or blood spotting noted on underwear or sanitary protection
2. Minor haemorrhage – blood loss less than 50 ml that has settled
3. Major haemorrhage – blood loss of 50–1000 ml, with no signs of clinical shock
4. Massive haemorrhage – blood loss greater than 1000 ml and/or signs of clinical shock

It is a major contributor to maternal and perinatal morbidity and mortality with several possible consequences or sequelae. Patients who experience APH are generally at risk of oligohydramnios, premature rupture of membranes, preterm labor, labor induction, cesarean delivery, puerperal pyrexia, sepsis, shock, disseminated intravascular coagulation, anemia, retained placenta, postpartum haemorrhage. Also,include small for gestational age, congenital anomalies, intrauterine growth restriction, intrauterine fetal death, birth asphyxia and early neonatal mortality. (1)

Therefore, authors proposed to conduct a prospective study, to evaluate the consequences of antepartum haemorrhage, their maternal and perinatal outcome, so as to outline the important causes and proper management of patient in order to improve both maternal and perinatal morbidity and mortality and specify as to what areas required improvement in a developing countries. The data collected from this prospective study will be used to gauge the severity of this problem so that management and preventive protocol could be established to avert possible pregnancy outcomes.

The aim of this study

The study aimed to elucidate the outcomes with the associated morbidities, which will help define the magnitude of the problem posed by antepartum haemorrhage in order to better the management measures available to promptly tackle and alleviate this condition. The scope of the study is todetermine and compare fetal & maternal morbidity and mortality among cases of placenta previa and accidental hemorrhage.

Patients & Methods

This study was prospective observational study conducted in Department of Obstetrics and Gynecology, Faculty of Medicine, Fayoum University Hospital and El-Sahel Teaching Hospital. All cases of antepartum hemorrhage admitted to emergency unit at maternity hospital after the age of 28 week of gestation during the period from (1st of August 2019 to end of November 2020) were included in this study.

Inclusion criteria:

All cases of ante partum hemorrhage after 28 week of gestation due to either placenta previa or accidental hemorrhage and cases of placenta previa without antepartum hemorrhage after 28wks of gestation (after establishment of diagnosis both clinically & or by ultrasound).

Exclusion criteria:

1. Cases presented with vaginal bleeding before 28 week of gestation.
2. Cases presented with vaginal bleeding after 28 week of gestation due to other causes rather than placenta previa or accidental (Vasa previa-cervicitis- cervical neoplasm- cervical polyp-rupture uterus).
3. Cases associated with hemorrhagic diseases like hemophilia and ITP and patients on full anticoagulation therapy like metallic valve replacement, patient with DVT in the pregnancy and thrompophilic patient on therapeutic dose of anti coagulation.

Data analysis in the form of: Estimation of fetal &maternal mortality & morbidity among cases of placenta previa & accidental hemorrhage in the following:

1. Comparison between patients of Accidental Hemorrhage and placenta previa concerning:

a- mode of delivery

b- Time of delivery concerning gestational age.

c- Duration of delivery.

d- Intra operative blood loss by visual estimation method.

e- Amount of blood transfusion

f- Postpartum hemorrhage if occurred.

g- ICU admission and its causes.

h- Urinary tract injury if occurred.

2. I-Hysterectomy if done
   2.Comparison between mortality rates of both causes of APH.
3. Comparison between fetal outcome of both causes of APH by using:

a- gestational age at time of delivery.

b- APGAR score at 1 and 5 min

c- Birth weight.

d- NICU admission.

e- Rate of stillbirth or IUFD.

4. Identification of risk factor of both causes.
5. Determining avoidable factors contributing to mortality & morbidity between both cases.
6. Evaluating the standard of care (antenatal care, ante-partum or post-partum). All cases of APH were observed as regard history, physical examination and relevant laboratory investigations as subsequently discussed.

History taking

Clinical examination

Investigations:

- Blood group & Rh type.
- Complete blood picture (hemoglobin level, hematocrit, platelets count).
- Coagulation profile (prothrombin time,

prothrombin concentration and partial thromboplastin time).
- Liver function test (liver transaminase level, serum albumin level).
- Kidney function test (creatinine level, urea, and serum uric acid).
- Urinary albumin.
- Obstetric ultrasonography to assess fetal presentation, gestational age, AFI, placental localization, adherence of placenta to uterus and its degree (accreta, increate, percreta), presence or absence of retroplacental hematoma and its size, fetal congenital malformation and whether living or dead.
- Fetal monitoring test in the form of non-stress test.

Delivery circumstances:

1. Mode of delivery : vaginal or CS
2. Timing of delivery :elective or urgent
3. Intra operative findings: Couvelaire uterue in cases of abruption & placental site & adherence to uterine wall in cases of placenta previa & its type.
4. Amount of blood loss intra-operative,the blood loss was measured by recording the fluid in the suction apparatus before and after placental separation, keeping in mind that most fluid in the apparatus before fetal extraction was amniotic fluid and therefore was deducted from the total. The net amount of blood in the suction apparatus was added to the volume of blood collected from blood-soaked sterilized towels used after fetal extraction, and the under buttocks drapes placed under the patient. The volume of blood collected in soaked materials was calculated according to the following equation (WET Item Gram Weight DRY Item Gram Weight ¼ milliliters of blood within the item
5. Maneuvers done intraoperative (devascularization, uterine artery ligation, internal iliac artery ligation, internal iliac ballooning, compression of placental bed , hysterectomy)

Estimation of maternal outcome:

1. Amount of blood transfusion
2. Hysterectomy
3. Pulmonary edema
4. Renal failure
5. DIC
6. Postpartum haemorghe
7. Urinary tract injury
8. Maternal mortality & its cause

Estimation of fetal outcome:

1. Timing of delivery (term or preterm)
2. Fetal birth weight
3. APGAR score (at 1 minute & at 5 minutes)
4. Congenital fetal malformation
5. Intra uterine growth restriction
6. Neonatal ICU admission
7. Still birth, intra uterine fetal death, early neonatal death

Statistical Analysis:

All data were analyzed using SPSS 21.0 for windows (SPSS Inc., Chicago, IL, USA) &MedCalc 13 for windows (MedCalc Software bvba).Frequency tables were drawn and Chi square analysis was used for categorical variables and p-value ofp-value of<0.5 was considered significant.

Results

Table (1) Comparison between Group I (placenta previa) patients & Group II (accidental hemorrhage) patients as regard sociodemographic data.

	Group I (Placenta previa) (n=67)		Group II (Accidental hemorrhage) (n=44)		Test	p
	No	%	No	%		
Age (years)					**t**	
Mean ± SD	30.03 ± 4.74		28.43 ± 5.92		1.572	0.119 (NS)
Median (Range)	30 (21 – 39)		28 (18 – 40)			
Gravidity					**MW**	
Mean ± SD	3.66 ± 1.21		3.11 ± 1.85		1192	0.082 (NS)
Median (Range)	4 (1 – 8)		3 (1 – 8)			
Parity					**MW**	
Mean ± SD	2.3 ± 1.01		1.68 ± 1.55		1079.5	0.015 (S)
Median (Range)	2 (0 – 5)		1 (0 – 5)			
Abortion					**MW**	
Mean ± SD	0.37 ± 0.77		0.45 ± 0.73		1351	0.350 (NS)
Median (Range)	0 (0 – 4)		0 (0 – 3)			
Antenatal care					χ^2	
No	28		41.8%		2.404	0.121 (NS)
Yes	39		58.2%			

Table (2) Comparison between Group I (placenta previa) patients & Group II (accidental hemorrhage) patients as regard predisposing factors

	Group I (Placenta previa) (n=67)		Group II (Accidental hemorrhage) (n=44)		Test	p
	No	%	No	%		
Previous CS					χ^2	
No	14	20.9 %	36	81.8 %	37.398	<0.001 (HS)
Yes	53	79.1 %	8	18.2 %		
1	13	24.5 %	4	50 %	9.354	0.052 (NS)
2	21	39.6 %	2	25 %		
3	15	28.3 %	0	0 %		
4	2	3.7 %	2	25 %		
5	2	3.7 %	0	0 %		
Previous abortion					χ^2	
No	50	74.6 %	29	65.9 %	0.934	0.321 (NS)
Yes	17	25.4 %	15	34.1 %		
					MW	
Mean ± SD	0.37 ± 0.77		0.45 ± 0.73		1351	0.350 (NS)
Median (Range)	0 (0 – 4)		0 (0 – 3)			

In vitro fertilization					χ^2	
No	65	97 %	44	100 %	1.338	0.247 (NS)
Yes	2	3 %	0	0 %		
Previous placenta previa					χ^2	
No	61	91.1 %	44	100 %	2.598	0.107 (NS)
Yes	6	8.9 %	0	0 %		
Previous accidental hemorrhage					χ^2	
No	67	100 %	29	65.9 %	26.410	<0.001 (HS)
Yes	0	0 %	15	34.1 %		
Presence of PET & HTN					χ^2	
No	67	100 %	18	40.9 %	51.701	<0.001 (HS)
Yes	0	0 %	26	59.1 %		
Trauma					χ^2	
No	67	100 %	40	90.9 %	6.319	0.012 (S)
Yes	0	0 %	4	9.1 %		

Table (3) Clinical, Ultrasound & intraoperative findings in studied patients

	No	**%**
Ultrasound findings in Group I (Placenta previa) patients (n=67)		
Type of placenta previa		
Marginalis	4	6 %
Incomplete centralis	5	7.5 %
Complete centralis	58	86.5 %
Adhesion		
Not accrete	32	47.8 %
Accreta	35	52.2 %
Degree of adhesion		
Accreta	24	68.5 %
Percreta	6	17.1 %
Increta	5	14.3 %
Intra-operative finding in Group I (placenta previa) patients (n=67)		
Adhesion		
Not accrete	43	64.2 %
Accreta	24	35.8 %
Degree of adhesion		
Accreta	14	58.3 %
Percreta	6	25 %
Increta	4	16.7 %

Ultrasound findings in Group II (Accidental hemorrhage) patients (n=44)		
Retro placental hematoma		
No	10	22.7 %
Yes	34	77.3 %
Mean ± SD	76.42 ± 57.33	
Median (Range)	61.5 (8 – 255)	
Type of accidental hemorrhage		
Revealed	9	20.5 %
Concealed	12	27.3 %
Mixed	23	52.3 %

Table (4) Comparison between Ultrasound findings & intraoperative findings in group I (placenta previa) patients

		Intraoperative findings		χ^2	P
		Accreta (n=24)	Not accreta (n=43)		
Ultrasound findings	**Accreta (n=35)**	**20 (30 %)**	**15 (22.3 %)**	**5.263**	**0.021 (S)**
	Not accreta (n=32)	**4 (6 %)**	**28 (41.7 %)**		
Sensitivity	**Specificity**	**PPV**		**NPV**	**Accuracy**
83.33 % (62.62-95.26)	65.12 % (49.07-78.99)	57.14 % (39.35-73.68)		87.50 % (71.01-96.49)	69.71 % (53.92-84.81)

Table (5) Comparison between Group I (placenta previa) patients & Group II (accidental hemorrhage) patients as regard laboratory findings

	Group I (Placenta previa) (n=67)	Group II (Accidental hemorrhage) (n=44)	Test	p
Hemoglobin level (g/dl)			**t**	
Mean ± SD	9.24 ± 1.52	8.40 ± 1.74	2.659	0.009 (S)
Median (Range)	9.5 (5 – 12)	8.4 (5 – 13)		
Platelet count ($\times 10^3/mm^3$)				
Mean ± SD	---	160.93 ± 72.88	---	---
Median (Range)	---	189 (22 – 273)	---	---
Serum creatinine (mg/dl)				
Mean ± SD	---	1.40 ± 1.28	---	---
Median (Range)	---	1 (1 – 8)	---	---
INR				
Mean ± SD	---	1.53 ± 0.84	---	---
Median (Range)	---	1.1 (0.7 – 5)	---	---

Table (6) Comparison between Group I (placenta previa) patients & Group II (accidental hemorrhage) patients as regard maternal outcome

	Group I (Placenta previa) (n=67)		Group II (Accidental hemorrhage) (n=44)		Test	p
	No	**%**	**No**	**%**		
Mode of delivery					χ^2	
Vaginal delivery	0	0 %	13	29.5 %	22.421	<0.001 (HS)
CS	67	100 %	31	70.5 %		
Indication of delivery					χ^2	
Elective	18	26.9 %	0	0 %	12.201	<0.001 (HS)
Urgent	49	73.1 %	44	100 %		
Amount of blood transfusion (units)					**MW**	
Mean ± SD	4.31 ± 3.27		3.57 ± 3.08		1180.5	0.073 (NS)
Median (Range)	4 (1 – 18)		3 (0 – 15)			
Maternal morbidity					χ^2	
Hysterectomy	21	31.3 %	3	6.8 %	9.426	0.002 (HS)
Shock	29	43.3 %	22	50 %	40.482	0.487 (NS)
ICU admission	14	20.9 %	10	22.7 %	0.053	0.819 (NS)
DIC	0	0 %	17	38.6 %	27.662	<0.001 (HS)
Renal failure	0	0 %	7	15.9 %	8.843	0.002 (HS)
Pulmonary edema	0	0 %	4	9.1 %	3.973	0.046 (S)
Urinary injury	5	7.5 %	0	0 %	1.922	0.165 (NS)
Maternal mortality					χ^2	
No	66	98.5 %	42	95.5 %	0.941	0.332 (NS)
Yes	1	1.5 %	2	4.5 %		

Table (7) Comparison between Group I (placenta previa) patients & Group II (accidental hemorrhage) patients as regard fetal outcome.

	Group I (Placenta previa) (n=67)		Group II (Accidental hemorrhage) (n=44)		Test	p
	No	%	No	%	χ^2	
Congenital fetal malformation						
No	65	97%	44	100 %	0.182	0.669 (NS)
Yes	2	3%	0	0 %		
Intrauterine fetal death					χ^2	
No	67	100 %	24	54.5 %	34.134	<0.001 (HS)
Yes	0	0 %	20	45.5 %		
Abnormal presentation					χ^2	
No	45	67.2 %	40	90.9 %	7.077	0.007 (S)
Yes	22	32.8 %	4	9.1 %		
Preterm labor					χ^2	
No	36	53.7 %	11	26.2 %	7.984	0.005 (S)
Yes	31	46.3 %	31	73.80%		
Gestational age (weeks)					**MW**	
Mean ± SD	36.01 ± 2.26		34.57 ± 2.85		1001	0.004 (HS)
Median (Range)	37 (30 – 40)		35 (28 – 40)			
Birth weight (gm)					**MW**	
Mean ± SD	2894.03 ± 622.77		2500 ± 641.33		496	0.005 (S)
Median (Range)	3000 (1200 – 3700)		2600 (1200 – 3500)			
Neonatal ICU admission					χ^2	
No	44	65.7 %	11	45.8 %	2.138	0.143 (NS)
Yes	23	34.3 %	13	54.2 %		
Neonatal death					χ^2	
No	64	95.5 %	4	100 %	0.151	0.698 (NS)
Yes	3	4.5 %	0	0 %		

Discussion

In this study cases of placenta previa were more than cases of accidental & this differ from other studies that showed that Causes of APH include placenta previa and abruptio placentae with almost equal contribution (4), while according to (5) one third only of all antepartum hemorrhage occurs due to placenta Previa. This difference can be explained in that, our hospital is a tertiarycenter where almost all cases of placenta previa even low lie placenta when diagnosed outside hospital are referred, while some of cases of mild abruption presented in labor with slight bleeding that may be mistaken as heavy show so managed outside hospital without referral.

Majority of patients in this study were in the age group (20-30) years for both placentaprevia andabruption. This is in contrast to their traditional association with advanced maternal age (6)

In a comparison of maternal risk factors for placenta previa and placental abruption, abruption is more likely to be related to condi-

tions occurring during pregnancy and placenta previa is more likely to be related to conditions existing prior to pregnancy (6).

In this study risk factors associated with placenta previa were mainly : previous delivery by CS, 53 case (79.1 %) & according to number of previous deliveries by CS frequency was as following : One CS 13 case(24.5%), 2 CS 21 case(39.6%), 3CS 15 case (28.3%), 4 CS 2 cases (3.7%), 5 CS 2 cases(3.7%). These findings are consistent with (5) who suggested that incidence of placenta praevia is increasing due to increased rate of Caesarian section & The risks increase 1.5- to 5-fold with a history of cesarean delivery. A meta-analysis showed that the rate of placenta previa increases with increasing numbers of cesarean deliveries, with a rate of 1% after 1 cesarean delivery, 2.8% after 3 cesarean deliveries, and as high as 3.7% after 5 cesarean deliveries (7).

In this study risk factors associated with abruption were previous abruption 15 case (34.1 %), presence of PET or gestationalHTN 26 case (59.1%), history of trauma 4 cases (9.1%). These results differ from (8) who reported that abruption recurs in 19–25% of women who have had two previous pregnancies complicated by abruption.

In this study there were 4 cases (6%) of placenta previa marginalis & 5 cases (7.5%) of placenta previa incomplete centralis &58 cases(86.5%) of placenta previa complete centralis . this differ with (9) who reported, the frequency of complete placenta previa ranges from 20 to 45%, partial placenta previa accounts for approximately 30%, and marginal placenta previa accounts for the remaining 25-50%. this increased frequency of complete centralis in this study can be explained that we are tertiary center where risky cases can be referred while less risky cases as marginalis or incomplete centralis can be managed at other hospitals.

In this study when comparing results of US &intra operative findings US was a good negative test as it has sensitivity 83.33 %, specificity 65.12 %, positive predictive value 57.14 %, negative predictive value 87.50 %, accuracy 69.71 %. These results are close to (10) with a reported sensitivity of 77%–87%, specificity of 96%–98%, a positive predictive value of 65%–93%, and a negative predictive value of 98%. In addition, a recent Cochrane review reported a sensitivity and specificity of 90.30% and 93.81%, respectively(11). This difference is due to the fact that US is operator dependent so there were definite false positives and negatives in this study as well as others.

Maternal morbidities due to placenta previa in this study include increased number of deliveries by CS 67 case (100%), number of units of blood transfusion (1-18) unit with a mean 4.31±3.27, hysterectomy 21 case (31.3%), Shock 29 case (43.3%), ICU admission 14 case (20.9%), Urinary injury, either bladder or ureteric injury 5 cases(7.5%) all of them were percreta .

Maternal morbidities due to abruption in this study include number of CS 31 case (70.45%), number of units of blood transfusion (1-18) with a mean 3.57 ± 3.08, hysterectomy 3 cases (6.8 %), shock 22 case(50%), DIC 17 case (38.6 %), renal failure 7 cases (15.9%), Pulmonary edema 4 cases (9.1%), ICU admission 10 cases (22.7%).

So in this study maternal out come with abruption more severe than previa as regarding DIC, pulmonary edema, renal failure, ICU admission, shock while placenta previa associated with increased rate of emergent hysterectomy, urinary tract injury. These results are consistent with results found in another local study (12). But these results not consistent with the results of another study made by (13) in which the median PRBCs transfusion required was 6 units (mean 7.7 units, Cesarean hysterectomy was done in 24 patients (18%), Forty patients (32%) were admitted to the maternity high-dependency unit and 12 (9.8%) were admitted to the intensive care unit. Urinary tract injuries occurred in 12 patients (9.8%). This

difference can be explained that rate of hysterectomy in our study is more than other studies due to failure of trials of devascularization of uterus &compression at placental site bleeding in cases of accreta ,less incidence of urinary injury due to less incidence of percreta in our study(5 cases only).

