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Acknowledgments

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

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

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

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Contents :

Letter from the Editor:

Dear esteemed colleagues,

Greetings

Very interesting subjects are included in this issue. The importance of an audit of hysterectomies is confirmed. The indication for hysterectomy should be carefully evaluated, so that the audit results can be used for improvement in the quality of health services. Another study concluded significant increase of caesarean section rates in the last few years which imply increased risks to the mother and neonate. The addition of prednisolone to clomiphene citrate (CC) in CC-resistant PCOS infertile lean patients was significantly associated with a higher ovulation rate and pregnancy rate. Maternal serum ferritin may be a useful test in the prediction of asymmetric IUGR. Poorer IVF clinical outcomes may be expected if unexplained infertility is associated with elevated inflammatory indices obtained from complete blood count, including white blood cell and platelet counts, neutrophil-to-lymphocyte-ratio. We welcome your comments.

Best regards.

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An audit of hysterectomies at Aswan University hospital in upperEgypt

Dr. Laila Ezzat

Abstract

Introduction: Hysterectomy is the removal of uterus and it is the commonest major surgical procedure performed in gynecology. It is the 2nd most common operative procedure performed on women in world after lower segment caesarean section(LSCS). Its incidence varies between 6.1 to 8.6 per 1000 women of all ages.

Like all other major surgical procedures it is not free of complications. There may be intra or post-operative complications. Rates of various complications vary from 0.05% to 43%.

Methods: This study involved all women who underwent hysterectomy at Department of Obstetrics and GynaecologyAswan University, Aswan, EgyptThis was a retrospective study and included all cases of hysterectomy from 1st Jan 2017 to 31st Dec 2017. Records from register and case sheets of patients were collected.

Results: A total of hysterectomies 53 were performed in the study period. Out of these 7(13.2%) were obstetrical and 46(86.79%) were gynaecological. Out of 46 gynaecological hysterectomies were 5(10.86%) were vaginal hysterectomy and 41(89.13%) were abdominal hysterectomy. Rupture uterus was most common cause represents 5 cases (71.42%) followed by abnormal placentation 2 cases (28.57%)

indications for abdominal hysterectomy were 27 cases (65.85%) underwent hysterectomy as a consequence of abnormal uterine bleeding (AUB), and among them the most common was leiomyoma (fibroid uterus) 18 (66.6%) . and postmenopausal bleeding represents 14 cases (34.14%).

Conclusion: Hysterectomy is a major surgery and it may be associated with complications during and after surgery. Therefore, the indication for hysterectomy should be carefully evaluated. Hence reporting of all hysterectomies should be made mandatory so that the audit results can be used for improvement in the quality of health services.

INTRODUCTION

Hysterectomy is the removal of uterus and it is the commonest major surgical procedure performed in gynaecology.(1)

Hysterectomy can be done by abdominal or vaginal route and with help of laparoscopy. Hysterectomy is the effective treatment option for many conditions like fibroid, abnormal uterine bleeding(AUB), endometriosis, adenomyosis, uterine prolapse(UP), pelvic inflammatory disease(PID) and in some cases ofmalignancies of genital tract.(2)

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It is the 2nd most common operative procedure performed on women in world after lower segment caesarean section(LSCS). Its incidence varies between 6.1 to 8.6 per 1000 women .(3)

In obstetrics it becomes a lifesaving procedure when all other methods fail to control post-partum haemorrhage(PPH).(4)

Like all other major surgical procedures it is not free of complications. There may be intra or post-operative complications. Rates of various complications vary from 0.05% to 43%.(5)

The complications may include risk of iatrogenic premature menopause, surgical and anaesthetic complications(6).

The aim of this audit is to evaluate the various types, indications and routes associated with all hysterectomies performed at tertiary care centre in upper Egypt at Aswan University hospital.

METHODS

This study involved all women who underwenthysterectomy at Department of Obstetrics and GynaecologyAswan University, Aswan, Egypt.

This was a retrospective study andincluded all cases of hysterectomy from 1st Jan 2017 to31st Dec 2017. Records from register and case sheets ofpatients were collected.

Case records were collected from medical recordsdepartment. Patients were identified by medical recordtracking.

There were no exclusion criteria. Patients were identified by medical record tracking. Data collected regarding age,parity socioeconomic status, clinical profile, chiefcomplaints, major medical history, any previousoperative history, indications, routes of hysterectomy, duration of hospital stay.

Baseline investigations including complete blood count , Blood sugar,urine complete examination, ECG, X ray chest, ultrasouned, viral markers were noted.

All cases of abdominalhysterectomies(AH) and vaginal hysterectomies(VH)were included.

- (AH) included total abdominalhysterectomy (TAH), total abdominal hysterectomy with unilateral salphingo oophorectomy (TAH,USO), total abdominal hysterectomy with bilateralsalphingo oophorectomy (TAH, BSO) andhysterectomy done as a part of staging laparotomy.
- (VH) included vaginal hysterectomywith pelvic floor repair (VH with PFR) for uterovaginal prolapse
- Vaginal hysterectomy without pelvic floor repair(VH) and non-descent (VH) done forindications other than uterovaginal prolapse(NDVH).
- Caesarean hysterectomy(CS H).

After collecting data variousindications were reviewed. Special emphasis was givenon indication of hysterectomy < 35 years of age. Maximum patients were discharged between 3rd and5thpost-operative day. The data was analyzed using percentages..

RESULTS

A total of hysterectomies 53 were performed in the study period. Out of these 7(13.2%) were obstetricaland 46(86.79%) were gynaecological. Out of 46gynaecologicalhysterectomies were 5(10.86%) werevaginal hysterectomy and41(89.13%)wereabdominal hysterectomy.

About Caesarean hysterectomy subjects were between age 25-34 years. As regard indications for Caesarean hysterectomy,Rupture uterus was most common cause represents 5 cases (71.42%) followed byabnormal placentation 2 cases (28.57%) .