Despite increased morbidities, mortality was not high in this study; this is attributed to prompt blood products replacement, timely ventilatory support and intensive care management.

All cases of PP in this study delivered by CS, while in abruption 13case (29.5 %) by VD & 31 case (70.5) by CS, despite that 24 case (54.5%) of abruption presented in labor, only 9 of them delivered vaginally & 15 of them delivered by CS, while other studies showed relatively lower CS rate (32.6%) in cases of accidental (14) and 27% (12). This is in significant contrast to CS rates of 91% by Tikanen et al., (8).

This difference in this study can be explained by unstable general condition of mother on admission (shock), presence of fetal distress, abnormal presentation of baby, previous deliveries were by CS all these causes necessitating termination by CS , also high CS rates in PP are attributable to greater number of PP major (86.5%) in this study .

Fetal morbidities due to placenta previa& accidental hemorrhage in this study include congenital fetal malformations: 2 cases only were observed during this study& both cases were in association with placenta previa one of them was cardiac anomalies & other was bilateral renal agenesis &cardiac anomalies. Preterm delivery, in cases of placenta previa 31 case (46.3 %) with mean gestational age 36.01±2.26 & mean birth weight 2894.03 ± 622.77 gm in contrast to 31 case (73.8 %) in cases of abruption with mean gestational age 34.57±2.85 & mean birth weight 2500 ± 641.33 .Neonatal ICU admission: 23 case (34.3 %) for placenta previa in contrast to 23 case (54.2 %) in case of abruption.

Other studies showed that rate of fetal abnormality is doubled in female with placenta previa; however, the mechanism of the association is not known (15). While other studies showed that Lethal congenital anomaly rate was not significantly different, 1.37% (n=3) in abruption versus 1.39% (n=2) in placenta previa (16).

As regarding fetal mortality in this study abruption was associated with 20 case (45.5 %) of intrauterine fetal death due to severe degrees of abruption causing impaired placental circulation, while in cases of placenta previa fetal losses occurred in 3 cases only (4.5 %) which occurred during early neonatal period due to respiratory distress from prematurity.

So, in this study fetal outcome in accidental is worse than placenta previa this is explained by most cases of accidental in this study are of severe type in which placental separation is severe causing sudden IUFD , most cases are terminated prematurely due to severe bleeding with low birth weight & low Apgar score. Similarly significantly higher perinatal mortality in abruption as compared to placenta previa is consistent with result of a one-year study from Lahore (17).

In this study there were some factors that may worsen maternal & fetal outcome in cases of placenta previa such as presence or absence of placenta accreta . In this study Fetal outcome is better in placentaprevia accreta than placenta previa withoutaccreta in contrast to maternal outcome, which is better in placenta previa withoutaccreta than placenta previa accreta.

Maternal outcome associated with accreta are hysterectomy (87.5%), shock(45.8%), ICU admission (54.2%), urinary injury (20.8%), blood transfusion with mean of (6.79 ± 4.16) maternal mortality (4.2%), in contrast to placenta previa without accreta, hysterectomy (0%), shock (41.9%), ICU admission (2.3%), urinary injury (0 %), blood transfusion with mean of (2.93 ±1.37) maternal mortality (0%).

Fetal outcome associated with pp without accreta include prematurity (58.1 %), mean birth weight 2739.53 ± 650.32, mean gestational age 35.53±2.54 , neonatal ICU (34.9 %), congenital fetal malformation (4.7 %), neonatal death (7 %), in contrast to placenta accreta, prematurity (25 %), mean birth weight 3170.83 ± 464.83, mean gestational age 36.88±1.26, neonatal ICU (33.3 %), congenital fatal malformation (0%), neonatal death (0%).

These results are consistent with other studies that indicates that maternal morbidity is significantly increased if PP is complicated by accreta which is already described in the literature (18), but in relation to fetal outcome there was no sufficient data about the difference in between both types but one study was done at Department of Obstetrics and Gynecology, Neonatal Intensive Care Unit, King Abdul-Aziz Specialist Hospital, Taif, Kingdom of Saudi Arabia from December 2009 to December 2012 which revealed no significant difference in neonatal outcome in placenta previa with or without accreta .

This difference in this study may be explained that in cases of accreta most cases are identified pre operatively (US over estimate rate of accreta), no or minimal to mild attacks of vaginal bleeding so most of them presented at stable general condition allowing them to be opened electively (50% of accreta are opened electively at or near term in contrast to 14% of placenta previa without accreta) after administration of corticosteroids so improving neonatal outcome, while maternal outcome is worse in accreta due to trials of manual separation of placenta aiming to conserve uterus& delayed decision of hysterectomy due to issues of fertility especially that most of patients of placenta previa in this study are of middle age resulting in severe bleeding from placental bed with consequent more blood transfusion, DIC & finally failed trials of serving uterus ending in hysterectomy. While in previa without accreta most of them presented by severe attack of vaginal bleeding necessitating termination of pregnancy pre- maturely.

Also, In this study both maternal & fetal outcome is better if placenta previa opened electively as following: decreased frequency of maternal shock (11.1 % VS 55.1 %), decreased ICU admission (14.3 % VS 38.9 %), decreased frequency of preterm delivery (5.6 % VS 61.2 %), Increased mean gestational age at time of delivery (37.39±0.60 VS 35.51 ± 2.43), Increased mean birth weight (3266.67 ± 295.05 gm VS 2757.14 ± 656.69 gm), While in contrary, increased frequency of hysterectomy (66.7 VS 18.4 %).This can be explained that urgent cases were admitted with severe bleeding, shock necessating termination of pregnancy prematurely while elective cases mostly are of accreta type that rarely present with bleeding, most of them receive corticosteroids preoperatively. But, being accreta most elective cases undergo hysterectomy (66.7%). These results are consistent with results of (19) greater blood loss &complications in emergent cesarean hysterectomy versus planned cesarean hysterectomy.

Also, presence or absence of antenatal care in cases of abruption may affect out come as maternal & fetal outcome is better in presence of ANC. decreased number of CS (52.6 VS 84 %), decreased amount of blood transfusion with a range of (0-4) & mean of 1.68 ± 1.0 VS range of (1-15) & mean of 5.0 ± 3.36, decreased frequency of Shock (10.5% VS 80%), decreased ICU admission (0% VS 40%), decreased DIC (10.5% VS 60%), decreased Renal failure (0% VS 28%), decreased frequency of IUFD (21.1 % VS 64%), Increased mean gestational age at labor (35.93±2.70 VS 33.9 ± 2.47)

Conclusion

- Previous CS was found to be the most important risk factor for Placenta Previa and accreta.
- Pre-eclampsia& previous abruption were the most important risk factors for abruption.

- Fetal morbidities associated with both placenta previa & abruption were prematurity, low birth weight, low Apgar score, admission to NICU.
- Both placenta previa & abruption were associated with perinatal mortality but more with cases of abruption & mostly occur due to intrauterine fetal death in abruption in contrast to early neonatal death in placenta previa due to respiratory distress from prematurity.
- Maternal morbidities specific to placenta previa are hysterectomy & urinary tract injury while abruption associated with DIC, pulmonaryedema, renalimpairment, ICU admission.

Recommendation

- Trying to reduce number of unjustified CS &increase number of vaginal deliveries after CS when it is available &safe.
- Programs of adequate ANC should be targeted for patients with pre eclampsia & previous history of abruption to early detect and prevent their progression to severe cases.
- Elective opening of placenta previa at 37 or 38 week is better than leaving it until become urgent.
- Administration of corticosteroids is better to avoid neonatal losses from respiratory distress.
- Not to delay in decision of hysterectomy if needed to avoid massive post-partum bleeding & subsequent mortality.

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Establishing a Successful IVF Program: Experience from a Tertiary Public Health Institution in North- Central, Nigeria

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Abstract

Background: The World Health Organization (WHO) has considered infertility a major problem in reproductive health. For millions of couples around the world, the ability to have children is a personal tragedy. For a significant proportion of them, the private agony is compounded by a social stigma, which can have serious and far—reaching consequences. It is not surprising therefore that the demand for Assisted Reproduction Technologies (ART) is growing in all regions most especially in the infertility belt of Africa with highest prevalence rates of infertility. The provision of such a highly esoterictechnology in a resource limited countries like ours with poorly developed health services, falling gross domestic product (GDP)coupled with unresolved ethical issues and limited awareness of ART is a great challenge. Hence the need for provision of effective, safe and affordable ART services through collaboration between government and non-governmental agencies via Public Private partnership for the benefit of the teeming population of infertile couples in Nigeria.

Keywords: Establishing, Assisted Conception, Infertility, Collaboration.

Introduction

Infertility is a serious problem with devastating social, cultural, emotional, economic, and medical consequences for affected couples [1].Worldwide it is generally estimated as occurring in 8-12% of all couples[2]. However, the incidence varies from one region of the world to the other, being highest in the 'infertility belt' of Africa that includes Nigeria [3]. In contrast to an average prevalence rate of 10-15% in the developed countries, the prevalence of infertility has been notably highly variable in sub-Saharan Africa ranging from 20-46% [3].This has been attributed to high rate of sexually transmitted diseases, complications of unsafe abortions, and puerperal pelvic infections [3]. About 30% of infertility is due to female problems, 30% to male problems, and 30% to combined male/female problems, while in 10%, there is no recognizable cause [3].Globally, there is evidence of worsening semen parameters in all regions[1].

Infecundity from irreparable tubal disease and suboptimal semen parameters can potentially be effectively treated by Assisted Reproduction Technology being the highest breakthrough in the medical treatment of infertility in the world over[4]. Therefore, the need for setting up an efficient fertility services cannot be overemphasized.

Establishing such a highly specialized service in our country with huge infrastructural deficit coupled with low levels of income are a daunting task; [1] particularly the unresolved ethical issues arising from the new innovation could limit its utilization in Nigeria where it is most needed. We seek to highlight some of these challenges in running a successful in vitro fertilization (IVF) program in Nigeria and indeed other low-resource settings and proffers probable solutions as applied to our unit.

Challenges

Establishing an IVF centre is capital intensive worldwide, this is even more in the third world countries due to infrastructural deficit and poor access to capital. Access to bank loans is highly restrictive as the interest rate of 25-30% is far beyond the reach of most investors. Also, currency exchange rate of almost 500 naira to a dollar could be frustrating as most equipment, drugs, consumables and sometimes personnel are foreign- sourced. Therefore, the few subsisting centers have to rely on foreign collaborations for survival. This informed our partnership with Apollo Hospital; Chennai: India, Chennai Fertility Centre; India, World laparoscopic Hospital; Delhi: India, Morula IVFJakarta; Indonesia and many others abroad in the area of procurement of IVF equipment, training and re-training of our personnel aiming at domestication of IVF program.

Worthy of note is the lack / poor awareness of infertility/ IVF program in Nigeria [5] and other African countries as beliefs in supernatural causes of infertility such as witchcraft or the belief that the infertile woman has taken a vow in her earlier life not to bear children are widespread [6]. In the situation of protracted infertility, western religious beliefs may give way to traditional beliefs [6].This will however limit access to modern fertility treatment as it stands as a source of inertia on the part of the couple

A major challenge to setting up and running a sustainable IVF program in Nigeria is lack of regulation to guide practices of assisted conception as most regulatory bodies are at infancy as against age- longed regulatory bodies in the developed countries [7]. Lack of regulation puts the couple at risk of several exploitative practices [8].A bill for the establishment of the "Nigerian Assisted Reproduction Authority" [9] has been presented by the Association for Fertility and Reproductive (AFRH) to the Nigerian parliament for consideration and if passed will be a good starting point for regulation of ART practice in Nigeria [4].

Another major issue is personnel recruitment and training. There are no training centres for the various IVF personnel in Nigeria. Also, there are few properly trained fertility specialists, embryologists and specialist IVF nurses. Therefore, obstetrician and gynaecologist resorts toshort training courses in India and elsewhere to acquire expertise in this field. The story is similar for clinical embryologist and IVF nurses and the expertise acquired may not be enough to ensure complete mastery of the field before setting up an IVF centre[1]. Hence the justification for national and international collaboration as obtained in our centre.

Supply of drugs and consumables is another concern. Drugs and IVF consumables are imported and delay in supplies sometimes affect planning of cycles. The pharmaceutical companies in the country do not produce IVF drugs due to the cost, limited demand and fear of sustenance of cold chain as a result of erratic power supply in Nigeria.

Stable power supply is a necessary requirement to maintaining optimal conditions inin vitro fertilization laboratory for embryo culture and development [1]. This is, however, extremely difficult to achieve in Nigeria due to incessant power failure with resultant reversion to a comprehensive power back-up system thereby increasing overhead cost with attendant effect on cost of care. In our centre, we made provision for 4 standby generators, 10 kVA UPS system, solar panels and fifteen (15) 200 amp wet cell batteries for power storage for at least 6 to 8 hours in case of power outage from the national grid. This serves as an intermediary between power source from the national grid and generators without compromising laboratory conditions. As stated earlier, acquisition and maintenance of these power back-up systems is another cost-burden borne toward establishing a functional IVF program that will eventually impact on the cost of service delivery.

Undoubtedly, the need for servicing and maintenance of IVF equipment on routine basis is of utmost importance towards achieving sustainable results. This poses a great constraints as the engineers are usually foreign-sourced and the few indigenous engineers have not achieved proficiency on the job. Thus, we are left with no other option than to seek for foreign-based expertise at higher cost. Nigerian government must therefore rise to the challenge of providing effective, safe, and affordable IVF services to its population. Innovative ways should be found to make ART services affordable in low-resource settings [10, 11, 12].

Nigerian governmentshould make efforts in improving infrastructures particularly in the area of stable power supply. Public financing of IVF must be considered as infertility is a disability of the reproductive system just as diseases of other body systems. This can be done by integrating the investigation and treatment of infertility into the existing reproductive health services. Government can support the private sector firms who are currently the main providers of IVF services by way of reducing or even waiving taxes on equipment for IVF, drugs, and consumables. This could significantly reduce the cost of treatment thereby making IVF services more affordable. There is also the need for strong regulatory bodies to regulate every aspect of ART practice to make the service efficient and safe.

To address the personnel and training needs, IVF centers should seek accreditation and begin training programs for the various categories of personnel needed in this highly specialized field of medicine. Nigerian universities offering postgraduate programs in obstetrics and gynecology should consider starting subspecialty training in infertility. Collaboration with local IVF centers and foreign universities should be sought so that trainees can spend valuable time in such centers to improve their knowledge and skills in ART services.

Conclusion

There is a great demand for ART services in Nigeria where tubal blockage resulting from STIs is still very high. Tubal factor infertility can be effectively treated by in vitro fertilization with embryo transfer. Unfortunately, ART services are inaccessible to large sections of the population mainly due to high treatment costs. While preventive strategies will play an important role in the overall prevention of infertility, more innovative, effective, safe, and low-cost ART strategies are the need of the hour in these low-resource settings.

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Risk factors for occurrence of placenta accrete spectrum following primary cesarean delivery

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Abstract

Background: Many maternal and fetal complications were reported during delivery for patients diagnosed to have placenta accrete spectrum (PAS). Cesarean delivery (CD) is considered to be the most common risk factor for developing PAS disorders during pregnancy specially with increasing rate of CD in developing countries.

Methods: A cross- sectional study over 12 months in a tertiary care obstetric unit between January 2020 and January 2021.

Results: 47 pregnant females having history of previous one CD were divided into 2 groups after ultrasonic and intraoperative evaluation of placenta site and invasion to the uterine wall.14 cases were in the low risk group ad 33 cases were in the high-risk group. The mean ages of patients were (27.6 ± 4.6 & 27.6 ± 4.6, p value =0.961) respectively. The median gravidity was (3 & 2) in both groups. We found that 36.4 % of case in the high-risk group had unreliable indications of the primary CD. Emergency caesarean deliveries were done in about 18 % of cases in the high-risk group either due to failure to progress in labour or foetal distress. We reported successful conservative management in both groups using either cervico-isthmic compression suture or step wise approach. There was statistically significant in the mean amount of intraoperative blood loss (1000 ml (850-1200) &1600 ml (850-2500), p<0.001) in the low and high-risk groups respectively. We reported 3 cases of intraoperative pulmonary embolism, urinary bladder injury and

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HELLP (hemolysis, elevated liver enzymes and low platelet count) syndrome among patients in the high-risk group. There was statistically significant longer hospital stay duration among patients in the high-risk group ranging from 2 days up to 21 days. On the other hand, there were no serious complications reported in the low risk group.

Conclusion: Decreasing rate of primary CD and optimizing obstetric care are mandatory to prevent maternal of fetal complication that could happen due to having future PAS. Fertility sparing surgery is feasible during surgical management of PAS disorders.

Key words: Placenta accrete spectrum, caesarean delivery, primary, fertility sparing, planned surgical management, maternal. neonatal complications

Introduction

Placenta accrete spectrum (PAS) is considered to be an epidemic in our locality. It is also thought to be due to the high rate of caesarean delivery (CD) [1]. Most of the primary elective caesarean delivery in our locality is performed due to unjustified indications and in private practice.

Patients withPAS have different degree of placental invasion either through the endometrium (accreta), myometrium (increta)or the serosa of urinary bladder(percreta)[2]. Those patients are at risk of havingsignificant blood loss during delivery and massive blood transfusion according to the degree of PAS [3].

Other risk factors are studied and well known to be associated with increased risk of PAS includingpresence of uterine scar due to evacuation of abortion, curettage of endometrium, Myomectomy, septal resection, assisted reproductive techniques, Uterine artery embolization, pelvic inflammatory diseases ,Intra-uterine device and presence of some uterine abnormalities as (Bicornuate uterus, Adenomyosis, Submucous fibroids) [4, 5].Also, the chance of recurrence is very high after having complicatedpregnancy with PAS.

Many surgical techniques were used to preserve fertility in such group of patient like step wise approach after pelvic devascularisation (ligation of uterine arties and internal iliac arteries) [6]however, with presence of morbidly adherent placenta and more invasion uterine wall the need for hysterectomy is increasing[7].

We conducted a hospital based cross sectional study on a group of patients undergoing repeat elective caesarean delivery due to abnormal placentation aiming to study the association betweenplace, indications of the primary caesarean delivery and the degree of PAS.

Patients and methods

This study was conducted in the period between (January 2020 till January 2021)on a group of patients admitted for repeat elective caesarean delivery (CD) at Mansoura university hospital (MUH). All patients had history of previous one CD.