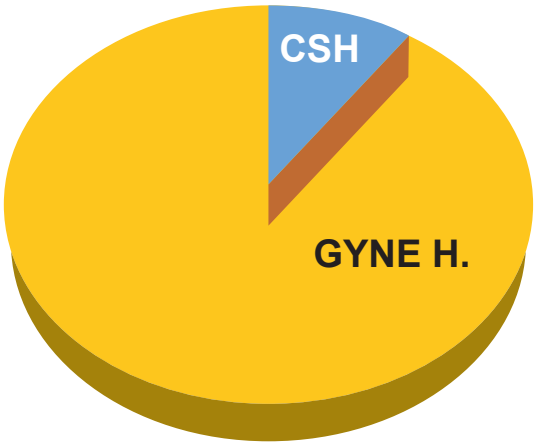
As regard gynaecologicalhysterectomiessubjects were between age 42-70 years and the most common type of hysterectomy is abdominal hysterectomy and the indications for abdominal hysterectomy were 27 cases (65.85%) underwent hysterectomy as a consequence of abnormal uterine bleeding (AUB), and among them the most common wasleiomyoma (fibroid uterus) 18 (66.6%) . and postmenopausal bleeding represents 14 cases (34.14%).

About the type of operation ofabdominal hysterectomy, the most common were total abdominal hysterectomy with bilateralsalphingo oophorectomy (TAH, BSO) 35 cases (85.36%) then total abdominal hysterectomy with unilateral salpingoooophorectomy (TAH,USO) 6 cases (14.63%), and Vaginal hysterectomy included vaginal hysterectomy with pelvic floor repair (VH with PFR) for

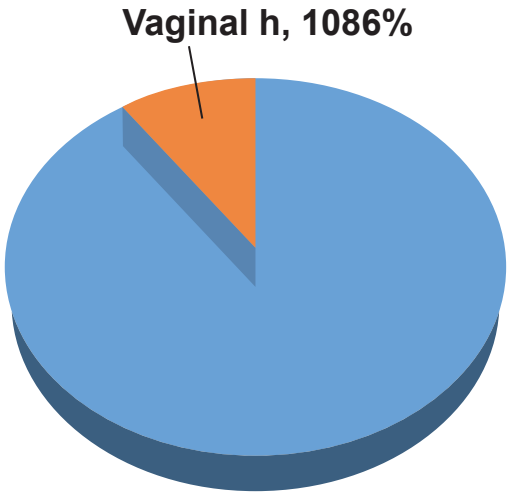
uterovaginalprolapse 5 Cases of total number of hysterectomies (10.86%).

Table 1: showing different types of hysterectomies.

Type	Number	Percentage
Caesarean hysTotal	7	13.2% of total number of hysterectomy
vaginal hysterectomy	5	10.86%
abdominal hysterectomy	41	89.13%
Total	53	



This chart showing different types of hysterectomies



This chart showing different types of gynaecological hysterectomies

Table 2: showing age wise distribution ofsubjects who had Obstetrical hysterectomy.

Age	Number	Percentage
25-29	3	42.85%
30-34	4	57.14%

Table 3: showing indications of Obstetrical hysterectomy.

Indication	Number	Percentage
Rupture uterus	5	71.42%
Abnormal placentation	2	28.57%
Total	7	100

Table 4: showing indications of abdominal hysterectomy.

Indication	Number	Percentage
abnormal uterine bleeding	27	65.85%
fibroid uterus	18	66.6% of cases of AUB
postmenopausal bleeding	14	34.14%

Table 5: showing types of abdominal hysterectomy.

Type	Number	Percentage
TAH, BSO	35	85.36%
TAH,USO	6	14.63%

DISCUSSION

Hysterectomy is the surgical removal of uterus. Hysterectomy is the most common operation performedby the gynecologist, and it is the 2nd most commonmajor surgical procedure after (CS) (7) (8).

Hysterectomy can be done using any of a variety oftechniques and approaches, including abdominal, vaginal,laparoscopic or robotic surgery(9).

Factors that may influence the route ofhysterectomy for benign causes include the size of thevagina, the size and shape of uterus; accessibility to theuterus; extent of extra uterine disease; the need forconcurrent procedures; surgeon training and experience; available hospital technology, devices and support;emergency or scheduled cases; and preference of thepatient (9).

There are variousindications of hysterectomy but when it comes toobstetrics it becomes a lifesaving procedure. In our study at Aswan university hospital 7 Obstetric hysterectomies were done in a span of 1 year. Age group was 25-34 years. This is in different with Vandanaetal(10) who found that the patient age group was 26-30 years. 35(33.3%) were P3, 31(29.6%) were P2 and one patient (0.9%) was P1 .

In our study most common indication forobstetric hysterectomies was rupture uterus(71.42%) followed byabnormal placentation (28.57%)patients were referred to our hospital as it's a tertiary care-hospital. Placenta accrete and percreta led to obstetric hysterectomy in (28.57%) patients because of uncontrolled haemorrhage, this is in agree with Vandanaetal(10)who found that Most common indication was rupture uterus (40%)followed by abnormal placentation (38.1%).

In our study , morbidly adherentplacenta was the second most common indication forEOH (Emergency Obstetric Hysterectomy). This wasalso the case in Turkey and the UK contributing to 40% and 38% of cases, respectively(10).

In this study46gynaecological hysterectomies were done during the study period of one year. Age of the patient studied inthis particular study ranged from 42 years to 70 years. This is in comparison with Vanithamanietal.(6) who found in his study 198 hysterectomies were done during thestudy period of one year. Age of the patient studied ranged from 32 years to 75 years.Most common age group was 41-50 years. Similar agegroup was observed in other studies conducted bySaravana A et al (2), Perveen S et al (11), Medhi P et al(12), DomblaeV et al (13), Patil H et al(14), Sharma C et al(15).

In this study Most common type of hysterectomy done was total abdominal hysterectomy with bilateral salphingooophorectomy

This is in agree with Vanithamanietal.(6) who found in his study most common type of hysterectomy done was total abdominal hysterectomy with bilateral salphingooophorectomy. Similar observation was made in studies conducted by Verma D et al (1), Patil H et al(14), Sharma C etal (15).