Inclusion criteria

1. Patients with history of previous one caesarean delivery
2. Patients who refused trail of vaginal birth after caesarean section
3. Patients with permanent indication for caesarean section like contracted pelvis
4. Patient without any history of complicated medical diseases

Exclusion criteria

1. Patients with history of more than one previous caesarean delivery
2. Patients with medical disorders necessitating emergency termination of the current pregnancy.
3. Patients with known uterine abnormalities eg (Bicornuate uterus, Adenomyosis and submucous fibroids)

4. Patients who are candidates for vaginal birth after caesarean section

Methods

ll the sociodemographic data were collected including age, residency, job, special habits and body mass index (BMI). Also, a detailed Past obstetric history was taken from all patients i.e. (Gravidity, parity, number of full-term normal delivery, number of full-term assisted vaginal deliveries, number of preterm deliveries, number of stillbirth deliveries, number of previous abortions, previous pregnancy complication and complication during puerperium). Details about the primary CD were taken including (Indication, duration, place of delivery, results of delivery, complication during labour, complication during puerperium). Any significant past medical, surgical and gynaecological histories were taken including the used methods for contraception. According to our protocol of management all the diagnosed pregnant females were admitted between 34-36 weeks of gestation and elective termination of pregnancy was planned at completed 37 weeks of gestation. All patients were evaluated by Trans abdominal ultrasound [7] and trans vaginal ultrasound (TVS)before hospital admission and a diagnosis of placenta praevia was made if the lower edge of the placenta is less than 2 cm form the internal os of the cervix[8].Placenta accretaspectrum (PAS) was considered according to the presence of at least 2 of the following signs[9, 10]:

- Multiple placental lacunae with turbulent blood flow
- Loss or irregularity of the retroplacental hypoechoic space
- Irregularities of myometrial–bladder interface
- Myometrial thickness at placental bed less than1 mm
- Bulging of placenta into a nearby organ
- Increased uterovesical vascularity with multiple bridging vessels
- Multiple placental lacunae feeder vessels

All the collected data by ultrasound were compared to intraoperative findings and final grading system was put according to FIGO classification 2019 [11]:

- **Grade 1: Placenta accreta**

Intraoperative assessment:there is no placental bulge at the site of implantation, absent or very low vascularity

- **Grade 2: Placenta Increta**

Intraoperative assessment: bluish placental bulge at the site of implantation, increased vascularity (multiple vessels running over the serosa of the uterus), no placental invasion to the serosa of the uterus, no placental separation after cord traction

- **Grade 3: Placenta Percreta**

- **Grade 3a:** Limited to the uterine serosa Intraoperative assessment: placental bulge over uterine serosa and presences of a clear dissection plane between the urinary bladder and uterine serosa
- **Grade 3b**: With urinary bladder invasion Intraoperative assessment: Placental invasionto urinary bladder only and non-identifiable dissection plane between the urinary bladder and uterine serosa
- **Grade 3c:** With invasion of other pelvic tissue/organs Intraoperative assessment: Placental invasionto other pelvic structures e.g. (vagina, broad ligament and lateral pelvic walls)

The data were analysed after classifying patients into 2 groups: low risk group (diagnosed to have grade 1)and high-risk group (diagnosed to have grade 2 or more) after ultrasound and intraoperative assessment.

Outcome measurements

Primary outcome

The primary outcome was the correlation-

between the indication for the primary CD (elective without labour or emergency after some labour) and the degree of placenta accrete spectrum.

Secondary outcome

Secondary outcomes were correlation between parity, gravidity, place of the primary CD, assisted conception and the degree of PAS.

Assessment of the maternal and foetal complication during and after delivery for patients with different grades of PAS.

Statistical analysis and data interpretation

Data were analysed using IBM SPSS Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. Qualitative data were described as number and percent. Quantitative data were described as median (minimum and maximum) & inter quartile range for non-parametric data and mean, standard deviation for parametric data after testing normality using Kolmogrov-Smirnov test. Significance of the obtained results was judged at the (0.05) level. For Qualitative data Chi-Square test was used tocompare≥2 groups.Monte Carlo test as correction for Chi-Square test when more than 25% of cells have count less than 5 in tables (>2*2).Fischer Exact test was used as correction for Chi-Square test when more than 25% of cells have count less than 5 in 2*2tables.Student's t-test was used to compare 2 independent groups for parametric data. Mann-Whitney U test was used to compare 2 independent groups for non- parametric data.

Results

We conducted a cross sectional study over 12 months to assess the correlation between degree of placenta invasion after primary caesarean delivery and the indications of this delivery. Also, we tried to specify the most frequent area of referral in our locality. Out of 267 cases were referred to our centre for elective termination of pregnancy due having different degrees of PAS only 47 cases fulfilled our inclusion criteria. After intraoperative assessment we found that 29.8% of cases were copping with low risk group and 70.2% of cases were copping with high risk group (figure 1).

There were no statistically significant differences between both groups regarding base line and sociodemographic data (table1). The mean ages of patients were (27.6 ± 4.6 & 27.6 ± 4.6, p value =0.961) respectively. The median gravidity was (3 & 2) in both groups. Only 4 cases had history of surgical evacuation of abortion in the low risk group and 6 cases in the high-risk group. On the other hand, 35.7% of cases had history of passive cigarette smoking in the low risk group and about 15.5% in the high-risk group. Intrauterine device was used as a method of contraception in about (78.6% & 57.6%) of low and high-risk groups respectively (table 1).

After assessing the different indications of the primary caesarean delivery in both groups, we found that 36.4 % of case in the high-risk group had unreliable indications of the primary CD. Reliable indications for caesarean deliveries were found in about 18 % of cases in the high-risk group either due to failure to progress in labour or foetal distress (table 2). Only 4 cases in the high-risk group had elective termination of pregnancy due to either foetal malpresentations or cephalopelvic disproportions (table 2). Although it is not recommended nowadays, we found that (7.1% & 15.2) of cases in the low and high-risk groups were requesting elective CD respectively (table 2).There was no statistically significant difference between both groups in the duration of previous CD (2.3 & 2.2 years) in the low and high-risk groups respectively.

Many recommendations were published regarding the plan of management of patients with PAS [12]. According to our local protocol of management we admit patients be-

tween 34-36 weeks of gestations and we arrange for elective termination of pregnancy at 37 weeks of gestation unless there is another indication of earlier termination of pregnancy. We reported successful conservative management in both groups using either cervico-isthmic compression suture or step wise approach (table 3). However serious complications were reported during management of high-risk group (table 3). There was statistically significant in the mean amount of intraoperative blood loss (1000 ml (850-1200)&1600 ml (850-2500), $p<0.001$) in the low and high-risk groups respectively. Transfusion of ≥ 4 units of packed RBCs were present in 18 % of cases in the high-risk group. Also, pulmonary embolism was diagnosed in one case in the high-risk group and administration of thrombolytic therapy was done to save her life. Urinary bladder injury also was reported in one case in the high-risk group which was in need for urological consultation and management. In spite of being rare condition to coexist with PAS, we reported one case of sever preeclampsia and HELLP (haemolysis, elevated liver enzymes and low platelet count) syndrome among patients in the high-risk group (table 3). There was statistically significant longer hospital stay duration among patients in the high-risk group ranging from 2 days up to 21 days according to the complication and plan of management after surgery. On the other hand, there were no serious complications reported in the low risk group (table 3).

There were no statistically significant differences in the foetal and neonatal outcomes between both groups (table 4). The mean foetal weights were (2968.6 gm ± 705.6& 2766.7 gm ± 607) in the low risk and high-risk groups respectively. Only 3 cases in the high-risk group were in need for neonatal intensive care unit after delivery and discharged after supportive treatment in a good health. Also 3 cases were diagnosed with intrauterine foetal death and it was an indication for early termination of pregnancy.

We studied the geographical distribution of the sites of primary CD and we found a higher incidence at certain areas and cites (figure 2) which could target a future research to assess the obstetric care and practice at such centres and to recommend more auditing of their practice.

Discussion

Placenta accrete spectrum is considered to be one of the high-risk conditions during pregnancy. Caesarean delivery also considered to be one of the major risk factors for having PAS. This is mostly due to abnormal trophoblastic invasion at the site of previous CD scar. With increasing incidence of CD nowadays, PAS complicated pregnancies are increasing specially in the developing countries. The CD rate in Egypt is increasing in the past decade[1, 13]. In our centre we perform between 250-300 delivers annually for pregnant ladies with different grades of PAS. We conducted this cross-sectional study to correlate the degree of severity of PAS with different indications of primary CD and trying to specify if there are certain areas of higher incidence in our locality. Out of 47 cases with PAS after only one CD we found 70.2 % of cases had high grade of PAS (≥ grade 2). It is known that the risk of having PAS is going to be about 3% after one CD[14].In another study about 22 % of 46 pregnant females with PAS were following the first CD[15]. In our study we classified our patients into low risk group and high-risk group after ultrasonic assessment this is due to the increased risk of anaesthetic, intraoperative, and post-operative complications in the high-risk group. In the high-riskgroup,about (18.2%, 15.2% and 57.6%) of cases had past history of surgical evacuation of abortion, passive smoking and using IUCD as a method of contraception respectively. This in comparison to a German study in which about 52% of cases had history of uterine curettage[15]. Being performed in private facilities, most of indications of the primary CD were not scientifically sound. Only (21.4% & 30.3 %) of primary CD were

done due reliable indications in the low and high-risk groups respectively. In spite of being recommended by WHO to justify the indications for primary CD[16], still there is unjustified obstetric practice in the developing countries. Maternal request was an indication of primary CD for 15% of cases in a study comparing the elective and emergency primary CD[17]. In another study the CD rate was 18.7% in a multicentric study conducted at Bhutan[18].

Different management protocols were put in order to guide clinical practice during treatment of PAS disorders[12]. We adopted hospital admission 1-2 weeks before delivery and termination of pregnancy after completing 37 weeks of gestation. As we were treating young age patients (mean age 27 years) with low parity we were trying to perform fertility sparing surgery during delivery. This was done after counselling of patients and arranging multi-disciplinary team (MDT) to lead the plan of management. As it was known also that many techniques were safe and effective during doing fertility preservation surgery [19]. Starting from the choice of type of anaesthesia [20] , till the choice of surgical technique .intraoperative and post-operative care are crucial parts in our plan. We succeeded to preserve fertility in all cases after using cervico-isthmic compression technique[21] and stepwise approach [6]. We preferred to do pelvic devascularization before placental delivery during performing the stepwise approach in high risk cases as it was expected to have more bleeding (1600ml (850-2500) in high risk group) and more need for blood transfusion (≥3 unites of packed RBCs in 36.4% of high-risk cases). The median hospital stay was (2 and 4 days) in the low and high-risk groups respectively. We reported 3 cases of intraoperative pulmonary embolism, urinary bladder injury and HEELP syndrome. All cases were in the high-risk group. In another study the median blood loss was (1600 ml (1100-2750)) and the median hospital stay was 6 days , also they reported one case of urinary bladder injury [15]. However, this study was conducted on patients with different grades of PAS and not restricted to only previous one CD. Our foetal and neonatal outcomes were comparable in both study groups and only 3 cases were diagnosed antenatally with IUFD and it was an indication of early termination of pregnancy. The estimated foetal weights (EFW) at delivery were (2968.57 gm ± 705.75&2766.67 gm ± 606.96) in the low and high-risk groups respectively. Only 3 neonates in the high-risk group were in need of NICU admission and were discharged in a good health. The mean EFW was 2750 gm (2235, 3156) and 41% of neonates were in need for NICU admission[15]. This could be due to adopting different protocol of management and different indication for termination of pregnancy. We performed a geographical distribution analysis for the place of primary CD in our study (figure 2) and we found that only 2 cases had the primary CD delivery at a governmental hospital while 45 cases were done at private facilities. Also, we localized certain cities in our locality with higher rates of primary CD. We recommend a future research to focused in such cites and trying to optimize the obstetric practice in such cities so as to minimize these high rates of primary CD and to prevent its future maternal and foetal complications which could life threatening.

In conclusion it is mandatory to decrease the rate of primary CD specially in low resource countries and optimize the obstetric care in our locality to prevent the major maternal of foetal complication that could happen due to having future PAS. Also, fertility sparing surgery is feasible during delivery of patients having different grades of PAS after one CD. MDT is mandatory during surgical management of PAS disorders.

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We acknowledge that all authors have contributed significantly, and that all authors agree with the content of the manuscript.

Disclosure

All authors disclose no conflict of interest.

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Table (1): Socio-demographic characteristics and past obstetric history among studied pregnant females.

Age/years **Mean ± SD**	27.64 ± 4.63	27.73 ± 5.58	t=0.05 p=0.961
BMI (kg/m2) **Mean ± SD**	26.49 ± 3.02	27.60 ± 3.52	t=1.04 p=0.305
Gravidity **Median (min-max)**	3 (2.0-5.0)	2.0 (2.0-14.0)	z=0.727 p=0.467
Parity **Median (min-max)**	1.0 (1.0-3.0)	1.0 (1.0-3.0)	z=0.212 p=0.832
Abortion **Median (min-max)**	0.0 (0.0-3.0)	0.0 (0.0-10.0)	z=0.133 p=0.894
History of Uterine surgery n (%) **No** **MVA**	 10 (71.4) 4 (28.6)	 27 (81.8) 6 (18.2)	χ^2=0.33 P=0.426
Smoking n (%) **No** **passive smoker**	 9 (64.3) 5 (35.7)	 28 (84.8) 5 (15.2)	χ^2=2.48 P=0.115
IUD usage n (%) **No** **Yes**	 3 (21.4) 11 (78.6)	 14 (42.4) 19 (57.6)	χ^2=1.88 P=0.171

Z: Mann Whitney U test , IQR: Interquartile range , t:Student t test χ2=Chi-Square test
BMI: body mass index, MVA: manual vacuum aspiration, IUD: Intrauterine device
*statistically significant (if p<0.05)

Table (2): Different indications and duration of primary Caesarean delivery among studied females.

Indications of CD			
Unreliable	8 (57.1)	12(36.4)	χ^2=1.74 p=0.19
EROM	2 (14.3)	3 (9.1)	FET P=0.59
Patient demand	1 (7.1)	5 (15.2)	FET P=0.45
ICSI	0	1 (3.0)	FET P=0.51
Failure to progress	2 (14.3)	3 (9.1)	FET P=0.62
Fetal distress	1 (7.1)	3 (9.1)	FET P=1.0
Cephalopelvic disproportion	0	1 (3.0)	FET P=1.0
Breech presentation	0	3 (9.1)	FET P=0.54
APS	0	2 (6.1)	FET P=1.0
Duration of previous CS median (min-max)	2.3 (1.0-5.0)	2.2 (0.5-6.5)	z=0.548 p=0.583

FET: Fischer exact test, χ2=Chi-Square test,EROM: Early rupture of membranes, ICSI: Intracytoplasmic sperm injection, APS: Antiphospholipid syndrome, CD: Caesarean delivery *statistically significant (if p<0.05)

Table (3): Intraoperative management and hospital stay and maternal complications of patients with different grades of placenta accrete spectrum.

Gestational age at delivery/week Median (min-max)	37.0 (25.0-39.0)	37.0 (19.0-38.0)	t=0.960 p=0.342
Used surgical techniques n (%) **Cervico-isthmic suture** **Stepwise approach**	14 (100) 0	27 (81.8) 6 (18.2)	χ^2=2.92 P=0.09
Units of blood transfusion n (%) **1** **2** **3** **4** **5**	 13 (92.9) 1 (7.1) 0 0 0	 7 (21.2) 14 (42.4) 6 (18.2) 5 (15.2) 1 (3.0)	 MC P<0.001*
Intraoperative blood loss/ ml median (min-max)	1000 (850-1200)	1600 (850-2500)	z=4.24 p<0.001*
Duration of hospital stay /days median (min-max)	2.0 (2.0-4.0)	4.0 (2.0-21.0)	z=3.79 p<0.001*
Maternal Complications n (%) **No** **Pulmonary embolism** **Paralytic ileus** **HELLP syndrome** **Bladder injury**	 14 (100.0) 0 0 0 0	 29 (87.9) 1 (3.0) 1 (3.0) 1 (3.0) 1 (3.0)	 p=0.762

Used tests: Chi-Square test, z: Mann Whitney U test, FET:Fischer exact test, MC:Monte Carlo test HELLP: Haemolysis, Elevated Liver enzymes and Low Platelet count *statistically significant (if p<0.05)

Table (4): Foetal and neonatal outcomes of patients with different grades of placenta accrete spectrum.

Foetal weight/gm Mean ± SD	2968.57 ± 705.75	2766.67 ± 606.96	t=0.994 p=0.326
NICU# n (%) **No** **Yes**	 13 (100.0) 0 (0.0)	 28 (90.3) 3 (9.7)	 FET P=0.544
Ordinary ICU n (%) **No** **Yes**	10 (76.9) 3 (23.1)	16 (51.6) 15 (48.4)	χ2=2.43 P=0.119

3 cases with IUFD

Used tests: Chi-Square test, z: Mann Whitney U test, FET:Fischer exact test, MC: Monte Carlo test

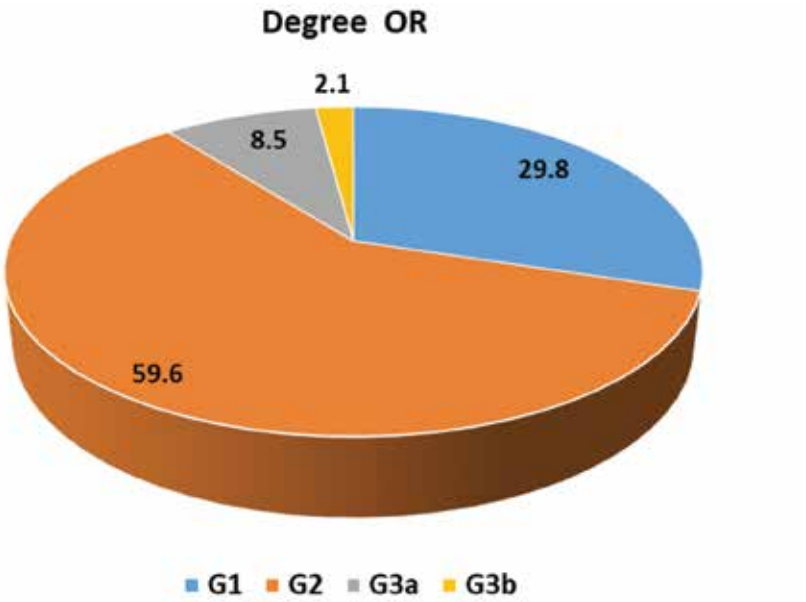
NICU: Neonatal intensive care unit, ICU: intensive care unit

*statistically significant (if p<0.05)

Legends of figures

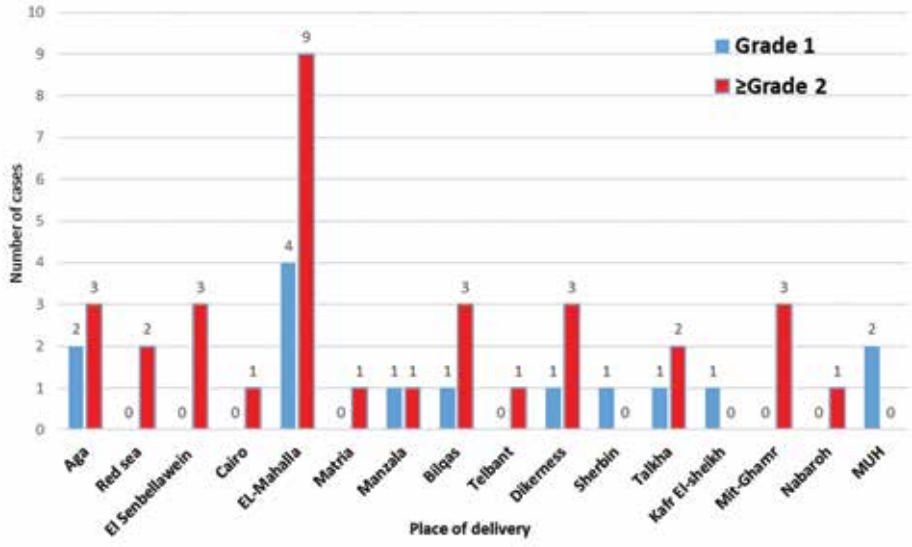
Figure (1)Different grades of placenta accrete spectrum after intraoperative (OR) assessment according to the International Federation of Gynaecology and Obstetrics (FIGO) 2019 classification.
Figure (2)Geographical distribution of primary caesarean delivery places among the studied pregnant females. Patients were classified into low risk (grade 1) and high risk (≥ grade 2) according to the International Federation of Gynaecology and Obstetrics (FIGO) 2019

Figure (1)



Data were presented as percentage (%).
Abbreviations degree OR: degree after intraoperative assessment, G: Grade
Total number (n) = 47 cases

Figure (2)



Data were presented as numbers.
Low risk cases (Grade 1) total number = 14 cases
High risk cases (≥ Grade 2) total number = 33 cases
Abbreviations; MUH: Mansoura university hospitals

Estimation of IL-1β, Osteoprotegerin, and YKL-40: A Diagnostic Array for Prediction of Gestational Diabetes Mellitus complicated by Gestational Hypertension

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Keywords: Gestational insulin resistance (GIR), hypertension (GHT) and diabetes mellitus (GDM).