In this study most common indication was abnormal uterine bleeding,next common indication was leiomyoma.This is in agree with Vanithamanietal.(6) This is comparable to studies conducted by Perveen S et al(11) and Sharma C et al(15).

CONCLUSION

In primi patient indicationof (CS) should be very clearand justified to avoid further LSCS and development ofplacenta accrete and percreta and reduses the number of obstetric hysterectomies. We should sensitize thegeneral population regarding the long-term complications of Caesarean Delivery on Maternal Request to reduce the number of LSCS.

Hysterectomy is a major surgery and it may be associated with complicationsduring and after surgery. Therefore, the indication forhysterectomy should be carefully evaluated. Hencereporting of all hysterectomies should be mademandatory so that the audit results can be used forimprovement in the quality of health services.

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Increased Rates of Caesarean Delivery at Mansoura University Hospital; an infuriating concern concluded from a prospective observational study

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Abstract

Background: Caesarean section (CS) rates increased nowadays all over the world. This increase raises the concern of un-necessary CS that increases maternal and fetal morbidity and mortality.

Objective: This study aimed to assess the rates, the indications of caesarean delivery and to find out why the rates are continuously increasing at a tertiary health care hospital in Egypt.

Methods: A prospective observational design was selected for this research. A cohort of 750 hospital deliveries were prospectively followed up intra-partum in the obstetric unit of Mansoura university hospital (MUH).Cases' history, labor anddelivery events were prospectively recorded bythe study team who just observed provided no intervention.

Results: an overall CS rate of 65.2% (489/750) was recorded in this cohort, vaginal delivery in the remaining (34.8%). Most CS were antepartum 59.1% (289/489) vs. (40.9%) intra-partum and those done intra-partum were mostly in the latent phase 78% (156/200) vs. (22%) in the active phase .This high CS rate (65.2%) has significantly exceeded the previous CS rate published from the same unit in 2013 47.25% (16348/34598). Compared with the previous study published from the same unit the CS rate increased significantly: odds ratio & 95% CI is 2.092 (1.797 to 2.434). Relative Risk & 95% CI is 1.380 (1.308 to 1.456, P<0.0001). Although we found no significant differences in maternal and neonatal morbidity and mortality between vaginal and CS deliveries in this study (probably due to small sized cohort) .The risks of maternal and neonatal morbidity has been emphasized in larger studies.

Conclusion: The study concluded that CS rates in our hospital has significantly increased in the last few years which implies increased risks to the mother and neonate and also imply a burden on the forecoming pregnancy(ies).

Key words: Cesarean section, rates, Mansoura university hospital.

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Introduction

Caesarean section(CS) is a surgical procedure in which fetus, placenta and membranes delivered throughoutan abdominal and uterine incision **(BasketteteaL.,2007)**. The first modern CSwas performed by German gynecologist Ferdinand Adolf Kehrer in 1881 **(BasketteteaL.,2007)**. Historically, this surgery has always been performed to save the baby rather than the mother; but indeed when it

is adequately indicated, it can prevent poor obstetric outcomes and be a life-saving procedures for both the mother and her fetus. However, at a time when the caesarean delivery rate; as a percentage of live births, is poved to be rising globally there is a growing concern about un-necessary caesarean sections and attention shuld be made **(SouzaJP & Gülmezoglu A 2010)**. Un-necessary CS rates already increase the risk of maternal morbidity, maternal mortality, neonatal admission to an intensive care unit, and even neonatal deaths **(Villar J, 2006)**. There is no consensus on the "optimal" rate of caesarean delivery at the population level although values between 5% and 15% of live births have been suggested **(Aelvoet et al., 2008)**. The basis on which these thresholds have been proposed is not clear despite the World Health Organization (WHO) has suggested that a caesarean delivery rate of 15% should be taken as a threshold that should not be exceeded, rather than a target to be achieved **(Aelvoet et al., 2008)**. Little research exists on trends of caesarean section delivery for any country in the Arab world. A descriptive study in Egypt carried out by **(Khawaja, et al., 2004)** supported the view that a significant risein caesarean deliveries occurred for all births, from alow of 4.6% in1992 to10.3% in 2000. Although the caesarean section rate was slightly higher in private hospitals, the rate also increased consistently in public hospitals. Also the high and exceptional increase in caesarean section rates may be partly resorted to non medical indications of CS suggesting physician practice patterns, financial incentives or other profitability factors, and patient preferences. This study aimed to assess the rates and the indications of caesarean delivery and to find out why the rate is continuously increasing at a tertiary health care hospital compared with previous years.

Patients & Methods

A prospective observational design was selected for this research. Such design fits the nature of the study under investigations, in which we assess the rates,the indications of caesarean delivery and to find out why the rateis continuously increasing at atertiary health care hospital at Mansoura Univeresity, Egypt.The hospitals are considered as tertiary care center that serves the population inthe middle of Delta, Egypt. The study was approved by the University Ethics Committee and the Institutional Research Ethical Committee of the concerned hospital. Permissions were granted from the head of the obstetrics & gynecology department as well as the Director of Mansoura University Hospitals. The objectives of the study were explained to the study subjects and their verbal and written consents were obtained from all. Patientsin the study were observed during the period from October 2015 to October 2016 and data were recorded. No intervention would be under taken, only data collection including personal data as name, age, menstrual history last menstrual period (LMP) and expected date of delivery, obstetrical history as parity, prior vaginal births, as well as full details about the previous CS, past medical history, family history, present history. A special sheet was settled by the researcher to collect the previous data and also for that belonging to the findings of the general, abdominal, local and per vaginal examinations of the participants. This was necessary to obtain the diagnosis of labor and any problem that might be encountered on woman admission to the Labor and DeliveryUnit.The partograph should theoretically be used for every participant to record data about fetal condition, labor progress "cervical dilatation and fetal descent", uterine contractions as well as the drugs and intravenous (IV) fluidsgiven and the maternal condition; vital signs and the results of laboratoryinvestigations performed. Evaluation of the neonates was achieved by neonatologists after delivery, who decided if neonates well or needed Neonatal Intensive Care Unit (NICU). Then; rates of CS, indications, spontaneous and assisted vaginal deliveries were recorded as well as neonatal fetal conditions. These are compared with national and international standards.

Statistical analysis

A prospective observational design was selected Data were analyzed with SPSS version 21. The normality of data was first tested with one-sample Kolmogorov Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-squaretest. Continuous variables were presented as mean±SD (standard deviation) for parametric data and Median for non-parametric data. The two groups were compared with Student t test (para-

metricdata) and Mann–Whitney test (non parametric data). For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value). sgnificant when the probability of error is more than 5% ($p > 0.05$). Significant when the probability of erroris less than 5% ($p < 0.05$). Highlysignificant when the probability of error is less than 0.1% ($p < 0.001$). The smaller thep-value obtained, the more significant are the results.

Results

Table (1) sho w that the mean age of CS was significant older in CS group, and the CS rate was higher among older than younger population and the same with gravidity also. Gravidity is higher among CS population. Gestational age tends to be lower in CS, preterm delivery also is associated with high CS ($p \leq 0.001$ in both). Degree of parity show no significant difference in either groups (p 0.104).

In table (2) method and time of delivery in those delivered by CS were estimated. It shows that overall CS rate 65.2%, of which 59.1% was performed in the ante-partum period and 40.9% was emergent CS, 78% was during the latent phase of the first stage, and 22% during the active phase. No CS cases were performed during the second stage of labor. Vaginal delivery rate was 34.8%, 81.2% was spontaneous delivery, 10.3 was induced and 8.5% was vaginal birth after casaerean section (VBAC).

Table (3) is setteled to compare between overall CS rates from same obstetric unit in Mansoura in 2 different dates, our study and Helal et al (2013); our study showed very high and percentage of CS rate compared by the previous one despite our cohort involved lower number of patients (489 "65.2%" vs 16347 "47.25%" respectively and $p < 0.0001$).

Table (4) shows the indications of CS. It reveals that repeat CS was the commonest indication, which represented 62.4%, followed by medical disorders affecting the mother 13.3%. Mal presentations 10.6%, placenta praevia 6.3% , multiple pregnancy 3.9%, Cephalo-plevic disporportion "CPD" 5.3%, antepartum haemorrhage "APH" 2.45%,and failure to progress (2.04%) and finally unreported causes which represented 5.7%.

Table (5) showed again a significant difference between our study and the previous one by Helal etal 2013 as regard to repeat CS rate, CS for antepartum hemorrhage and CS for failure to progress ($p<0.0001$).

Regarding the neonatal outcome between vaginal and caesarean delivery, our data showed that there is no significant difference (p 0.76) as evidenced in table [6].

Discussion

Over the last years, there has been rapid increase in CS rates all over world. Wide variations are present among countries, regions and even hospitals within the same area and with similar socioeconomic status and patient characteristics. This suggests that there are no sharp rules to do CS, with a consequent overuse of this surgical obstetric intervention **(Aelvoet et al., 2008)**. Egypt,being a part of the world, is affected by thisand a significant rise in CS rates is observed and recorded in recent years.(Khawaja et al.,2004). Lastly, the Egyptien Demographic and Health Survey (EDHS) 2008 showed that more than 25% of deliveries in the five-year period before that survey were by CS and about 37% percent of urban births were CS compared to 22% of rural births **(El-Zanaty and Associates, 2009)**. Our present hospital based cohort study showed even higher rate of CS compared to many published data reporting CS rates ranging between 24 and 38 % **(Dobson 2001, Khawaja, et al.2004 , Helal etal, 2013)**. This increase in CS rate is even higher than the increase in CS rates allover world & even higher than CS rate (47.25%) reported from the same institute in a previous study **(Helal et al, 2013)** (table 3). However Helal et al (2013) study was retrospective, and missing data were significant defect in the records which were the source of that study. Our study, which is prospective observational cohort, revealed higher rates of CS in older, higher gravidity females and in those with preterm babies (table 1). This comes in agreement with some previous studies **(Gomes ,et al.1999, Freitas et al.2005, Baskett et al 2007).**

The significantly higher gravidity and not parity among CS deliveries compared to vaginal deliveries in our study refers to the probability of higher

early pregnancy losses among CS deliveries which renders these fetuses (precious babies) a common label for CS delivery. The higher ratio of preterm deliveries can be explained by the indications of CS deliveries (antepartum haemorrhage due to placenta previa, hypertensive disorders, diabetes mellitus all of which are significantly associated with preterm delivery). In this study 59.1 % of CS cases were done ante-partum and the remainder (40.9%) were done intra-partum (table 2) .

So most of CS cases had been ante-partum. This high percentage of ante-partum CS may be explained by the fact that Mansoura University hospital is a tertiary care center in Egypt delta serving large area of population and receiving referrals from private and publichospitals . These referrals are mostly high risk cases that needed intervention before the onset of labor. On the other hand 40.9% of CS were intra partum, in the first stage of labor. Since repeated CS comprise 62.4% of the overall indications of CS in our cohort (table 2) it is logic that ante-partum CS well be higher than intra-partum ones. All intra-partum CS in this cohort were done in the first stage mainly in the latent phase (table 2) . This comes in accordance to results of **Zhang, et al. (2010)** reporting that 53% of inductions were terminated by CS due to failure of progress very early in labour (before 5 cm cervical dilation). That the minority of CS deliveries were intra-partum and also early in labour was reported by **Boyle Reddy et al; (2013)**; who found that only 1 in 3 (35%) of cases of CS had been done due to failure of labor progress very early in labor.

We can speculate that most of the intra-partum CS performed in this study were performed for doctor convenience not in the patient or fetus interests. The decision of CS is initiated by an over worked resident who wants to finish his shift with minimum effort and minimum complications. A notification about (failed of progress) is an accepted label to gain approval on CS via phone call with the senior staff on duty.