Abstract

Objectives: Estimation of serum levels of interleukin (IL)-1β, Osteoprotegerin (OPG) and YKL-40 early in pregnancy of normotensive normoglycemic women to find early predictors for development of gestational insulin resistance (GIR), hypertension (GHT) and/or diabetes mellitus (GDM).

Patients & Methods: The study included 255 pregnant women who were evaluated during their 1st antenatal visit (ANV-1) for age, body mass index (BMI), baseline systolic (SBP) and diastolic blood pressure (DBP) and gave blood samples for estimation of serum levels of insulin and the studied cytokines. Then, all women underwent 75-Oral glucose tolerance test (OGTT) for diagnosis of GDM and the homeostasis model assessment of IR (HOMA-IR) score at 6th and 24th GW.

Results: 32 women developed GIR that progressed to GDM, 38 women developed GHT, 13 developed GIR and GHT and 7 women developed the triad of GIR, GDM and GHT. There are significant differences in serum IL-1β, YKL-40 and OPG levels in women free and with these disorders. Serum levels of IL-1β showed positive, while serum OPG levels showed negative significant correlations with the incidence of pregnancy-induced disorders, while high serum YKL-40 was positively correlated with the incidence of GIR with GHT or GDM or both. ROC curve and Regression analyses defined combination of high DBP and serum YKL-40 as the significant early predictors for development of the three disorders, while high HOMA-IR score, SBP and serum IL-1β as the significant predictors for GHT, high BMI, DBP and serum YKL-40 as early predictors for GIR and GHT and high serum YKL-40 and low serum OPG levels as early predictors for GIR and DM.

Conclusion: Estimation of the studied array of cytokines at the 1st ANV has a high diagnostic value for the upcoming pregnancy-induced disorders single or combination with

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higher predictive value than the reliance on clinical data.

Keywords: Pregnancy, Insulin resistance, Diabetes mellitus, Hypertension, Cytokines, Early prediction.

Introduction

Preeclampsia (PE) is a hypertensive disorder that develops during pregnancy and adversely affects both the mother and the fetus. PE is characterized by hypertension and proteinuria, and affects about 5% to 8% of pregnancies and causes maternal and perinatal mortality and morbidity (1). Maternal inflammatory and vascular endothelial dysfunction is important factors in the pathogenesis of PE (2).

Normally, during pregnancy, insulin resistance (IR) is increased as an adaptation process to enhance maternofetal nutrient transfer to meet the nutritional needs of the developing fetus, especially to glucose requirements (3). Increased proinflammatory cytokines during pregnancy are associated with hyperglycemia and IR and could be useful for predicting the development of gestational diabetes mellitus (GDM) (4).

Diabetes mellitus (DM) could be classified according to etiology and pathology as type 1, type 2 DM and GDM, which is characterized by hyperglycemia during pregnancy (5). Some studies have indicated that DM was related with inflammation (6).

Early studies suggested a reciprocal relationship between abnormal glucose metabolism and development of hypertension depending on the observation that individuals with abnormal glucose and insulin metabolism have higher incidence of hypertension, and patients with untreated essential hypertension have higher than normal plasma insulin concentrations and are resistant to insulin-stimulated glucose uptake (7). Recently, **Morikawa et al.** (8) found pregnant women with DM are at high risk for hypertensive disorder of pregnancy.

Hypothesis

The current study supposed a certain relation between development of GIR, DM and hypertension in pregnant women and these disorders may have a common pathogenic stack.

Design

Prospective comparative clinical trial

Setting

Benha University Hospitals in conjunction with some private centers

Objectives

Estimation of serum levels of interleukin (IL)-1β, Osteoprotegerin (OPG) and YKL-40 early in normotensive normoglycemic pregnant women in trial to find an early predictor for any of these pregnancy-induced disorders.

Patients & Methods

The study was conducted since June 2019 till Aug 2020 when the last enrolled case had reached her 36th gestational weeks (GW). The study protocol was approved by the Local Ethical Committee to include all women who attended the Antenatal (AN) Outpatient Clinics (OPC) at Benha University Hospitals for assurance of being pregnant, the 1st AN visit (ANV-1), were eligible for evaluation. At ANV-1, the collected demographic data included age, weight and height, and body mass index (BMI) was calculated in kg/m2 as weight (kg)/ height (m2) (9). The collected baseline obstetric and clinical data included number of previous pregnancies, deliveries and living children in multiparous women and medical history with special regard to essential hypertension, diabetes mellitus and kidney diseases.

Exclusion criteria include manifest DM, previous GDM in multipara women, morbid obesity with BMI>35 kg/m2 (10), essential hypertension, history of treatment for DM, hypertension, or other diseases treated by drugs well known to induce DM, hypertension or kidney affection, liver or renal diseases.

All pregnant women were asked to attend the OPC overnight fasting on the next day to the ANV-1 to give fasting blood samples for estimation of fasting blood glucose (FBG), fasting serum insulin (FSI), the study biomarkers, and to undergo the 75-Oral glucose tolerance test (OGTT) that entails estimation of FBG and postprandial blood glucose (PPBG) levels at one and two hours after taking a 75-gm oral glucose diet. Then, all women were allowed to relax while they were laying down and blood pressure was estimated on two occasions 4-hr apart and the median value of systolic and diastolic blood pressures (SBP & DBP) was determined and considered as the baseline measures. Only women with normal OGTT and median values of SBP and DBP and signed written fully informed consent, and free of exclusion criteria were enrolled in the study. All women were asked to attend the OPC at the 12th, 24th, 32nd, 36th GW for determination of SBP and DBP and at the 24th GW women must attend fasting to give blood samples for re-estimation of FSI and to repeat the 75-OGTT. Women who completed their pregnancy free of pregnancy induced disorders were grouped as control group; while those who developed these disorders were labelled according the type and multiplicity of disorders.

Diagnosis of Insulin resistance (IR) and gestational DM (GDM)

Insulin resistance (IR) was evaluated using the homeostasis model assessment of IR (HOMA-IR) score that was calculated according to the formula: fasting serum insulin (μU/ml) x [FBG (mg/ml)/18])/22.5; HOMA-IR score of >2 is considered abnormal (11). HOMA-IR score was determined twice at 6th and 24th GW. The results of the 75-OGTT were interpreted for diagnosis of GDM according to the recommendations of the International association of diabetes and pregnancy study groups (12) as follows: FBG ≥92 mg/dl, 1-h BG ≥180 mg/dl and 2-h BG ≥153 mg/dl indicate GDM.

Diagnosis and categorization of pre-eclampsia (PE)

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

Investigations

Sampling: Venous blood samples (5 ml) were collected from the antecubital vein under complete aseptic conditions and were divided into two parts:

1. The first part was put in a tube containing sodium fluoride (2 mg sodium fluoride/ml blood) to prevent glycolysis for estimation of blood glucose levels.

2. The second part was collected in plain tube, allowed to clot, centrifuged at 1500×g for 15 min and the serum samples were collected in clean dry Eppindorff tube to be stored at –70°C until assayed.

Estimated parameters

a. Blood glucose levels were estimated using glucose oxidase method (17).
b. Serum levels of IL-1β, OPG and YKL-40 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 insulin was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab200011, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique (18).
2. Human IL-1β was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46052, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique (19).
3. Human OPG was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab189580, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique (20).
4. Human YKL-40 was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab255719, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique (21).

Statistical analysis

Obtained data were presented as mean, standard deviation (SD), numbers and percentages. Results were analyzed using One-way ANOVA with post-hoc Tukey HSD Test and Chi-square test (X2 test). Possible relationships were investigated using Spearman's linear regression for non-parametric data. Sensitivity & specificity of estimated parameters as predictors for PREGNANCY-INDUCED DISORDERS 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 and then were verified using the Regression analysis, Stepwise method. Statistical analysis was conducted using the IBM SPSS (Version 23, 2015) for Windows statistical package. P value <0.05 was considered statistically significant.

Results

The study included 268 pregnant women eligible for evaluation, 13 women were excluded and 255 women were included in the study (Fig. 1). At the end of the study duration 87 women developed pregnancy-induced disease, while the remaining 168 women completed their pregnancy free (Control group). Sixty-two women (24.3%) developed GIR with HOMA-IR score of ≥2, unfortunately, 32 women (12.5%) of those had GIR gave positive OGTT and were considered GDM. Thirty-eight women developed GHT, 16 developed early PE and 22 had late PE; 10 women had severe PE and 28 had late PE. Seven women had GIR, GDM and GHT, while 13 women had GIR and GHT and 18 women had GHT without GIR or GDM. At ANV-1, women who developed pregnancy-induced diseases showed significant differences in comparison to those completed their pregnancy free of diseases concerning enrolment data as shown in table 1.

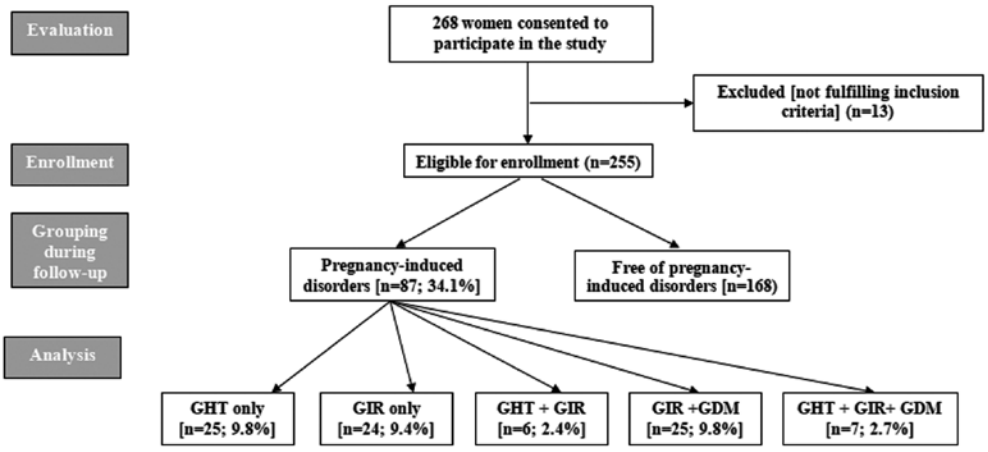

Figure 1: Consort Flow sheet

Table (1): Enrolment demographic and clinical data of studied women

Data		Control group (n=168)	Gestational diseases						P value
			GHT only	GIR only	GHT +IR	GDM+ GIR	GHT+ IR+GDM	Total	
Number (%)		168 (68.6%)	25 (9.8%)	24 (9.4%)	6 (2.4%)	25 (9.8%)	7 (2.7%)	87 (34.1%)	
Age (years)		28.3±2.5	28.7±2.3	26.6±2.9	28±3.9	28.1±3.2	27.7±4.2	27.5±3	0.022
BMI (kg/m^2)		28.2±1.7	29.1±0.8	28.4±1.9	31±2.6	28±2.6	28.1±3.9	28.8±2	0.009
Obstetric history	Primigravida	56 (33.3%)	7 (28%)	24 (100%)	6 (100%)	11 (44%)	6 (85.7%)	54 (62.1%)	0.000022
	Primipara	60 (35.7%)	8 (32%)	24 (100%)	6 (100%)	11 (44%)	6 (85.7%)	55 (63.2%)	0.000029
75-OGTT	FBG (mg/dl)	80.3±5.8	81.5±6.8	80.8±4.2	81.2±2.3	79.9±7.9	78.1±11.2	80.7±5.6	0.615
	PPBG (mg/dl)	118.1±6.8	119.6±4.8	120.6±4.5	121.5±4.2	117.6±10.6	115.9±16.2	119.8±5.5	0.045
HOMA-IR	FSI (µU/ml)	3.65±0.86	3.66±0.98	5.84±0.84	6.23±0.63	4.16±1.66	4.4±1.97	5.9±1.94	<0.0001
	HOMA score	0.74±0.18	0.75±0.21	1.18±0.17	1.27±0.15	0.84±0.34	0.87±0.4	1.2±0.39	<0.0001
Blood pressure	SBP (mmHg)	104.5±5.9	117.6±9	104.1±5.5	124±6	104.4±9.4	103.5±14.8	111.9±9.8	<0.0001
	DBP (mmHg)	68.8±2.4	74.7±5.2	69.8±2.8	79.5±4.9	68.6±5.4	68.3±9.4	72.7±5.3	<0.0001

Data are presented as mean; standard division (SD), numbers & percentages; BMI: Body mass index; 75-OGTT: 75-oral glucose tolerance test; FBG: Fasting blood glucose; PPBG: Postprandial blood glucose; FSI: Fasting serum insulin; HOMA-IR: Homeostasis model assessment of insulin resistance; SBP: systolic blood pressure; DBP: Diastolic blood pressure; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P value indicates significance of difference between free and diseased group; P<0.05: indicates significant difference

At the 24th GW, women of control group had significantly lower FBG in comparison to women who developed GIR and GDM with or without GHT, while the differences were non-significant versus FBG of women who developed GHT or GIR only. Women who developed only GHT or GIR had significantly lower FBG levels compared to women who developed GIR and GDM with or without GHT. As regards PPBG levels estimated at the 24th GW, women of control group had non-significantly lower levels than women who developed GHT only, while was significantly lower than levels estimated in all women of other groups. Women developed GHT only had significantly lower PPBG level in comparison to women who developed GHT and GIR with or without GDM. Estimated FSI and calculated HOMA scores were significantly lower in women of control group and women who developed GHT in comparison to women of other groups (Table 2).

Table (2): Results of 75-OGTT and HOMA-IR score calculation at the 24th GW studied women categorized according to the diagnosed pregnancy-induced disorder

Group Variables		Control group (n=168)	GHT only (n=25)	GIR only (n=24)	GHT +GIR (n=6)	GDM+ GIR (n=25)	GHT+IR+ GDM (n=7)
75-OGTT	FBG (mg/dl)	86.4±6	88.2±7.4	87.2±2.9	88±2	110.2±9	109.6±10.9
	P1		0.179	0.539	0.518	<0.0001	<0.0001
	P2				0.949		<0.0001
	P3				0.509	<0.0001	<0.0001
	PPBG (mg/dl)	127.7±9.4	129.8±9.8	140.5±6.6	142.7±8.5	190.4±17.5	211.6±17.8
	P1		0.295	<0.0001	0.0002	<0.0001	<0.0001
	P2				0.0065		<0.0001
	P3				0.494	<0.0001	<0.0001
HO-MA-IR	FSI (μU/ml)	5.21±0.85	5.39±0.92	9.55±0.5	10.15±0.8	10.4±1.29	11.4±1.39
	P1		0.327	<0.0001	<0.0001	<0.0001	<0.0001
	P2				<0.0001		<0.0001
	P3				0.0278	<0.0001	<0.0001
	HOMA score	1.13±0.2	1.19±0.25	2.11±0.11	2.24±0.15	2.87±0.42	3.1±0.27
	P1		0.142	<0.0001	0.0002	<0.0001	<0.0001
	P2				<0.0001		<0.0001
	P3				0.248	<0.0001	<0.0001

Data are presented as mean; standard division (SD); 75-OGTT: 75-oral glucose tolerance test; FBG: Fasting blood glucose; PPBG: Postprandial blood glucose; FSI: Fasting serum insulin; HOMA-IR: Homeostasis model assessment of insulin resistance; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P1 indicates significance of difference versus women free of pregnancy induced diseases; P2 indicates significance of difference versus women developed GHT only; P2 indicates significance of difference versus women developed GIR only; $P<0.05$: indicates significant difference

Estimated blood pressure measures in patients who developed GHT alone or with GIR and/or GDM throughout the study duration were significantly higher in comparison to measures estimated in control women, or developed GIR with or without GDM. Moreover, estimated blood pressure measures during pregnancy course were significantly higher in women developed GHT and GIR with or without GDM in comparison to measures of women who developed IR only. Women who developed GIR and GDM had significantly higher blood pressure measures at the 12th GW in comparison to women who completed their pregnancy of control group, but the differences became non-significant thereafter. Moreover, the differences in blood pressure measures between women who developed GIR only versus those who developed GIR and GDM were non-significant (Table 3).

Table (3): Blood pressure measures during pregnancy course in studied women categorized according the associated pregnancy-induced disorders

	Control group (n=168)		GHT (n=25)		GIR only (n=24)		GHT +GIR (n=6)		GDM+ GIR (n=25)		GHT+GIR+ GDM (n=7)	
	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP	SBP	DBP
ANV	105±6	69±2	118±9	75±5	104±6	70±3	124±6	79±5	104±9	69±5	104±15	68±9
12th	108±5	71±2	124±8	80±5	107±5	73±2	131±8	85±6	111±7	72±3	128±5	85±4
P1			<0.0001	<0.0001	0.616	0.018	<0.0001	<0.0001	0.027	0.037	<0.0001	<0.0001
P2							0.064	0.091			0.272	0.028
P3							<0.0001	<0.0001	0.072	0.498	<0.0001	<0.0001
24th	112±5	74±3	138±18	91±14	112±5	76±3	147±19	98±16	114±7	75±3	142±15	94±11
P1			<0.0001	<0.0001	0.735	0.008	<0.0001	<0.0001	0.107	0.197	<0.0001	<0.0001
P2							0.278	0.269			0.648	0.566
P3							<0.0001	<0.0001	0.201	0.342	<0.0001	<0.0001
32nd	116±5	77±3	133±7	87±4	115±4.9	78±3	140±3	90±4	117±6	78±3	136±4.8	90±3
P1			<0.0001	<0.0001	0.169	0.387	<0.0001	<0.0001	0.391	0.112	<0.0001	<0.0001
P2							0.067	0.194			0.528	0.124
P3							<0.0001	<0.0001	0.125	0.539	<0.0001	<0.0001
36th	120±5	81±3	146±10	95±9	120±3.8	81±3	145±9	96±6	120±5	82±3	144±14	94±8
P1			<0.0001	<0.0001	0.921	0.492	<0.0001	<0.0001	0.825	0.247	<0.0001	<0.0001
P2							0.863	0.688			0.699	0.842
P3							<0.0001	<0.0001	0.925	0.685	<0.0001	<0.0001

Data are presented as mean; standard division (SD); ANV: First antenatal visit; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P1 indicates significance of difference versus women free of pregnancy induced diseases; P2 indicates significance of difference versus women developed GHT only; P2 indicates significance of difference versus women developed GIR only; P<0.05: indicates significant difference

Mean serum levels of IL-1β and YKL-40 were significantly lower, while serum OPG levels were significantly higher in control women in comparison to women who developed pregnancy-induced disorders. Also, women who develop GIR had significantly higher serum lower IL-1β and YKL-40 levels, while had significantly lower serum OPG levels in comparison to those developed GIR in addition to GHT and/or GDM. Serum IL-1β showed non-significant differences between women who developed GHT only or with GIR and GDM. On contrary, serum levels of OPG were significantly higher and serum YKL-40 were significantly lower in women developed GHT only in comparison to women who developed additional disorders (Table 4).