We failed to document intra-partum monitoring data to document "protracted cervical dilation". Defensive obstetrics has now become a common reason for high rates of CS. It has been observed that 82% of physicians performed CS to avoid negligence claims **(Birchard K.1999)**. This is closely related to daylight obstetrics for the obstetrician's convenience. It takes usually 20-30 minutes to perform a CS while conducting a vaginal birth may need 12 hours or more heavily taxing on the obstetrician's time and patience. Litrop et al (2015) reported in a semi-structured individual in-depth interview study that residents often missed support from their senior colleagues when making decisions, and felt that midwives pushed them to perform CS. Many care givers stated that their fear of blame from colleagues and management in case of poor outcomes made them advocate for, or perform CS on doubtful indications.

In our study; no CS had been done in the second stage of labor.This disagrees with **Gifford DS, et al.(2000)** who found that one-fourth of the primary CS were performed in the second stage of the labor . This difference may be partially explained by the fact that most cases in our study were high risk cases that cannot sustain prolonged trial of vaginal delivery. Also our data showed tha VBAC comprises 8.5% of vaginal births (table 3) and this rate does not much differ from the figure reported by **Mc Dorman, et al 2011**. It is clear that even Mansoura University Hospitals was unable to adopt strategy of VBAC although it has more facilities, more knowledge and provide more observational care than many other institutes as most of patients are unable to provide records for description of their previous CS.This may be the main explanation why VBAC is not strongly recommended in our hospital. So, the author's openion is that; reduction of primary CS rate should be our main strategy to reduce repeat CS.

Looking to the commonest indication of CS we reported repeat CS(62.4%) whilest failure to progress was the lowest one (2.04%) as listed in table (4). This coincides with some other study **(Baskett et al 2007)** who considered 4 major indications accounting for more than 90% of indications of CS including previous CS, dystocia, fetal distress and lastly breech. In our study, there are no cases reported to be fetal distress because all intra-partum CS were done early in labor under the label of failure to progress. This verifies to our previous observation that most intra-partum CS were done for system convenience. The use of electronic fetal monitoring had been employed in 85% of labors in united states **(Martin et al, 2003)** and was implicated in the increased the rate of CS to as much as

40%, a method which is not adopted in our hospital and agin this may partially explain absence of acute fetal distress as an indication for intra- partum CS. Medical disorders are the next common indication of CS in our study (13.3 %.). This does not agree with **Baskett, 2007** who reported that medical disorders are not common indications for CS. Our explanation is that medical disordersare commonly reffered to our center,being a tertiary care hospital, from private clinics and general hospitals. Malpresentations comes as the third most common indication (10.6%) in our cases; table (4) and this approves the openion taken by (Young Johanson, 2001 and Levry et al,2005) who stated malpresentation, especially breech, in frequently meets criteria of a trial of vaginal delivery and be risky for the mother and baby, so it is an accepted indication for CS when facilities exist. Our results also documented that APH accounts for 6.3% (table 4) of the indications, which is a well known indication except in the very minor degree conditions as previously stated by **(Lukas et al, 2000)**. Cephalopelvic disproportion represents 5.3% of cases (table 4) and it is one of the undeniable indications of CS according to **Baskett et al (2007)**.

Comparing our study to the previous one in the same unit **Helal et al (2013)**; there is a significant difference as regard to increased ratio of repeat CS and CS for antepartum hemorrhage and less use of trial of vaginal delivery as attested to by significantly lower proportion of CS for failure of progress of labor in the current study table (5). Again, the decreasing rate of VBAC may be due to lack of experience of in new generations, less monitoring facilities, fearing of complications.

In our study there is no significant difference ($p > .05$, table 6) as regard to neonatal outcome between vaginal and CS delivery. There is general consensus that CS is associated with less risk of neonatal morbidity and this in many instances influence the choice of CS despite the associated maternal risks **(Cunnigham et al 2014)**.The relatively small sample size of our cohort may be insufficient to illicit differences in neonatal morbidity between CS and vaginal delivery groups. Some long term follow-up studies of infants have linked CS with some problems, for example, Josef Neu and **Jona Rushing, (2011)** found increased risk of bacterial colonization and repeat gastroenteritis as well as immune system troubles in CS than vaginal deliveries. Moreover; **Debbey et al (2005)** found increased childhood asthma for babies delivered by CS meanwhile Renz-Polster, et al 2005 found increased risk of allergic disorders in childhood for babies delivered by CS.

In our study we had only clinical data about very early neonatal status of the fetus. We had no data about later neonatal, infant development, or their microbiological culture, so we can not compare our cases with other studies in this respect.

This study revealed no cases of maternal morbidity or mortality after caesarean or vaginal delivery, this disagrees with other studies **(Sally C. Curtin, et al 2013)** which revealed that maternal morbidity was higher for Cesarean than vaginal deliveries. **Cunnham etal 2014**; reported maternal complications associated with low- risk planned CS compared with the planned vaginal deliveries among healthy women in Canada. The results showed that casarean delivery was associated with significantly higher complications than vaginal typeas regard, hysterectomy, anesthesia, cardiac arrest, venous thromboembolism, puerperal infection, wound disruption, wound hematoma. The same report showed however that vaginal delivery group had higher transfusions and higher maternal mortality compared to CS group. Absence of maternal morbidity & mortality in our study may be explained by the fact that most of our casarean deliveries were ante-partum (elective) with every thing (personnel and others) prepared and ready from the start to deal with the possible complications. Our patients did not reveal early or late maternal morbidity as the vaginal deliveries, as the the rule is discharging the case from the emergency unit within hours. The same thing occurs with low risk CS which are also discharged next morning. Definitely such complications do exist but are probably under reported or dealt with elsewhere; may be at a private or outpatient clinics.

Conclusions

There is an international and national rise in the rates of CS and our study witnessed significant increase in CSrate (47.25% in 2013) to (65% in 2016). Most of the operations were done ante partum (60%) while the remainders (40%) were intra

partum early in labor. Repeat CS rate is continuously increasing and efforts should be expended to incarse VBAC or decrase primary section in our unit.

Conflict of interest

Authors stated that there is no conflict of interest.

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Results

Table (1): Patient features in CS vs. Vaginal delivery groups:

Items	Cesarean section group (n=489)		Vaginal delivery group (n=261)		Test of sig.	p-value
	No	%	No	%		
Age	27.67±5.32		26.75±4.16		t=2.42	0.016*
<35y	451	92.2	258	98.9	X^2=14.43	≤0.001**
≥35y	38	7.8	3	1.1		
Gravidity	2.69±1.53 2(1-11)		2.31±1.23 2(1-9)		Z= 2.47	0.013*
≤3	381	77.9	223	85.4	X^2= 6.14	0.013*
>3	108	22.1	38	14.6		
Parity	1.42±1.16 1(0 -7)		1.19±1.11 1(0-6)		Z=1.62	0.104
≤3	721	96.1	255	97.7	X^2= 2.64	0.104
>3	29	3.9	6	2.3		
G.A	37.77±2.03		38.06±1.91		t=1.92	0.055
≤37w	151	30.9	54	20.7	X^2= 8.89	≤0.001**
>37w	338	69.1	207	79.3		

ZforMann–Whitney test: ***significance≤0.05** ****high significance ≤0.001**
Data presented as mean (SD), $p < 0.05$ is considered significant.
Abbreviations: GA; gestational age.

Table (2): Method of delivery in the study cohort:

Method of delivery	Study cohort (n=750)	
	No	%
Cesarean section	489	65.2
Ante-partum	289	59.1
Intra-partum	200	40.9
First Stage	156	78%
Latent phase	44	22%
Active phase	No cases	
Second stage		
Vaginal delivery	261	34.8
spontaneous	212	81.2
induced	27	10.3
VBAC	22	8.5

Data presented as number (%), $p < 0.05$ is considered significant.
Abbreviations: VBAC; vaginal birth after cesarean delivery.

Table (3): Comparison between overall CS rates from same obstetric unit in Mansoura in 2 different dates:

	Current study (n = 750)	**Helal et al (2013) (n =34598)**	**P value**
Cesarean Section	489 (65.2%)	16347 (47.25%).	*P<0.0001 OR ,95% CI 1.67 (1.44 -1.95)
Vaginal delivery	261 (34.8%)	18251 (52.75%)	

*Fisher exact test
Data presented as mean (SD), $p < 0.05$ is considered significant.

Table (3): Indications of CS:

Indications of CS	**Study cohort (n=750)**	
	No	%
Repeated cs	305	62.4
Medical diseases	65	13.3
Malpresentation	52	10.6
Placenta previa	31	6.3
Multiple pregnancy	19	3.9
Cephalo- Plevic disporportion (CPD)	26	5.3
Other APH	12	2.45
Failure to progress	10	2.04
Unreported indication	43	5.7

Data presented as number (%), $p < 0.05$ is considered significant.
Abbreviations: CS; Caesarean Section; CPD, cephalo pelvic disproportion

Table (5): Comparison between CS indications in 2 different dates in the same obstetric unit in Mansoura:

	Current study (n = 489)		Helal et al (2013) (n = 16348)		P value*
	No	%	No	%	
Repeated CS	305	52.1	5849	35.78	<0.0001
Medical diseases	65	13.3	2330	14.25	0.549
Malpresentation	47	9.6	1618	9.9.0	0.891
Placenta previa	31	6.3	420	2.57	<0.0001
Multiple pregnancy	19	3.9	893	5.46	0.129
Other APH	12	2.45	239	1.46	0.074
Failure to progress	10	2.04	1696	10.4	P<0.0001
Other unreported	0		3303	20.2	

*Fisher exact test
Data presented as number (%), $p < 0.05$ is considered significant.
Abbreviations: CS; Cesarean Section; APH; antepartum hemorrhage

Table (6): Comparison between CS and VD regarding Neonatal Outcome:

Items	Cesarean Section		Vaginal delivery		Test of sig.	p-value
	No	%	No	%		
well	442	89.3	233	89.3	X^2= 0.53	0.76
NICU	52	10.5	28	10.7		
dead	1	0.2	0	0		

Data presented as number (%), $p < 0.05$ is considered significant.
Abbreviations: CS; Caesarean Section, VD; vaginal delivery, NICU; neonatal intensive care unite

Adding Prednisolone During Ovulation Induction with Clomiphene Citrate in Lean Women with Clomiphene Citrate Resistant Polycystic Ovarian Syndrome

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Abstract

Background: polycystic ovarian syndrome (PCOS) is a common cause of chronic anovulation; insulin resistance is considered an accepted mechanism for anovulation in PCOS especially in obese patients. Excess adrenal androgens are observed in patients with PCOS. An inverse relationship exists betweenDehydroepiandrosterone sulfate (DHEAS) the body mass index (BMI). The use of corticosteroids could improve ovulation in PCOS by decreasing serum androgen level.

Objective: Evaluation of the efficacy of concomitant administrationof prednisolone and clomiphene citrate (CC) for the ovulation inductionin infertile lean women having CC-resistant polycystic ovarian syndrome(PCOS).

Methods: Three hundred infertile lean women with clomiphene citrate (CC) resistant PCOS were randomly divided into two groups. Group 1:150 patient received clomiphene citrate (5 consecutive days of 150mg daily starting from the second day of the cycle) and prednisolone tablet (10 consecutive days of 10mg daily starting from the second day of the cycle). Group 2:150 patientreceived the same protocol of CC plus placebo (10 consecutive days of 0.5mg folic acid daily starting from the second day of the cycle). All patientsshowed clinical manifestations of Hyperandrogenism (variable degree of hirsutism and/or acne) where 18 patients were dropped out, and data on all relevant outcomes were available for 282 women and data were analyzed from 143 women in the CC-Prednisolone group and 139 in the CC-placebo group. The main outcome was the ovulation rate. Secondary measures included a number of follicles 18 mm or more, endometrial thickness on day of HCG administration and clinical pregnancy rate. Ovarian follicular response was monitored by transvaginal ultrasound and mid-luteal phase serum progesterone. HCG 10000 IU was given when at least one follicle measured 18 mm, and timed intercoursewas advised.