Table (4): Serum levels of IL-1β, OPG and YKL-40 estimated at time of enrolment of studied women

Group Parameters		Control group (n=168)	GHT only (n=25)	GIR only (n=24)	GHT +GIR (n=6)	GDM+ GIR (n=25)	GHT+- GIR+ GDM (n=7)
IL-1β (ng/ml)	Level	45.7±7.12	139.3±11.9	115.2±17.2	138.5±6.7	118.2±19.1	143.1±19.3
	P1		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P2				0.979		0.514
	P3				0.0032	0.567	0.0009
OPG	Level	2.21±0.09	1.95±0.07	1.94±0.04	1.85±0.07	1.85±0.11	1.82±0.1
	P1		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P2				0.0055		0.0006
	P3				0.0005	0.00048	0.00008
YKL-40	Level	36.79±11.2	49.34±9.5	67.2±10.8	82.45±3.76	75.1±5	88.6±4.5
	P1		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	P2				<0.0001		<0.0001
	P3				0.0022	0.0018	0.00002

Data are presented as mean; standard division (SD); IL-1β: Interleukin-1β; OPG: Osteoprotegren; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P1 indicates significance of difference versus women free of pregnancy induced diseases; P2 indicates significance of difference versus women developed GHT only; P2 indicates significance of difference versus women developed GIR only; P<0.05: indicates significant difference

Spearman' correlation of demographic, clinical and laboratory data obtained at ANV-1 for the possibility of development pregnancy-induced disorders showed significant correlation between maternal age and previous gravidity, HOMA-IR score, blood pressure measures and serum levels of studied parameters and the possibility of development of GHT, IR and DM. While there was significant correlation between BMI, previous gravidity and parity, HOMA-IR score and studied parameters and the possibility of development of GIR with HT or DM (Table 5).

Table (5): Spearman's correlation coefficient for the possibility of development of pregnancy-induced disorders and demographic, clinical and laboratory data determined at ANV-1 of studied women

Pregnancy-induced disorders / Variable	Possibility of development of GHT+-GIR+GDM		Possibility of development of GHT		Possibility of development of GIR+GHT		Possibility of development of GIR+GDM	
	Rho	p	Rho	p	Rho	p	Rho	p
Age	-0.153	0.014	0.039	0.540	0.010	0.872	-0.028	0.659
BMI	0.114	0.069	0.067	0.495	0.166	0.008	0.156	0.013
Gravidity	-0.121	0.047	-0.265	0.039	-0.164	0.009	0.039	0.688
Parity	-0.115	0.068	0.048	0.443	-0.159	0.011	0.038	0.698
FBG	-0.037	0.554	0.080	0.203	0.076	0.229	-0.102	0.553
2-hr PPBG	-0.016	0.799	0.052	0.412	0.008	0.893	-0.054	0.387
HOMA-IR score	0.247	<0.001	-0.123	0.049	0.184	0.003	0.473	<0.001
SBP	0.238	<0.001	0.369	<0.001	0.233	<0.001	0.068	0.280
DBP	0.241	<0.001	0.300	<0.001	0.218	<0.001	0.025	0.693
Serum IL-1β	0.239	<0.001	0.449	<0.001	0.214	0.001	0.326	<0.001
Serum OPG	-0.237	<0.001	-0.302	<0.001	-0.208	0.001	-0.440	<0.001
Serum YKL-40	0.281	<0.001	0.113	0.073	0.242	<0.001	0.427	<0.001

ANV: First antenatal visit; BMI: Body mass index; FBG: Fasting blood glucose; PPBG: Postprandial blood glucose; HOMA-IR: Homeostasis model assessment of insulin resistance; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; IL-1β: Interleukin-1β; OPG: Osteoprotegren; GHT: Gestational hypertension; IR: Gestational insulin resistance; GDM: Gestational diabetes mellitus; P indicates the significance of the correlation; -: indicates negative correlation

For prediction of the possibility of development of the triad of GHT, IR and DM, the correlated variables were studied using the ROC curve analysis which excluded the gravidity as an early predictor (Fig. 2); then the Regression analysis for verification of variables with significant AUC on ROC analysis defined combination of high DBP and serum YKL-40 are the significant early predictors for the development of the three disorders.

On the other hand, the ROC curve analysis excluded the gravidity as an early predictor for the development of GHT alone (Fig. 3); but Regression analysis defined combination of high HOMA-IR score, SBP and serum IL-1β as the significant early predictors for the development of GHT.

Regarding the possibility of development of GIR and HT, ROC curve analysis assured the early predictability of the variables that showed positive correlation with the development of these two disorders (Fig. 4), Regression analysis defined high BMI, DBP and serum YKL-40 as the early significant predictor for development of combination of GIR and HT. Concerning the early prediction of GIR and DM, ROC curve analysis excluded BMI and DBP as early predictors (Fig. 5) and Regression analysis defined high serum YKL-40 and low serum OPG levels as the early predictors for GIR and DM.

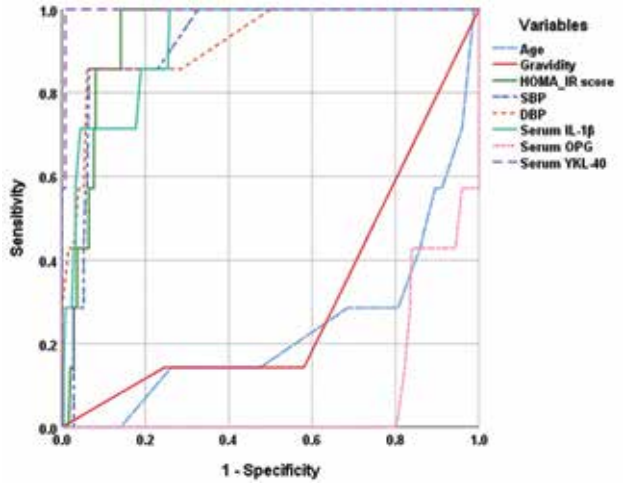

Fig. (2): ROC curve analysis for demographic, clinical and laboratory data determined at ANV-1 as predictors for development of the triad of GHT, GIR and GDM during pregnancy

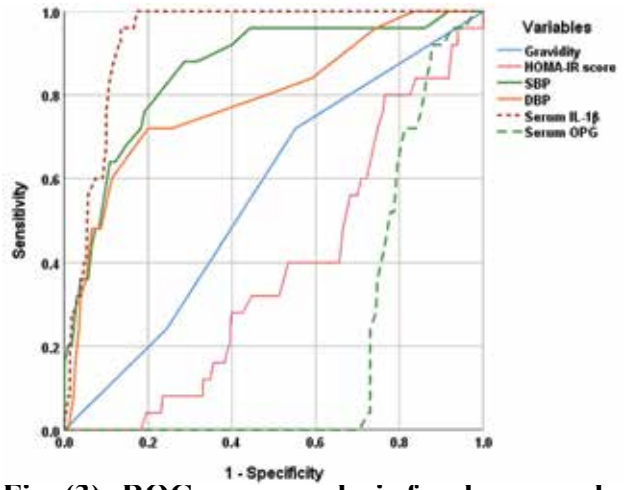

Fig. (3): ROC curve analysis for demographic, clinical and laboratory data determined at ANV-1 as predictors for development of GHT during pregnancy

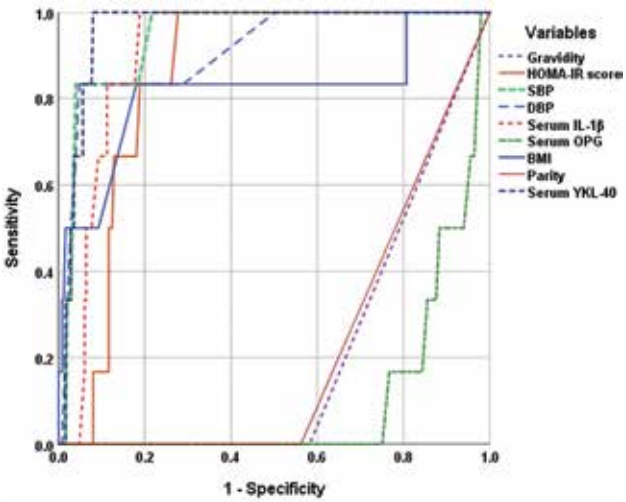

Fig. (4): ROC curve analysis for demographic, clinical and laboratory data determined at ANV-1 as predictors for the triad of GHT, GIR and GDM

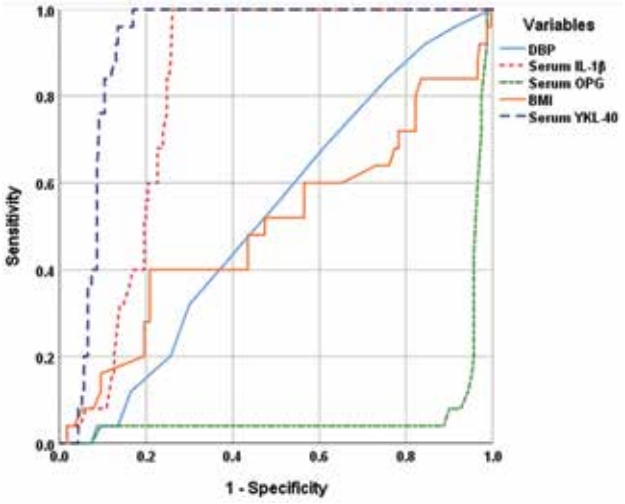

Fig. (5): ROC curve analysis for demographic, clinical and laboratory data determined at ANV-1 as predictors for development of GIR and GDM during pregnancy

Discussion

Pregnancy-induced disorders were detected in 87 pregnant women for a frequency of 31.4% of the studied population, 7 women (2.7%) had developed the triad of GIR, GDM and GHT, 31 women developed GIR with GHT or GDM. These figures point to a certain pathogenic mechanism underlying the development of these disorders alone or together and may suggest a reciprocal relation between the three disorders.

Multiple recent studied tried to explain the coincidence of this triad of disorders; Luo et al. (22) experimentally indicated that reduced dopaminergic neuronal activity at the area communicating with the hypothalamic suprachiasmatic nuclei contributes significantly to increased sympathetic tone with subsequent development of metabolic syndrome and hypertension. Clinically, Motta et al. (23) found acute physical and mental stress is associated with significant

increase in blood levels of free fatty acids and norepinephrine with elevation of DBP and increased peripheral vascular resistance of the large and small arteries in comparison to baseline and attributed pathological increase of blood pressure and metabolic changes to sustained increase in sympathetic activity. These explanations for coincidence of hypertension and metabolic syndrome could be a possible mechanism for that occurring during pregnancy which is documented as a state of continued physical and mental stress with an anxious or traumatic experience for some women that is closely associated with their psychological well-being (24) and is associated with several mood changes (25). As another explanation, Suárez-Cuenca et al. (26) attributed the coincidence of this triad to the effect of obesity and increased weight gain with subsequent change of adipocyte volume and suggested that hypertension-resistin-HbA1c interactions were associated with larger subcutaneous adipocytes; while potential insulin-adiponectin associations were observed for larger visceral adipocytes and thus suggested a relation between adipocyte morphology and source with cardiometabolic and atherogenic risk in population with obesity

These attributions could explain and support the reported data where the incidence of the studied disorders showed positive significant correlation with BMI and HOMA-IR scores that were determined at the 1st antenatal visit (ANV-1). Also, systolic and diastolic blood pressure measures determined at ANV-1 were positively correlated with BMI and with the oncoming GHT alone or with GIR.

The current study also reported significant differences in serum IL-1β, YKL-40 and OPG levels in women free and with PDI and serum levels of IL-1β showed positive, while serum OPG levels showed negative significant correlations with the incidence of PDI, irrespective of being single, double or triple disorders, while high serum YKL-40 was positively correlated with the incidence of GIR with GHT or with GDM or with both. These findings indicated a relationship between altered levels of these cytokines and the development and/or severity of disorders, irrespective of being a causal or resultant relation.

The obtained data go in hand with and support the results of previous studies evaluated the levels of these cytokines in pregnant women, despite being studied separately; wherein Nunes et al. (27) reported higher plasma concentrations of IL-1β, TGF-β1, and TNF-α in women with severe than mild PE, and in comparison to normotensive pregnant women, and all PE women showed decreased plasma levels of sCD163 and IL-10 and concluded that in PE women there is an impairment in the modulation of the systemic inflammatory response. Thereafter, Emara et al. (6) found serum level of inflammatory markers were significantly higher in diabetic patients than controls with a significant positive correlation between with FBG, serum creatinine, total cholesterol, LDL-C, HbA1c, and microalbumin/creatinine ratio. Also, Lin et al. (28) using PE-induced rat model found administration of E2 decreased inflammation, NO levels and altered the uterine angiogenic status and significantly suppressed the toll-like receptor 4 signaling pathway with attenuation of high BP, fetal weight, proteinuria, inflammatory response, oxidative stress and endothelial dysfunction and thus attributed these disturbances with subsequent development of PE to low maternal E2 levels.

Recently, Luo et al. (29) demonstrated that serum YKL-40 levels are increased in diabetics and are positively associated with the severe degree of albuminuria and suggested that YKL-40 could be a marker along with other inflammatory markers, if DM is suspected. Also, Huang et al. (30) experimentally detected glucose intolerance, decreased β-cell proliferation and serum insulin levels in placenta-specific OPG knockdown pregnant rat model and suggested that placen-

ta-derived OPG regulated glucose homeostasis during pregnancy via enhancement of β-cell proliferation and may be used as a potential therapy for GDM.

Conclusion

Pregnancy is a stressful condition and is associated with coincidence of pregnancy-induced disorders in large number of women and this may endanger both the mother and the fetus. Disturbed immune milieu in direction of inflammation, progressive increase of BMI with subsequent release of adipocytokines may be a common stack for development of pregnancy-induced disorders, so coincidence of more than one disorder is not infrequent event. Estimation of the studied array of cytokines at the 1st antenatal visit has a high diagnostic value for the upcoming pregnancy-induced disorders either single or combination. Predictive value of these cytokines was superior to the reliance on clinical data especially in communities where follow-up may be infrequent.

Limitations

The study is limited to one center serving certain locality, so multicenter wider-scale studies are advocated to evaluate the coincidence of multiple pregnancy-induced disorders and the diagnostic validity of the supposed diagnostic array.

Recommendation

Prophylactic physical training and interventions to reduce weight gain must be tried as a trial to reduce the incidence and severity of pregnancy-induced disorders.

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Role of Cerebro-Placental Doppler Ratio in prediction of perinatal outcome in Intrauterine Growth Restriction

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Abstract

Background: The Doppler examination of fetal and maternal blood vessels is one of the most important evaluation tools of the blood supply to the embryo specially when intrauterine growth restriction(IUGR) diagnosed during intrauterine life. We conducted an observational study to assess the role of Cerebro-Placental Doppler Ratio (CPR) in prediction of adverse perinatal outcome of IUGR.

Methods: This prospective observational cohort study was conducted at Mansoura University Hospital-department of obstetrics and gynecology among 100 pregnant women diagnosed with IUGR. We performed two-dimensionalultrasound examination of fetus and placenta associated with Doppler waveform analysis of umbilical and middle cerebral arteries (UA & MCA). Estimation of CPR was performed by the following equation (MCA pulsatility index/ UA pulsatility index).

Results: We divided our patients into two groups either with CRP <1 or with CRP ≥1.An emergencycesarean delivery (CD) was the dominant mode of delivery in 66.7% of cases with CPR<1, versus 35.4% in cases with CPR≥1. There was no statistically significant difference between both groups in the mean gestational age at delivery (35.38±1.53 & 36.15±1.77p=0.07) respectively. The mean neonatal weightswere (1497.6±227.2 gm& 1813.9±304.76 gm p<0.001) in both groups respectively. All neonates with CPR<1were bornewith Apgar score< 7 at 5 minutes and were admitted to neonatal intensive care units (NICU).

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Only 62% of neonates were admitted to NICU when CPR was ≥1.

Conclusion: Data from our study showed that the cutoff point for CPR is ≤1.1, and it has95.5% sensitivity in predicting adverse perinatal outcomes.

Key words: cerebro-placental Doppler ratio, intrauterine growth restriction, perinatal outcome.

Introduction

Fitzgerald and Drumm were the first to perform Doppler examination during intrauterine life. They found that there is low resistance to blood flow from maternal to foetal side through placental blood vessels (1). Nowadays it is very important to evaluate blood flow through uterine arteries, umbilical artery (UA) foetal middle cerebral artery (MCA) and foetal ductus venosus (DV) using Doppler signals (2).It was found to be of high importance specially with high-risk pregnancies (3).Intrauterine growth restriction (IUGR) can be diagnosed when the estimated intrauterine foetal weight is ‹ 10th percentile (4).Brain-sparing phenomenon is oneof the defensive mechanisms that protect vital brain centers in cases of IUGR. It helps in redistributing blood in the foetal circulation to prevent hypoperfusion to vital centers in the brain and other organs(5). It is recommended to do Doppler evaluation of foetal blood vessels as it is known to be affected earlier than other foetal well-being tests (6). This also allow early and rapid intervention to optimize the neonatal outcomes (7). Also, it is known that with IUGR there will be an increase in the diastolic blood flow in the MCA with decrease in this flow at UA(8).

Cerebroplacental ratio (CPR) can be calculated by (MCA pulsatility index / UA pulsatility index)It is considered to be a marker of the severity of the brainsparing effect(9). Doppler examination of the MCA and UA blood flow were studied to predict foetal and neonatal outcomes in cases of IUGR (10,11). We conducted this observational study to assess sensitivity and specificity between CPR and Doppler indices of UA and MCA in predictingthe adverse perinatal outcome of growth restricted foetuses.

Material and methods

This was a Prospective cohort observational study conducted at Department of obstetrics and gynaecology,Mansoura University Hospital In the period between January 2017 till March 2019. All participants in the study were pregnant women diagnosed to have foetal IUGR. The study protocol was submitted for approval by Mansoura Institutional Medical Research Board (IRB).There were 143 patients eligible for the study. eight patients refused to participate in the study and 35 patients were not meeting the inclusion criteria for the study. Only 100 cases were analysed and they were divided into2 groups according to the CPR (21 cases with CPR < 1 & 79 cases with CPR ≥ 1).

Inclusion criteria:

1. Pregnant women with singleton pregnancy
2. Pregnant women diagnosed to have IUGRand the estimated foetal weight (EFW)was found to be < 10th percentile for gestational age (GA).
3. Pregnant women with sure date of last normal menstruation which was not preceded by lactation or use of hormonal contraception

Exclusion criteria:

1. Pregnant women with unsure or uncertain date of last menstrual period
2. Pregnant women who did not have documented ultrasound scan of pregnancy at the end of 1sttrimester.
3. Pregnant women diagnosed to havefoetal congenital anomalies.

Method

IUGR was diagnosed at 30-32 weeks of gestation.Details about the technique and the possible side effects wereexplained for all participants. Each participant signed an informed written consent before their inclusion in the study. Detailed history taking, and obstetric abdominal examination were done for all patients including determination of fundal level and symphysis-fundal height.

Ultrasound examination of the placenta was done to evaluate it place and to detect any pathology. Foetal biometry was estimated by detecting biparietal diameter, femur length and abdominal circumference. The EFW was calculated by software in the ultrasound machine then it was put in the centile curve to compare it with the GA.Then the Doppler mode was turned on and waveform analysis of UA and MCA indices were done including; Peak Systolic/End Diastolic ratio (S/D). Resistance Index (RI). Pulsatility Index (PI). The inbuilt software of the machine will be use to give all the values of indices after tracing the waveforms. CPR was calculated as a simple ratio between MCA PI / UA PI considered abnormal if < 1.Ultrasound examination and Doppler indices were repeated every week from the time of admission till delivery.Neonatal assessment was done including estimation of foetal weight at delivery, GA at delivery, 5-minute Apgar score, neonatal intensive care unit (NICU) admission rate, NICU type and duration of NICUadmission.