Results: There were no statistically significant differences between groups as regards age, duration of infertility, BMI,the serum level of FSH, LH, TSH,and prolactin. The mediannumber offollicles ≥18 mm) at the time of HCG administration and the mean endometrial thickness wassignificantly higher in the prednisolone group than in the placebo group ($P < 0.001$).Similarly, there were significantly higher rates of ovulation (58.7% versus 23%) ($P < 0.001$) in the prednisolone group. The clinical pregnancy rate was significantly higher in the prednisolone group ($p = 0.006$).

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Conclusion: The addition of prednisolone to CC in CC-resistant PCOS infertilelean patients was significantlyassociated with a higher ovulation rate, number of ovarian follicles ≥18 mm and endometrial thicknesson day of HCG administration, and number of patients becameclinical pregnant.

Keywords: PCOS, CC-resistant, prednisolone, ovulation rate,and clinical pregnancy rate

Introduction

Chronic anovulation due to PCOS can be treated with CC as a first line of the treatment (1). However, resistance to CC, defined as ovulation failure after receiving of CC daily for 5 days in a dose of 150mg daily per cycle for at least 3 cycles, which may be occurred in 15 - 40% in women with PCOS. (2).Overweight and hyperandrogenism are the major factors involved in CC resistance (3).Hyperandrogenism adversely affects female fertility asthe increase in androgen concentrations will interfere with the development of ovarian folliclesthrough FSHaction down-regulationon the granulosa cells (3). It was reported that the ovarieswere considered to be the main source of patients with PCOS androgen excess,but also the increase in adrenal androgen levels have been observed in those patients (4). The adrenal glands have been reported to be the main source of dehydroepiandrosterone sulfate (DHEAS)(5), which was found to be high in 22–25% of PCOS patients (6). Kumar et al. (7) and Moran et al. (8) have been postulated that a negative co-relation between DHEAS and BMI or fasting insulin among PCOS patients, so the proportion of adrenal androgen excess may be higher in nonobese PCOS patients.Jones et al, 1953, is considered the first study refers to the using of corticosteroid in ovulation dysfunction treatment(9). The improvement in ovulation with corticosteroid treatment through its decreasing effect of androgens of the adrenal source on folliculardevelopment (10,11).

Patients and Methods

Thiswasa prospective placebo-controlled study that was conducted in Mansoura University Hospital(infertility care unit) andoutpatient's clinic fromFebruary 2015 to December2018. The study was approved by the local Institutional Research Ethical Committee "institutional research board". 384 infertile patients enrolled in our study,with PCOS as defined by the Rotterdam criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) (12), all of them were diagnosed as CC resistance (defined as failure of ovulation after receiving 150 mg/day of CC for 5 consecutive days per cycle, for at least 3 cycles),and showed clinicalhyperandrogenism (variable degree of hirsutism and/or acne), 84 patients were excluded (70 did not meet inclusion criteria, 8 were declined to participate, and 6 due to other reasons)(Figure 1). Our inclusion criteria were: 1) agebetween 20 and 35 years. 2) body mass index between 18.5 and 25. 3) normal serum Prolactin and TSH.Exclusion criteria included patients aged < 20 or > 35 years, body mass index (BMI) < 18.5 kg/m2 or > 25 kg/m2, presence of any infertility factor other than anovulatory PCOS, previous history of ovarian surgery or surgical removal of one ovary, previous exposure to cytotoxic drugs or pelvic irradiation, oral hypoglycemic drugs or hormonal therapy either currently or in the preceding 3 months, and metabolic or hormonal abnormalities. All patients underwent history taking, examination, basic laboratory investigations, and hormonal profile (Follicular stimulating hormone (FSH), luteinizing hormone (LH), prolactin, and (TSH) thyroid stimulating hormone. The remaining 300patients were selected randomly to receive CC and either prednisolone or placebo using closed dark envelopes, so patients classifiedinto two groups; 150 patients in each one. Women in the CC-Prednisolone group **(group 1)** received CC (5 consecutive days of 150mg daily starting from the second day of the cycle) and prednisolone tablet (10 consecutive days of 10mg daily starting from the second day of the cycle), women in the CC-placebo group **(group 2)** received the same protocol of CC plus placebo (10 consecutive days of 0.5mg folic acid daily starting from the second day of the cycle).Transvaginal sonographic (TVS) folliculometrywas performed regularly starting from day 10 of the stimulation and repeated every 2-3 days. When there was at least one follicle ≥18 mm in diameter, final oocyte maturation was induced by intramuscular administration of 10000 IU of human chorionic gonadotropin (HCG) and timed intercourse was advised. If there was no follicle ≥ 12 mm by day 16 of the cycle, monitoring of follicular growth was discontinued and the

cycle was presumed to be anovulatory. Ovulation was documented by TVS scan one week after triggering of oocyte maturation and was confirmed by assessing the mid-luteal serum progesterone level. Each woman was subjected to ovarian stimulation for a maximum of 3 consecutive cycles except if she became pregnant in the first or second cycle. The data collected to evaluate the effect of concomitant prednisolone administration with CCfor ovulation induction through registration of ovulation rate, number of ovarian follicles 18 mm or more and endometrial thickness on HCG administration day, and clinical pregnancy rate throughout 3 months follow up period.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Quantitative data were described using median (minimum and maximum) for nonparametric data and mean, the standard deviation for parametric data after testing normality using Kolmogorov-Smirnov test. The significance of the obtained results was judged at the 0.05 level and all tests were 2 tailed. Chi-square test and Fischer exact test were used for categorical variables, to compare between different groups as appropriate. Student-t-test was used for parametric quantitative variables, to compare between two studied and Mann Whitney test was used for nonparametric quantitative variables, to compare between two studied groups.