Outcome measurements

Primary outcome

Detraction of sensitivity and specificity of CPR in prediction of NICU admission after delivery.

Secondary outcome

Detraction of sensitivity and specificity of MCA & UA Doppler indices separately in prediction of NICU admission after delivery.

Detection of the perinatal complication rates after delivery.d

Statistical analysis and data interpretation:

Data were fed to the computer and analysed using IBM SPSS software package version 22.0. Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) & inter quartile range for non-parametric data and mean, standard deviation for parametric data after testing normality using Kolmogrov-Smirnov test. Significance of the obtained results was judged at the (0.05) level.

Data analysis

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

Quantitative data between two groups:

Parametric tests: Student t-test was used to compare 2 independent groups.

Non Parametric tests: Mann-Whitney U test was used to compare 2 independent groups.

Diagnostic accuracy

Receiver Operating Characteristic (ROC) curve analysis: The diagnostic performance of a test, or the accuracy of a test to discriminate diseased cases from non-diseased cases is evaluated using Receiver Operating Characteristic (ROC) curve analysis. Sensitivity and Specificity were detected from the curve.

Results

Data from our study revealed that CPR can predict admission to NICU with 81.4% sensitivity and 50% specificity. The mean gestational agesat delivery were not statically sig-

nificant different when the CPR was ‹ 1 or ≥1 (35weeks & 36 weeks) respectively.There was no statistically significant difference between both groups in the median gravidity (4&3) respectively. Also, there was higher caesarean delivery rate when the CPR was found to be ‹ 1 (table 1). About 85.7 % of cases underwent CD due foetal indication when CPR was ‹ 1. However vaginal delivery was feasible when the CPR was ≥1 (table 1).

The mean neonatal weight after delivery was significantly lower when the CPR was ‹ 1 (table 2). All neonates born with CPR ‹ 1 developed low Apgar score after 5 minutes in comparison to only 1.3% of neonates born with CPR ≥1 (table 2).There were statistically significant differences in the NICU admission rats in both groups (100% &62%) respectively (table 2). About 90% of NICU admissions were due neonatal respiratory distress syndrome (RDS) in the abnormal CPR group (table 2).Although high number of cases with normal CPR were scored >7 at 5 min, 62% of them admitted to NICU, 42.9% needed O2 therapy. In spite of having different indications for NICU admissions, the mean durations of admission were not statistically significant different in both groups (table 2).

There were statistically significant differences in the Doppler indices of the MCA 7 UA (table 3). Middle cerebral artery Doppler mean values (S/D, RI & PI) were significantly lower in cases with abnormal CPR (3.42±0.58, 0.74±0.13&1.16±0.13respectively), where UA Doppler mean values (S/D, RI & PI) were higher than normal values in the same group(4.35±.66, 0.77±0.08&1.38±0.23respectively) (table 3).

After studying the validity of Doppler indices in predicting low Apgar score (<7) at 5 minutes, we found that UA PI was the most sensitive index (90.9%) at cutoff ≥1.185 (table 4).While the sensitivity of UA S/D and RI indices were 86.4% and 81.8% respectively (table 4). Also, the UA RI has a specificity of 97.4% in predicting low Apgar score at cutoff ≥0.73 (table 4).The study of Middle cerebral artery Doppler indices revealed that MCA S/D at cutoff ≤3.95 can predict low Apgar score with sensitivity of 81.8%, while MCA RI at cutoff ≤0.715 was more specific (80.8%) (table 4).For CPR we found that at cut off point ≤1.125, it can predict cases with 5 minutes Apgar score <7 with 95.5% sensitivity and 61.5% specificity (table 4).

We found also that UA SD at cutoff point ≥3.69 was most sensitive and specific than other UA indices (77.1% sensitivity and 76.7% specificity) in predicting the need for NICU admission (table 5). However, for MCA Doppler indices,MCA S/D at cutoff ≤3.95 had sensitivity of 62.9%, but RI was most specific 70% at cutoff≤0.715. On other hands, MCA PI at cutoff ≤1.355 has 60% sensitivity and 66.7% specificity in prediction (table 5).CPR can predict admission to NICU with 81.4% sensitivity and 50% specificityat cutoff ≤1.17 (table 5)

Discussion

Foetal growth restriction is of the most important obstetric disorders.It can be predicted in 12-47% of cases (12).Multiorgan affection could happen due to foetal hypoxia which may predispose to late disabilities (13).Many defence mechanisms are present to protect against theses harmful effects. Vasodilatation of cerebral blood vessels will allow perfusion of more blood to the vital centers in the brain. This phenomenon will be reflected in decreases of MCA RI (14).Estimation of CPR during pregnancy was found to give good idea about the degree of foetal affection and also help in the management plan (15).

We conducted this study to determinesensitivity, specificity of UA and MCA Doppler values and assessing the role of CPR in prediction of adverse perinatal outcome among 100 pregnant females diagnosed to have IUGR.All patients were subjected to Colour Doppler study of UA and MCA after detailed clinical examination. Our outcome measures

wereneonatal weight at delivery, gestation age at delivery, mode of delivery, 5-minute Apgar score <7, NICU admission and period of admission.

Our study revealed that, there was higher incidence of emergency CD with abnormal CPR (66.7%) compared with 35.4% with normal CPR. This was mostly due to foetal indications in abnormal CPR group (85.7%) andit was done due to maternal indications in normal CPR group. Ropacka-Lesiak et al. (14) reported a significant increase in prevalence of emergency CD that was noted in the group with abnormal CPR ratios(<1.1). Gaikwad et al. (16), reported that labour induction failed and emergencyCD were performed in 67% of patients with abnormal CPR compared to 50% of those with normal CPR. However, Khalil et al. (17) reported that 15.6% of pregnancies were terminated by CDdue to suspected foetal compromise with abnormal CPR and 3.7% of the neonateswere admitted to the NICU.Gibbons et al. (18) reported that the mode of delivery was not statistically significant different in the study groups despite that the CPR <10th centile cohort. Foetaldistress was the main cause of CD in this study (18).

In our study, there was no significant difference between both groups at GA at delivery, but the mean neonatal weight was significant lower with CPR<1 than CPR≥1 group ($p<0.001$). Gaikwad et al. (16) study revealed that neonates who had lower CPR was averagely less in weight and 69% of them were delivered earlier than those whose CPR was higher.Gibbons et al. (18) also, reported that, when CPR was less than the 10th centile,it was significantly associated with a low birthweight and high rate of preterm birth.

Prior et al. (19); RJ Hui (20) and retrospective studies with Sabdia et al. (21); Khalil et al. (22) reports high perinatal complications, emergency CS, increasing incidence of lower Apgar scores and admission to NICUs with low CPR.Nassr et al. (23) conducted a meta-analysis and concluded that low CPR was associated with an increased risk of CS due tofoetal distress, low Apgar score and NICU admission.In our study, we found that all neonates had 5 min Apgar score <7 when CPR was abnormal and all of them were admitted to NICU with 90% on O2 therapy (RDS). However, 62% of neonates were admitted mostly to ordinary NICU for LBW 57.1% when CPR was normal. There was no significant difference between both groups in NICU admission period (table 2).Ebrashy et al. (24); Flood et al. (25) reported increased emergency CD rate, Apgar scores less than 7 at 5 minutes and higher NICU admission rate when CPR was low. Also, Flood et al. (25) reported 11-fold increased incidence of adverse perinatal outcomes, neonatal morbidity and mortality for small neonates with abnormal CPR in comparison to those with normal CPR.

We found that, for prediction of 5 min Apgar score <7, UA PI at cutoff ≥1.185 has highest sensitivity (90.9%) but UA RI at cutoff ≥0.73 was more specific than other indices (97.4%). CPR at cutoff ≤1.125 has sensitivity of 95.5% and specificity of 61.5% in prediction of low Apgar score.

Also, UA S/D at cutoff ≥3.69 has more predictive value than other indices with sensitivity of 77.1% and specificity of 76.7%in prediction of NICU admission. CPR at cutoff ≤1.17 has sensitivity of 81.4% and specificity of 50% in prediction NICU admission. Jain et al. (26) reported a negative correlation between abnormal CPR and incidence of foetaldistress, IUGR as well as the length of stay in the NICU. They also reported that CPR was the most reliable predictor of adverse neonatal outcomes.

Hershkovitz et al. (27); Baschat and Gembruch (28) reported that, the CPR was a more reliable predictor of adverse neonatal outcomes than the individually evaluated MCA & UA flow indices.Odibo et al. (29) based their prediction of perinatal complications on abnormal MCA that showed 35% sensitivity and 56% specificity. However, CPR value

less than 1.08 was reported to be more sensitive and specific than abnormal MCA indices. Khalil et al. (22) reported higher UA PI, lower MCA PI and CPR for the neonates requiring NICUadmission.Ropacka-Lesiak et al. (14) compared some Doppler blood flow indices and reported that CPR wasa highly sensitive predictor of the adverse neonatal outcome (87.8%).In contrast, Gaikwad et al. (16) found higher predictive value of MCA RI and MCA PI than UA S/D, UA RI and PI, and CPR in detecting adverse perinatal outcome.

Luong et al. (30) also reported that, the predictive value of UA-PI and MCA-PI, is not more reliable regarding perinatal complications. But, Lakhkar et al. (31) reported that MCA Pl was more sensitive in prediction of perinatal outcomes in comparison to the sensitivity of CPR.Interpretation of CPR values was commonly based on the cutoff value. Gramellini et al. (10) considered CPR less than 1.08 to be abnormal. While CPR less than1.05 was considered the most reliable predictor of perinatal complications according to Devine et al. (32). On the contrary, Odibo et al. (29) considered the reference ranges of CPR superior to the cut of value when predicting perinatal complications.

In conclusion,our present study reported the significance of Doppler ultrasound studies in patients with IUGR to identify compromised foetuses in utero and to take timely appropriate action. CPR at cutoff ≤1.1 was having high sensitivity in predicting adverse perinatal outcome.However, UA S/D and RI were more specific for prediction of preinatal outcomes.

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Disclosure

All authors disclose no conflict of interest.

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Table (1): Comparison of demographic data between cases with CPR <1 & ≥1.

	CPR<1 (abnormal CPR)	CPR≥1 (normal CPR)	Test of significance
	n = 21	n = 79	
Age /years Mean ± SD	27.05 ± 5.0	31.39 ±7.53	t=2.49 p=0.01*
Gravidity Median (Range)	4.0 (1.0 - 6.0)	3.0 (1.0 - 6.0)	z=0.22 p=0.83
Parity Median (Range)	1.0 (1.0 - 4.0)	3.0 (1.0 - 4.0)	z=2.58 p=0.01*
Previous CS number Median (Range)	1.0 (1.0 - 3.0)	2.0 (1.0 - 4.0)	Z=2.86 P=0.004*
GA at delivery by weeks Mean ± SD	35.38 ± 1.53	36.15 ± 1.77	t=1.82 p=0.07
Mode of delivery Vaginal delivery Elective CD Emergent CD	n (%) 0 (0.0) 7 (33.3) 14 (66.7)	n (%) 9 (11.4) 42 (53.2) 28 (35.4)	MC P=0.02*
Cause of emergent CD Maternal condition Fetal causes	n (%) 2 (14.3) 12 (85.7)	n (%) 28 (100.0) 0 (0.0)	FET P<0.001*

t:Student t test Z:Mann Whitney U test FET: Fischer exact test χ2:Chi-Square test MC: Monte Carlo test *statistically significant (p<0.05)
GA: Gestational age CRP: Cerebro-placental ratio SD: Standard deviation
CD: Cesarean delivery

Table (2): Comparison of neonatal outcome between cases with CPR <1 & ≥1.

	CPR<1 (abnormal CPR)	CPR≥1 (normal CPR)	Test of significance
Newborn weight/gm Mean ± SD	1497.6 ± 227.2	1813.9 ± 304.76	t=4.43 p<0.001*
5 minutes Apgar score n (%) <7 >7	21 (100.0) 0 (0.0)	1(1.3) 78 (98.7)	χ^2=39.87 p<0.001*
NICU admission n (%)	21(100.0)	49 (62.0)	χ^2=11.39 p=0.001*
NICU type n (%) Ordinary (LBW) O2 therapy (RDS)	2 (9.5) 19 (90.5)	28 (57.1) 21 (42.9)	χ^2=13.61 p=0.001*
Admission period/days Mean ± SD	14.0±6.26	14.53 ± 6.96	t=0.27 p=0.78

t:Student t test χ2:Chi-Square test
*statistically significant (p<0.05).
LBW: Low birth weight NICU: Neonatal intensive care unitSD: Standard deviation
CRP: Cerebro-placental ratio RDS: Respiratory distress syndrome

Table (3): Comparison of Doppler values of UA and MCA between cases with CPR <1 &≥1.

	CPR<1 (abnormal CPR)	CPR≥1 (normal CPR)	Test of significance
	n=21	n=79	
MCA S/D Mean ± SD	3.42 ± 0.58	3.83 ± 0.85	t=2.09 p=0.03*
MCA RI Mean ± SD	0.74 ± 0.13	0.79 ± 0.11	t=2.0 p=0.04*
MCA PI Mean ± SD	1.16 ± 0.13	1.46 ± 0.39	t=3.39 p=0.001*
UA S/D Mean ± SD	4.35 ± 0.66	2.77 ± 0.41	t=13.59 p=0.001*
UA RI Mean ± SD	0.77 ± 0.08	0.63 ± 0.07	t=7.59 p=0.001*
UA PI Mean ± SD	1.38 ± 0.23	1.19 ± 0.24	t=3.18 p=0.002*

t:Student t test *statistically significant (p<0.05)
CRP: Cerebro-placental ratio UA: Umbilical artery MCA: Middle cerebral artery
S/D : Peak Systolic/End Diastolic ratio RI : Resistance Index PI : Pulsatility index

Table (4): Validity of Doppler indices of UA, MCA and CRP in predicting 5 minutes Apgar score <7:

5 minutes Apgar score <7	AUC P	Cut off point	Sensitivity (%)	Specificity (%)
UA S/D	0.937 <0.001*	≥3.22	86.4	84.6
UA RI	0.86 <0.001*	≥0.73	81.8	97.4
UA PI	0.722 0.002*	≥1.185	90.9	55.1
MCA S/D	.621 0.08	≤3.95	81.8	50.0
MCA RI	.699 0.005*	≤0.715	72.7	80.8
MCA PI	.769 <0.001*	≤1.22	77.3	67.9
CPR (MCA PI/UA PI)	0.93 <0.001*	≤1.125	95.5	61.5

*statistically significant (p<0.05)
AUC: Area under curve
CRP: Cerebro-placental ratio UA: Umbilical artery
MCA: Middle cerebral artery S/D : Peak Systolic/End Diastolic ratio
RI : Resistance Index PI : Pulsatility index

Table (5): Validity of Doppler indices of UA, MCA and CPR in predicting NICU admission:

NICU admission	AUC P	Cut off point	Sensitivity (%)	Specificity (%)
UA SD	0.823 <0.001*	≥3.69	77.1	76.7
UA RI	0.83 <0.001*	≥0.625	71.4	70.0
UA PI	0.621 0.05	≥1.185	62.9	63.3
MCA S/D	0.56 0.06	≤3.95	62.9	56.7
MCA RI	0.28 0.05	≤0.715	31.4	70.0
MCA PI	0.71 0.054	≤1.355	60.0	66.7
CPR (MCA PI/UA PI)	0.738 <0.001*	≤1.17	81.4	50.0

*statistically significant ($p<0.05$)
AUC: Area under curve
NICU: Neonatal intensive care unit
CRP: Cerebro-placental ratio
UA: Umbilical artery
MCA: Middle cerebral artery
S/D : Peak Systolic/End Diastolic ratio
RI : Resistance Index PI : Pulsatility index

Comparison of Reproductive Outcomes on addition of GnRH agonist for Luteal phase Support in Antagonist IVF cycle

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Abstract

Background: The luteal phase supplementation was reported to be necessary in Controlled Ovarian Hyperstimulation cycles for IVF or ICSI, independently when agonists or antagonist were used for pituitary desensitization. The effectiveness of GnRH agonist in luteal phase supplementation remains controversial.

Aims and Objectives: This study aimed at comparing the clinical outcomes of addition of GnRH agonist for luteal phase support in antagonist IVF cycle.

Materials and Methods: A cross-sectional study of 150 eligible clients who underwent assisted reproduction program in two autonomous IVF centers between 1st January, 2017 and 31st December, 2020. Clients were divided into two groups; (I) Antagonist stimulation protocol with progesterone and oestradiol valerate and (II) Antagonist Protocol with a single bolus of buserelin in addition to progesterone and oestradiol valerate for luteal phase support. The primary outcome was live birth rates while the secondary outcomes were clinical pregnancy, miscarriage rates and the safety for OHSS.

Results: There were no statistically significant differences between the number of oocyte received, fertilized, embryos availability for transfer, duration of infertility, duration of FSH usage, endometrial thickness and OHSS risk between the groups ($p>0.05$). The clinical pregnancy and live birth rates were more in group II while the miscarriage rate was lower compared to group I. The differences were statistically significant ($p<0.005$).

Conclusion: From this study, buserelin addition to the luteal phase of antagonist cycles appears to improve pregnancy outcomes with no associated increase in OHSS risk. Further multi- centered studies with larger sample sizes are required to validate these findings.

Keywords: Comparison, Antagonist protocol, GnRH- agonist, luteal phase.

Introduction

Luteal phase support (LPS) is an integral part of assisted reproduction treatment (ART). Defective luteal phase in assisted reproduction cycles has been attributed to adverse effects of controlled ovarian stimulation, suppression of the pituitary luteinizing hormone (LH) release by gonadotropin releasing hormone (GnRH) analogs, and to depletion of granulosa cells due to follicle aspiration. (1) Controlled ovarian stimulation has been shown to advance endometrial maturation thus disrupting the delicate mechanism of embryo-endometrium interaction. (2)

It has been long recognized that supporting the luteal phase with progesterone or human chorionic gonadotrophin (hCG) is associated with higher pregnancy and delivery rates. (3) The initial agent of choice to support the luteal phase has been hCG, however, due to an increased risk of Ovarian Hyperstimulation Syndrome, it has been largely replaced by progesterone. Luteal phase support exclusively with progesterone might not always be sufficient to promote implantation, and other approaches can be attempted. (4, 5, 6)

Recently, the co-administration of a single dose GnRH agonist in the mid-luteal phase was reported to significantly increase implantation and live birth rates in women undergoing intracytoplasmic sperm injection (ICSI) and embryo transfer (ET). (5) It has been suggested that GnRH agonist may act both through an indirect stimulus to corpora lutea by gonadotropin discharge from pituitary gland, leading to a stimulus to corpora lutea, and via a direct effect on endometrium and embryo. (7) The data on donor cycles, obtained when agonist was added in the luteal phase in the absence of corpora lutea, suggested that the effect might be due to a direct effect, on the endometrium or the embryo. (4, 6)

The effects of GnRH agonist administration in the luteal phase has been the focus of different studies. Lemay et al. (8) suggested that GnRH agonist can act as a luteolytic agent due to desensitization of GnRH receptors. Furthermore, Dubourdieu et al. (9) and Herman et al. [10] reported deterioration of corpus luteum function with the administration of GnRH agonist. However, attempts to interrupt pregnancy or even prevent implantation have not been impressive. (11) On the other hand, a series of studies show that the inadvertent administration of GnRH-a in the luteal phase does not compromise the continuity of pregnancy, and suggested, to the contrary, a possible favorable effect on implantation. (12, 13, 14) Recently, different studies analyzing single (15, 16) or multiple administrations (17, 18) of medication have, in fact, suggested a beneficial effect in supporting the luteal phase.

We therefore seek to assess the impact of addition of GnRH agonist for luteal phase support on pregnancy outcomes in antagonist protocol IVF cycle in two independent IVF centers as compared to the conventional Progesterol and oestradiol valerate.

Materials and Methods

This study is a cross-sectional study of 150 eligible clients that underwent assisted conception program (IVF/ICSI) at two independent IVF centers between January 1, 2017 and December 31, 2020. Clients were recruited using a purposive sampling method and informed consent was obtained from selected clients. The case records and the theatre records of the clients were retrieved from IVF centers using a prepared proforma. Data extracted from the case notes are; biosocial variables, types of stimulation protocols, dose of FSH needed, duration of stimulation, risk for OHSS, and pregnancy outcomes.

All clients had a body mass index (calculated as weight in kilograms divided by the square of height in meters) [19] ranging between 18 and 30 with a mean of 24 ± 4 Kg/m^2. All had antagonist protocol for Controlled Ovarian Hyperstimulation. Their infertility evaluation results were normal. Furthermore, all had oral contraceptive pills for menstrual cycle

synchronization and pre-cervical assessment (trial/dummy transfer) on day 2/3 of menses prior to commencement of stimulation.[20]

Stimulation protocol

All patients were treated with the gonadotropin-releasing hormone (GnRH) antagonist protocol. They were commenced on 150 IU (2 vials) of recombinant follicle-stimulating hormone (FSH) Gonal F (Gonal F[R]; Merck Serono, Germany) and 75 IU (1 vial) highly purified FSH (Folliculin ®; Barrat pharmaceutical, India) on day 3 of menstrual cycle for 11–14 days. Transvaginal ultrasonographic scan was done at interval from day 5/6 of stimulation to determine the numbers, size of follicles, and endometrial thickness. Subcutaneous 2.5 mg daily GnRH antagonist (Cetrotide ®; Merck Serono, Germany) was administered whenever the follicles have grown to 14 mm size usually around day 6/7 of stimulation and was continued till the day of trigger to prevent premature luteinizing hormone surge. Eighty-three microgram (2000 IU) of recombinant human Chorionic gonadotrophin (hCG: Ovitrelle; Merck Serono, Germany) and 0.25 mg of buserelin (Supricur ®; Aventis Pharm, West Malling, UK) were administered subcutaneously for trigger whenever two or more follicles have grown to 18 mm or more. [20]

Oocyte retrieval

Oocyte retrieval was done at 35.5 h of hCG injection by transvaginal needle aspiration under ultrasound guidance using general anaesthesia (Propofol and Midazolam). [21] The aspirate in conical test tubes each containing 1 ml of Global Collect ® was then transferred immediately to the laboratory for oocyte screening and pickup. Oocytes were rinsed in oocyte handling medium (Global collect ®; LifeGlobal, Europe) and also rinsed and cultured in a center well dish (Oosafe, Denmark) of 1 ml fertilization media (Global total for fertilization, LifeGlobal, Europe) overlaid with 1 ml paraffin oil (LifeGlobal, Europe) which is then incubated for 4–6 h prior insemination.[20]

Sperm preparation

All semen samples were allowed to liquefy at room temperature for 30 min. Semen analysis was performed according to the World Health Organization (WHO) guidelines (WHO, 2010).[22] The density gradient centrifugation method of semen preparation was used. All Grad 90% and 45% (LifeGlobal, Europe) were overlayed with a maximum of 2 ml raw semen and centrifuged at 300 g for 20 min. The pellets were resuspended into Falcon tube containing 5 ml All Grad wash (LifeGlobal, Europe) and centrifuged again at 300 g for 10 min.

Insemination procedure

Postwash spermatozoa at a final concentration of 150,000 × 10^6/ml were added to the oocytes and incubated for 16–18 h. A maximum of eight oocytes were inseminated in a center well dish (Oosafe, Denmark) of global total for fertilization (LifeGlobal, Europe) overlaid with paraffin oil (LifeGlobal, Europe). Intracytoplasmic sperm injection was performed on metaphase II oocyte if indicated

Assessment of fertilization

Denudation of cumulus cells was performed by the use of glass denuding pipettes (Vitromed GmbH, Germany). The oocytes were then rinsed four times in a single-step medium (Global total, Life Global, Europe) and assessed for fertilization before further culture in global total (Life Global, Europe). The oocytes were considered fertilized when two distinct pronuclei and two polar bodies were visible.

Embryo culture

A maximum of six fertilized oocytes were cultured in a center well dish with 1 ml single-step media (Global total, Life Global, Europe) under oil (Paraffin oil; Life Global, Europe) at 37°C in a humidified atmosphere of 5% O_2 in air for day 3 or day 5.

Embryo grading

Assessment of cleavage stage embryos and grading was done on postretrieval day 3, based on the number of blastomeres, symmetry (evenness of blastomere size), and the degree of fragmentation using the Society for Assisted Reproductive Technology grading system.[23] Assessment of blastocyst and grading was also done postretrieval day 5, based on the expansion, inner cell mass, and Trophectoderm according to Gardner et al. [24]

Embryo transfer

The best quality blastocyst and expanded blastocyst were transferred on day 5 for all the groups. ET was done under transabdominal ultrasound guidance, and the transfer catheters were checked to ensure that all the embryos were transferred. In case of retained embryo (s), the embryo (s) were reloaded in a new transfer catheter and transferred immediately. The number of embryo transferred was individualized, 2 or 3 in most cases. All ET were performed with a soft catheter (Kitazato, Spain). [9]

Luteal phase support

Luteal phase support was conducted with progesterone (400 mg twice daily [cyclogest pessaries®; Cox, Brarnstaple, UK] and Intramuscular 100 mg twice weekly [Gestone®; Ferring, pharmaceutical, Mumbai, Maharashtra, India]) [9] and Oestradiol valerate 4mg daily [oestrafert ®; Mark Pharmaceutical; Gujarat, India] for group (I) while addition of a single bolus of subcutaneous 0.25 mg of buserelin (Supricure®; Aventis Pharm, West Malling, UK) was administered one week oocyte retrieval in Group (II)

Pregnancy test

Serum pregnancy test was carried out 2 weeks after ET and subsequently transvaginal ultrasound at 6th week for detection of gestational sac and/or viability of the fetus.

Statistical analysis

Statistical analysis was done using IBM SPSS (Statistical Package for Social Sciences) Version 20. Categorical data were expressed as numbers and percentages, while numerical data were expressed as a mean and standard deviation. Associations of categorical variables were tested using Chi-square test, while statistical significance was set at $P \leq 0.05$. Results were presented in table

Results

A total of one hundred and fifty (150) normoresponder clients were enrolled for the study. Clients' mean age and their spouses were 32.8 ± 4.0 and 37 ± 4.3 years respectively. Majority (98%) were Para 3 and below. Most (60.7%) had primary infertility. Their mean duration of infertility was 4.7 ± 2.9 years with the majority (95.3%) having experienced more than a year duration of infertility [Table 1]. All had antagonist protocol for Controlled Ovarian Hyperstimulation. The mean number of oocytes retrieved, fertilized, embryos available for transfer, duration of FSH use, ampoules of FSH and endometrial thickness were 11.2 ± 5.1, 7.3 ± 3.2, 4.9 ± 2.3, 13.6 ± 1.9 and 8.8 ± 2.0 respectively. The overall clinical pregnancy, miscarriage, OHSS and live birth rate were 45.3%, 13.3%, 12% and 32% respectively. Among whom were 8 triplets and 22 twins' births. [Table 2].

Table 3 shows the association between luteal phase support and pregnancy outcomes. The

clinical pregnancy rate in group II (60.3%) was higher than 39.7% in group I, live birth rate in group II (48%) was also higher than group I (16%), while the miscarriage rate was more in group I (20%) compared with group II (6.6%), the differences were statistically significant ($P < 0.05$). Conversely, the risk for OHSS was found not to be statistically significant between the two groups. There were no statistically significant differences between the number of oocytes received, fertilized, embryos availability for transfer, duration of infertility, duration of FSH usage, number of FSH ampoules, endometrial thickness and OHSS risk between the groups ($p>0.05$)

Discussion

The use of GnRH agonists to enhance embryo implantation has a relatively long history. As in many other cases of important scientific innovation, the concept of luteal phase support with a GnRH agonist was born of fortuitous observational findings rather than a clearly defined scientific project. [25] This study aimed at comparing the effectiveness of addition of GnRH agonist for luteal phase supplementation in antagonist IVF cycles. The primary outcome was live birth rates while the secondary outcomes were clinical pregnancy and miscarriage rates and the safety for OHSS respectively.

In this study, there were no statistically significant differences in the of number oocyte retrieved, fertilized and available embryos for transfer between the groups. This is in keeping with the findings of Isik et al [26] in a similar study which reported significantly higher rates of implantation and pregnancy rates in favor of GnRH agonist luteal phase support group. The clinical pregnancy and live birth rates were significantly higher in the GnRH agonist luteal phase group while the miscarriage rate is significantly lower. This is in consonance with the reports from previous studies [5 12-14, 16, 26] which documented a significant improvement in implantation, clinical pregnancy rates and live birth rates in GnRH agonist luteal support group. The beneficial effects of the GnRH agonist may be attributed to a direct effect on the embryo or to an effect on endometrium mediated by luteinizing hormone (LH), in accordance with previous observations on the effects of LH activity on endometrial receptivity, independent of ovarian function. [4] However,further studies also have shown a beneficial effect ofGnRH agonists on embryo implantation in ovarian stimulation cycles, particularly in those using a GnRH antagonist protocol. [5, 27] This informed the basis of adoption of antagonist protocol for Controlled Ovarian Hyperstimulation in this study.

On the contrary, other studies reported a significant reduction in implantation and ongoing pregnancy rates in luteal GnRH agonist group as compared with placebo. [8-10] However, clinical pregnancy rates were similar in the GnRH agonist and placebo groups. This may be related to the luteolytic effects of GnRH agonist due to desensitization of GnRH receptors resulting in deterioration of corpus lutea functions. [8-10, 28]

Although the safety of GnRH agonist in luteal phase is still at experimental phase, however there was no reported increase in embryonic or fetal malformations related to this treatment since its routine use after embryo transfer, which supports the safety of using GnRH agonists. [6,12-18] This is similar to the findings from our series.

Ovarian Hyperstimulation Syndrome (OHSS) which a potentially life-threatening iatrogenic complication of Controlled Ovarian Hyperstimulation during assisted reproductive therapy (ART). This study reported no significant increase in OHSS risk between the two groups despite addition of GnRH agonist for luteal phase supplementation in group II, thus affirming its safety.

Conclusion

Findings from this study demonstrate that the luteal-phase single-dose GnRH-a administration is effective in increasing pregnancy outcome in antagonist IVF cycle devoid of increase in OHSS risk. Further multi- centered studies with larger sample sizes are required before evidence-based recommendation can be provided.

Conflict of interest: Nil

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Table 1: Socio-demographic variables

Variables	Frequency	Percent (%) N=150
Age Wife (years)		
<25	1	0.7
25-34	100	66.7
≥35	49	32.6
Mean -	32.8±4.0	
Age Husband (years)		
<40	107	71.3
40-49	42	28.0
≥50	1	0.7
Mean-	37.7±4.3	
Parity		
0-3	147	98.0
>3	3	2.0
Mean		
Duration Infertility (years)		
≤1	7	4.7
>1	143	95.3
Mean-	4.7±2.9	
Type of Infertility		
Primary	91	60.7
Secondary	59	39.3

Table 2: IVF Parameters/Indices and Pregnancy outcome

Variables		Frequency	Percent (%) N=150
Number of oocyte retrieved			
<10		63	42.0
≥10		87	58.0
Mean-	11.2±5.1		
Number fertilized			
<10		117	78.0
≥10		33	22.0
Mean-	7.3±3.2		
Number of embryo			
<10		145	96.7
≥10		5	3.3
Mean	4.9±2.3		
Protocol			
Antagonist		150	100
Support			
Agonist		75	50.0
No Agonist		75	50.0
Pregnancy outcome			
Negative		82	54.7
Positive		68	45.3
Miscarriage n=68			
Yes		20	29.4
No		48	70.6
OHSS*			
No		132	88.0
Yes		18	12.0
Duration FSH (days)			
<15		107	71.3
≥15		43	28.7
Mean-	13.6±1.9		
FSH AMP			
<30		49	32.7
≥30		101	67.3
Mean-	32.3±4.8		
Endometrial Thickness			
<10		99	66.0
≥10		51	34.0
Mean-	8.8±2.0		

***Ovarian Hyper-stimulation Syndrome**

Table 3: Association between Use of support, parameters and outcomes of IVF

Variable p value	Agonist	Support (%)	No Agonist (%)	χ^2	df
Number of oocyte					
<10	30 (47.6)	33 (52.4)	0.25	1	0.741[a]
≥10	45 (51.7)	42 (48.3)			
Number Fertilized					
<10	54 (46.2)	63 (53.8)	3.15	1	0.114[a]
≥10	21 (63.6)	12 (36.4)			
Number of Embryo					
<10	72 (49.7)	73 (50.3)	0.21	1	1.000[a]
≥10	3 (60.0)	2 (40.0)			
Pregnancy outcome					
Negative	34 (41.5)	48 (58.5)	5.27	1	0.016
Positive	41 (60.3)	27 (39.7)			
Miscarriage n=68					
No	35 (72.9)	13 (27.1)	14.55	1	0.001
Yes	5 (25.0)	15 (75.0)			
Duration of Infertility					
≤1	3 (42.9)	4 (57.1)	0.15	1	1.000
>1	72 (50.4)	71 (49.6)			
OHSS					
No	67 (50.8)	65 (49.2)	0.25	1	0.802[a]
Yes	8 (44.4)	10 (55.6)			
Duration FSH					
<15	58 (54.2)	49 (45.8)	2.64	1	0.148[a]
≥15	17 (32.0)	36 (68.0)			
FSH AMP					
<30	24 (49.0)	25 (51.0)	0.03	1	1.000[a]
≥30	51 (50.5)	50 (49.5)			
Endom. Thickness					
<10	51 (51.5)	48 (48.5)	0.27	1	0.730[a]
≥10	24 (47.1)	27 (52.9)			

[a] **Fischer Exact**

Serum Kisspeptin-1 at time of Pregnancy Diagnosis is superior to serum βhCG for prediction of Early Pregnancy Loss

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Abstract

Objectives: Estimation of serum Kisspeptin-1 (Kiss-1) and beta human chorionic gonadotropin (βhCG) levels at time of pregnancy diagnosis (Booking time) and at the 12th gestational week; S1 and S2 samples, in trial to evaluate the predictability of these levels for the possibility of early pregnancy loss (EPL).

Patients & Methods: 283 women gave S2 samples, 76 women (26.9%) developed EPL (EPL group) and 207 women had viable fetus (Control group). Blood samples were obtained for ELISA estimation of serum Kiss-1 and βhCG. The study outcome was the predictability of S1 levels of both parameters for the possibility of oncoming pregnancy loss.

Results: S1 sample βhCG levels showed non-significant differences between both groups, while serum Kiss-1 levels were significantly lower in EPL than in control women. Serum levels of both parameters in S2 sample were significantly lower in EPL than both control women and levels estimated in S1 samples of EPL women. Incidence of EPL was negatively correlated with S1 sample serum levels of both parameters. Regression analysis defined 6500 IU/L and 318 pmol/L as cutoff points for βhCG and Kiss-1 to predict 50% hazard for EPL. These cutoff points defined 158 and 181 true cases, respectively with significant difference in favor of Kiss-1 value. ROC curve analysis assured the sensitivity of lower S1 levels of both parameters for prediction of 50% hazard of EPL, but area difference under ROC was significant in favor of low serum Kiss-1 as a significant sensitive predictor for EPL.

Conclusion: Estimation of serum kiss-1 at time of pregnancy diagnosis inversely correlates with incidence of EPL. Lower serum Kiss-1 levels had significantly higher predictability for EPL than βhCG. Lower serum Kiss-1 at the 12th GW or development of EPL assured the predictability of the result obtained at booking time and spared the need for sequential estimations.

Keywords: Early pregnancy loss, Early prediction, Kisspeptin-1, βhCG

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Introduction

Peptide hormones, including kisspeptin (Kiss) play a prominent role in controlling energy homeostasis and metabolism, and are implicated in controlling the reproductive functions[1].Kisspeptin is a potent stimulant of luteinizing hormone (LH) pulses and preovulatory surge in females, which is essential for fertility [2]. Kiss-stimulated LH release involves neurokinin B that acts through the hypothalamic neurokinin-3 receptor [3].

Early pregnancy loss (EPL) is defined as the spontaneous loss of a pregnancy before the 13th weeks of gestation [4]. EPL is one of the most common pregnancy complications that is mostly encountered early during pregnancy with an incidence up to 15% of all clinically recognized pregnancies [5]. A significant proportion of patients with EPL are unaware of their miscarriage especially when it occurs in the early stages of pregnancy [6], and so may be misdiagnosed as an expected menstrual cycle [7].

Although the actual mechanism underlying the development of EPL is undefined, multiple attributions were claimed; decidualization is essential for the successful pregnancy, and if it is dysregulated this may lead to EPL [8]. Regulation of nucleosome was found to be critical for the maintenance of genome stability and epigenetic information, and lack of this may lead to EPL [7]. Overexpression of IL-7 by decidua induces a proinflammatory environment that may induce EPL [9].As another mechanism, postovulatory apoptosis leads to oocyte aging, which could be considered as one of the major causes for human EPL [10].

Objectives

Estimation of serum levels of Kiss-1 and βhCG in newly pregnant women at booking time and at the 12thgestational week (GW) in trial to evaluate the predictability of at booking levels for the possibility of EPL

Setting

Departments of Obstetrics & Gynecology, Faculty of Medicine, Benha University

Design

Prospective observational comparative study

Patients & Methods

Throughout the duration of the study since March 2019 till Jan 2021, all newly pregnant women who attended the Antenatal Care Unit, Benha University Hospital for assurance of being pregnant were eligible to evaluation. At the 6th GW (Booking visit), general clinical evaluation including history taking with special regardtoparity, previous miscarriage, missed abortion, pregnancy-induced diseases during the previous completed pregnancies, hormonal disturbances, nutritional deficiencies, or lifestyle initiating or promoting EPL. Then, clinical and ultrasonographic examinationswere performed. Blood samples were obtained for routine laboratory investigations, ELISA estimation of serum Kiss-1 and estimation of serum levels of βhCG.

Exclusion criteria

Presence ofanembryonic sac, multiple pregnancy, renal, cardiac or hepatic disorders.

Inclusion criteria

Pregnant women with singleton fetus, free of exclusion criteria and accepted to sign the written fully informed consent to attend the follow-up visit at the 12th GW or on development of manifestations of EPL were enrolled in the study.

Investigations

At booking time, blood sample was drawn under complete aseptic conditions from the

antecubital vein. Blood samples wereput in a plain tube, allowed to clot, centrifuged at 1500×g for 15 min and the serum samples were divided into two parts:

1. The 1st part was used for immediate estimation of serum βhCG levels
2. The 2nd part was collected in clean Eppindorff tube and stored at -20oC forELISA estimation of Kiss-1 serum levels using ELISA kit (catalogue no. ab19028 abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique.

Study outcome

1. The primary outcome was the predictability of at booking levels of βhCG and Kiss-1 for the possibility of oncoming pregnancy loss.
2. The secondary outcome was the extent of change in serum levels of βhCG and Kiss-1 at the 12th GW or development of EPL, in relation to booking levels.

Statistical analysis

The obtained data were presented as mean, standard deviation (SD), numbers, percentages, median and interquartile ranges (IQR). The percentage of change was calculated as the level estimated in S2 minus the level estimated in S1 sample divided by the level estimated in S1 sample and multiplied by 100. Parametric data were compared using paired t-test and Mann-Whitney test. Non-parametric data were compared using Chi-square test. Kaplan-Meier regression analysis was used to determine the cutoff point that can predict 50% of getting EPL. Predictability of cutoff points was assured by the Receiver characteristic curve analysis. Statistical analysis was performed using SPSS software package, 2015. P value of <0.05 was considered significant.

Results

The study included 311newly pregnant women; 21 were excluded for not fulfilling the inclusion criteria and 290 women were enrolled in the study and gave S1 sample. During follow-up, 7 women were missed and 283 women gave S2 sample. Unfortunately, 76 women developed EPL for an incidence of 26.9% and were categorized as EPL group, 207 women had viable fetus at the 12th GW visit and were grouped as Control group (Fig. 1). Enrolment data of women of both groups showed non-significant differences (Table 1).

Table (1): Enrolment data of patients of both groups

Data	Group A (n=107)	Group B (n=116)	P value
Age (years)	25 (3.4)	24.6 (4)	0.441
Body mass index (kg/m2)	28.7 (2.1)	28.1 (2.8)	0.119
Gravidity*	2 [1-2]	2 [1-2]	0.184
Parity*	1 [0-1]	1 [0-1]	0.116
Systolic blood pressure (mmHg)	115 (6.6)	114 (5.8)	0.217
Diastolic blood pressure (mmHg)	75.6 (4.9)	77 (5.1)	0.098
Random blood glucose (mg/dl)	116.8 (6.9)	115.8 (7.7)	0.297

Data are presented as mean; standard deviation (SD); *median and interquartile range (IQR); P value indicates the significance of difference between both groups; P<0.05 indicates significant difference; P>0.05 indicates non-significant difference

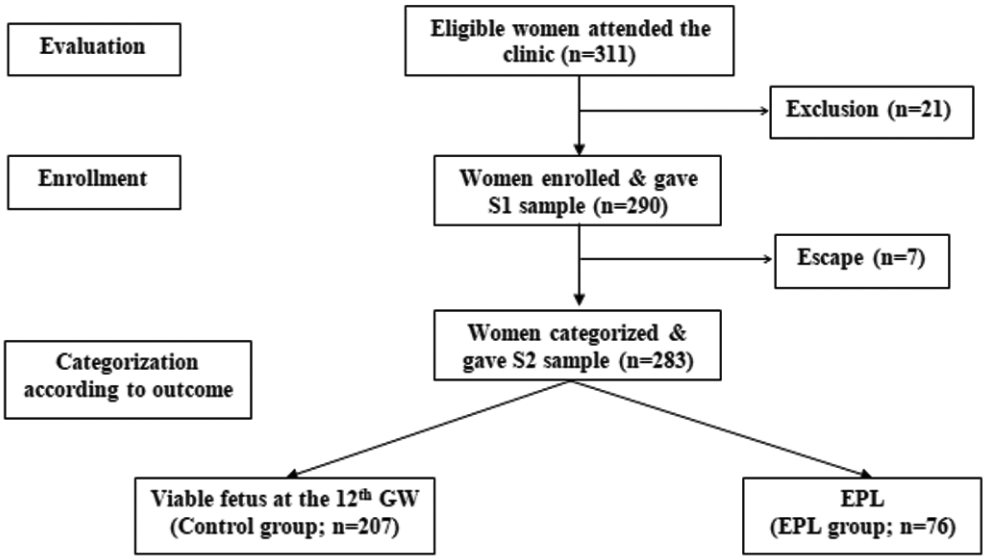

Fig. (1): Study Flow Chart

Estimated serum levels of βhCG at booking time (S1 samples) showed non-significant differences between samples of women of both groups. On contrary, at booking time mean serum levels of Kiss-1 were significantly (p=0.0057) lower in samples women of EPL group in comparison to levels estimated in samples of control women. On the other hand, mean serum levels of both βhCG and Kiss-1 estimated at the 12th GW (S2 samples) were significantly (p<0.0001) lower in samples of EPL women in comparison to samples of control women (Table 2, Fig. 2).

Serum levels of βhCG and Kiss-1 in S2 sample of EPL women were significantly (p<0.0001) lower in comparison to levels estimated in their S1 samples with a median percentage of decrease by 47.1% and 45% for βhCG and Kiss-1, respectively. However, serum levels of βhCG and Kiss-1 estimated in S2 samples of control women were significantly (p<0.0001) higher in comparison to levels estimated in their S1 samples with median percentage of increase of 53.3% and 93% for βhCG and Kiss-1, respectively (Table 2, Fig. 3).

Table (2): Serum levels of βhCGand Kiss-1 estimated in S1 and S2 samples of patients of both groups

Data	Group	Control (n=207)	EPL (n=76)	P value
βhCG (IU/L)	Mean S1 level	6854.6 (1462.3)	7023.7 (1432)	0.387
	Mean S2 level	10541 (1964.3)	3909.2 (1331)	<0.0001
	P1	<0.0001	<0.0001	
	Percentage of change*	53.3 [44-66.1]	-47.1 [35.8-55]	
Kiss-1 (pmol/L)	Mean S1 level	341.4 (156.5)	282 (164.5)	0.0057
	Mean S2 level	655.3 (221.1)	142.7 (62)	<0.0001
	P1	<0.0001	<0.0001	
	Percentage of change*	93 [64.9-123.6]	-45 [37.3-54.1]	

Data are presented as mean; standard deviation (SD); *median and interquartile range (IQR); P value indicates the significance of difference between both groups; P1 value indicates the significance of difference between levels estimated in S1 and S2 samples of each group; P<0.05 indicates significant difference; P>0.05 indicates non-significant difference

The incidence of EPL was negatively correlated with serum βhCG (Rho=-0.157, p=0.008) and Kiss-1 (Rho= -0.375, p<0.001) levels estimated at booking time. Kaplan-Meier regression analysis defined median level of βhCG that predict 50% cumulative hazard for getting EPL at 6500 with 95% CI of 6218-6782 IU/L (Fig. 4) and the median level of Kiss-1 for prediction of 50% cumulative hazard of EPL at 318 with SE of 15.7 and 95% CI of 287-349 pmol/L (Fig. 4). At the cutoff point for βhCG (6500 IU/L), there were 158 true cases and 125 false cases, while at cutoff point for Kiss-1, there were 181 true and 102 false cases with significant difference (p=0.0485) in favor of Kiss-1 value as determinant of 50% cumulative hazard for EPL. Using both variables at the same cutoff points, there were 114 true and 169 false cases with significantly lower predictability for the 50% cumulative hazard of EPL in comparison to βhCG alone (p=0.0002) or Kiss-1 alone (p<0.0001), (Table).

Table (3): Patients' distribution as true and false cases for oncoming EPL as the suggested cutoff points for βhCG and Kiss-1, and both

Variable	Cutoff point	True cases					
		Positive	Negative	Total	Positive	Negative	Total
βhCG	6500 (IU/L)	48 (16.9%)	110 (38.9%)	158 (55.8%)	97 (34.3%)	28 (9.9%)	125 (44.2%)
Kiss-1	318 (pmol/L)	56 (19.8%)	125 (44.2%)	181 (64%)	83 (29.3%)	19 (6.7%)	102 (36%)
Both				114 (40.3%)			169 (59.7%)

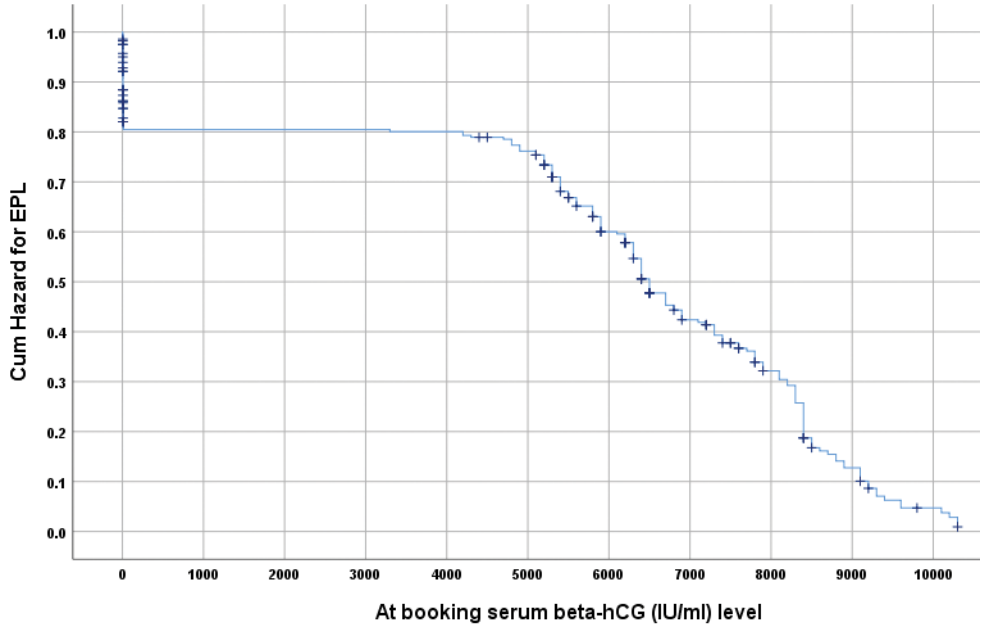

Fig. (4): Kaplan-Meier regression analysis of serum βhCG as predictor for the cumulative hazard for getting EPL

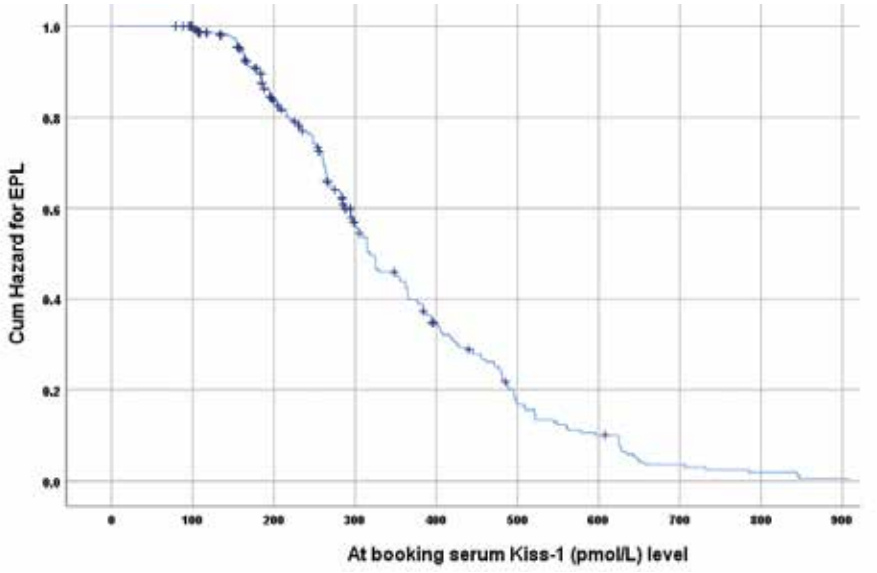

Fig. (5): Kaplan-Meier regression analysis of serum Kiss-1 as predictor for the cumulative hazard for getting EPL

ROC curve analysis assured the sensitivity of lower at booking levels of both parameters for prediction of 50% cumulative hazard of EPL (Fig. 2). However, paired-sample area difference under ROC analysis defined low serum Kiss-1 as a more significant sensitive predictor for EPL than βhCG with significant (p<0.001) difference in AUC (=0.164±0.262; CI= 0.072-0.257).

Table (3): ROC curve analysis for serum levels of βhCG and Kiss-1 as predictors for oncoming EPL

ROC curve analysis	Parameter	AUC	SE	P	95% CI
	βhCG	0.396	0.037	=0.008	0.323-0.470
	Kiss-1	0.232	0.031	<0.001	0.170-0.293
Paired-sample area difference under ROC analysis		AUC difference	SE difference	p	95% CI
		0.164	0.262	<0.001	0.072-0.257

ROC: Receiver characteristic curve; AUC: Area under curve; P indicates the significance of the results; P<0.05 indicates significant difference; p>0.05 indicates non-significant difference

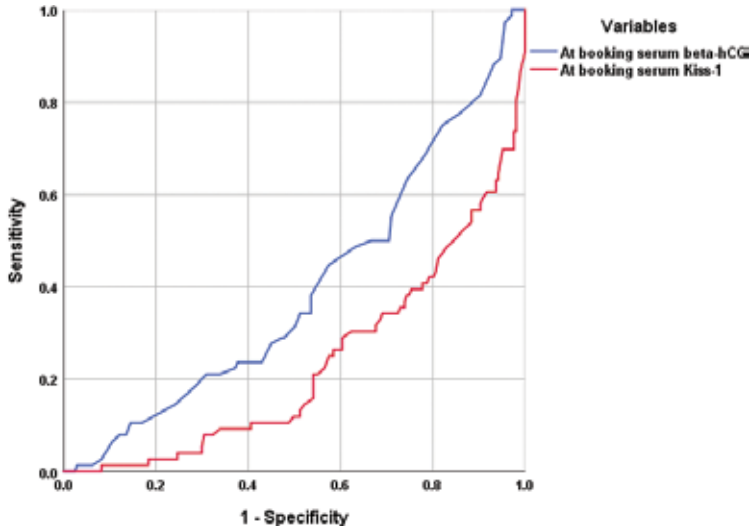

Fig. (6): ROC curve analysis of serum βhCG and Kiss-1 as predictors for getting EPL

Discussion

Control women showed significant increases of estimated serum levels of Kiss-1 in samples obtained at the 12th GW (S2 samples) in comparison to levels estimated in their S1 samples that were obtained at booking time. These data indicated source-related increases of Kiss-1 with progress of pregnancy and a possible relation between maintenance of pregnancy and serum Kiss-1 either as a factor for or as indictor of pregnancy progress and maintenance. In line with these findings, multiple previous in-vitro studies detected expression of Kiss gene in placental tissues of normal pregnancies [11,12,13] and with the afterward studies that assured placental source of plasma Kiss [14]. Moreover, the obtained results and the suggested assumption assured that previously detected by Hu et al., [15] who concluded that plasma Kiss levels significantly increase across pregnancy.

On the other side, serum Kiss-1 levels estimated in S1 sample of women of EPL group were significantly lower than that of S1 sample of control women. Considering the previously mentioned of the placental source of Kiss, these low serum levels of Kiss detected early on pregnancy diagnosis (Booking time) indicated defective placentation and was assured by the detected subsequent decreased Kiss levels. These results allow suggestion that estimated serum Kiss levels could be used as an early predictor for the possibility of oncoming pregnancy loss during the first trimester. In line with these findings, Sullivan-Pyke et al., [16] reported that serum Kiss levels differ between the pregnant and non-pregnant state and by viability, and Hu et al., [15]documented that plasma kiss levels could be used as a potential biomarker for the detection of miscarriage.

In support of the role of increased Kiss levels for maintenance of pregnancy, Bódis et al., [17] reported that in women undergoing IVF, serum kiss levels significantly increased in successful cases and regression analyses showed that these increases improved IVF outcome. Also, Rehman et al., [18]documented that increased levels of Kiss and estradiol in serum and Kiss in follicular fluid resulted in an optimum endometrial thickness, probability of fertilization of oocytes and chances of clinical pregnancy in ICSI cycles of unexplained infertile females. Recently, Qin et al., [19] found serum kiss levels estimated at the beginning of gonadotropin stimulation, 8-d thereafter and on the day of ovum pick-up in IVF/ICSI-treated infertile women were comparable in women had successful outcome, while in women had unsuccessful outcome serum Kiss levels on day of retrieval were significantly lower than levels estimated at the beginning of stimulation.

Statistical analyses detected positive significant correlation between serum levels of Kiss and βhCG and a negative significant correlation between S1 serum levels of both biomarkers and incidence of EPL. The ROC curve analysis defined both biomarkers as sensitive early predictors for EPL, but area under curve (AUC) difference was significant for Kiss. Moreover, detection of true cases at cutoff points determined by regression analysis was significantly higher with Kiss than with βhCG. These findings point to the superior predictability of serum levels of Kiss at booking time over that of βhCG and the possibility of dependence on single estimation of Kiss without the need for sequential estimations.

These findings support the previously reported by Jayasena et al., [20]who found Kiss had a higher diagnostic performance for miscarriage than hCG with significant AUC difference. Thereafter, Sullivan-Pyke et al., [16] detected that serum Kiss levels were positively associated with gestational age and hCG especially in spontaneous abortion and documented that stability of kisspeptin assay in serum and its potential clinical utility as a biomarker for early pregnancy viability. Recently, Abbara et al., [21] detected lower circulating Kiss and βhCG in samples from

women with miscarriages than in healthy pregnancies by 79% and 70% and the AUC for identifying 1st trimester miscarriage was 0.874 and 0.859, respectively, and detected improved performance of Kiss but worsened performance of βhCG in identifying miscarriage with increased length of gestation. Moreover, Qin et al., [19]found serum kiss estimated 8-d after gonadotropin stimulation and on retrieval day had positive correlations with serum E2 and hCG estimated at the same time and with the outcomes of IVF/ICSI treatment.

In line with the dependence on single estimation of Kiss early in pregnancy as predictor for EPL, Jayasena et al., [20]documented that single plasma kiss measurement during the booking visit could identify asymptomatic pregnant women at increased risk of miscarriage or EPL. Thereafter, Yu et al., [22]found single serum estimation of Kiss and βhCG concentrationswere correlated with different pregnancy outcomes and lower Kiss levels were detected in women who experienced biochemical pregnancy loss, but sequential measurements of serum Kiss levels are not effective in determining pregnancy outcome.

In trial to explain the relation between low serum Kiss and EPL, Wu et al., [23]detected a positive correlation between expression of Kiss and progesterone-induced blocking factor in syncytiotrophoblasts, cytotrophoblasts and deciduas and concluded that decreased kiss and progesterone-induced blocking factor are associated with recurrent spontaneous abortion. Also, Martino et al., [11]after in vitro proliferation of bovine primary placental cotyledon cell lines isolated at the 1st trimester detected involvement of the Kiss-1R/Kps system in the regulation of cell proliferation of bovine placenta but, it may not be involved in modulating placental progesterone secretion. Thereafter, Li et al., (24)using immunohistochemistry for detection of Kiss gene in placentas of women had EPL versus controls indicated that down-regulation of kisspeptin expression acts as an invasion-inhibitor gene with subsequent interference with normal homeostasis of trophoblast regulation, ultimately resulting in miscarriage.

Conclusion

Estimation of serum kisspeptin at time of pregnancy diagnosis correlates with pregnancy outcome concerning viability. Lower serum Kiss-1 at booking time had significantly higher predictibity for EPL than βhCG. Lower serum Kiss-1 at the 12th GW or development of EPL assured the predictivbility of the result obtained at booking time and spared the need for sequential estimations

Limitation

The small sample size limited the assurance of the suggested cutoff point for at booking serum Kiss-1 level as early predictor for EPL

Recommendations

Wider scale studies for assurance of the proposed cutoff point of serum Kiss-1 prior to defining its clinical utility.

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