RESULTS:

The study was carried out on 300 patients diagnosed as clomiphenecitrate-resistantPCOS, classified into two groups;CC-Prednisolone group and CC-placebo group (150 in each group), 7 women in the CC-Prednisolone group were lost to follow-updue to various reasons and 11 women in the CC-placebo groupwere dropped out, 8 of them due to difficult to follow up and 3 did not receive the allocated intervention. Data on all relevant outcomes were available for 282 women and data were analyzed from 143 women in the CC-Prednisolone group and 139 in the CC-placebo group (Figure 1). The study revealed that patient's mean age was 26.47±3.7 in CC-Prednisolone group and 26.97±4.1 for CC-placebo group. The mean BMI was 22.49±1.76 in CC-Prednisolone group and 22.53±1.77 in CC-placebo group with no statistically significant difference asregards demographic data between two groups as shown in table1. The data from table1 showed that there was no statistically significant difference in infertility duration and hormonal profile between the studied groups, whereas, the median follicle number >18 mm or morewas 2.0(0.0-5.0) in CC-Prednisolone group and 0(0.0-2.0) in CC-placebo group, in addition, the endometrial thickness median was 9.69±2.22 in CC-Prednisolone group and 8.01±1.7 in CC-placebo group with a highly statistically significant difference between CC-Prednisolone group and CC-placebo group as shown in table2.Regards the ovulation incidence was 84(58.7%) in CC-Prednisolone group, and 32(23.0%) in CC-placebo group, while the clinical pregnancy was 26(18.2%) in CC-Prednisolone group, and 10(7.2%) in CC-placebo group with a highly statistically significant difference between CC-Prednisolone group and CC-placebo group. There was no statistically significant difference between two groups in the occurrence of ovarian hyperstimulation syndrome as shown in table 2.

Discussion:

Resistance to clomiphene citrate (CC) during ovulation induction for PCOS patients is attributed to overweight and Hyperandrogenism (3).Although the ovaries are the main source of androgen excess in PCOS, excess adrenal androgen levels have also been observed in PCOS patients(4). Circulating adrenal androgens are converted into testosterone in the ovarian follicles. It was reported that circulating DHEA-Which is exclusively secreted by the adrenal glandacts as 48% of the testosterone precursor found in follicular fluid(13).High intraovarian androgens concentration interferes with ovulation as it impairs the selection of the dominant follicle (14). An inverse relationship between DHEAS and BMI or fasting insulin among PCOS patients has been reported by Kumar et al.(7) and Moran et al.(8), so the proportion of adrenal androgen excess may be higher in nonobese PCOS patients.In our study,the prednisolone groupwas associated with a significantly higher ovulation rate. In addition, the prednisolone groupwas associated with a significantly higher number of

follicles 18 mm or more at the time of HCG administration than in the placebo group. These results are agreed with Isaacs et al(11) and Reyes et al(15) studies. There are suggested mechanisms by which prednisolone could improve the ovarian function. Glucocorticoids decrease circulating adrenal androgens by nearly 40 % (16). The ovulation improvement can be achieved bydecreasing the effect of androgens of the adrenal source on follicular development (10,11). The endometrial thickness mean was significantly higher in the prednisolone group than in the placebo group. The adverse effect of CC on the endometrium is attributed to its relatively long half-life (5 days), which is not seen with prednisolone. The endometrial thicknessmean for prednisolone group was 9.65mm versus 7.9 mm for the placebo group. Casterlin et al. (17) demonstratedthat 7–11 mm endometrial thickness is the suitable thickness for the occurrence of pregnancy. The clinical pregnancy rate was significantly higher in the prednisolone group when compared to the placebo group. This may be attributed to higher ovulation rate and better endometrial thickness in the prednisolone group. This finding agreed with studies performed by Isaacs et al (11)and Reyes et al(15).The findings of our study are similar to those reported by other studies as Elnashar A et al. 2006(18), Parsanezhad ME et al. 2002(19), and Daly DC et al. 1984(20)through using dexamethasone in addition to clomiphene citrate. Prednisolones was recommended by Isaacs et al (11) because when compared with dexamethasone it has the advantage of shorter pharmacologic duration and less potency so can suppress adrenal androgens and not complete adrenal suppression

Conclusion

Prednisolone combined with CC for management of CC-resistant PCOS is economic and effective. It provides safety advantages when compared to other alternatives as gonadotropin therapy and laparoscopy and thus should be tried first.

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Table (1): Demographic characteristics and hormonal profile between studied groups

	Group A N=150	Group B N=150	Test of sig
Age /years Mean±SD	26.47±3.7	26.97±4.1	t=1.05 p=0.29
BMI Mean±SD	22.49±1.76	22.53±1.77	t=0.13 p=0.89
Infertility duration/ years Mean±SD	3.34±1.07	3.29±1.13	t=0.31 p=0.76
FSH Mean±SD	5.71±1.21	5.68±1.19	t=0.18 p=0.86
LH Mean±SD	7.16±1.64	7.09±1.66	t=0.33 p=0.74
TSH Mean±SD	2.28±0.52	2.36±0.60	t=1.9 p=0.28
Prolactin Mean±SD	14.77±4.45	14.59±4.38	t=0.35 p=0.73

t: Student t test SD: Standard deviation

Table (2): ET, Follicles number>18 mm, ovulation incidence, pregnancy,and OHSS between studied groups.

	Group A N=150(%)	Group B N=150(%)	Test of sig
Follicles number >18 mm median(min-max)	2.0(0.0-5.0)	0(0.0-2.0)	z=8.37 p<0.001*
ET/mm Mean±SD	9.69±2.22	8.01±1.7	t=7.17 p<0.001*
Ovulation -ve +ve	59(41.3) 84(58.7)	107(77.0) 32(23.0)	χ^2=37.14 p<0.001*
Clinical pregnancy -ve +ve	117(81.8) 26(18.2)	129(92.8) 10(7.2)	χ^2=7.64 p=0.006*
OHSS -ve +ve	139(97.2) 4(2.8)	138(99.3) 1(0.7)	FET P=0.37

χ^2: Chi-Square test FET: Fischer exact test *statistically significant (if p<0.05) t: Student t test SD: Standard deviation.

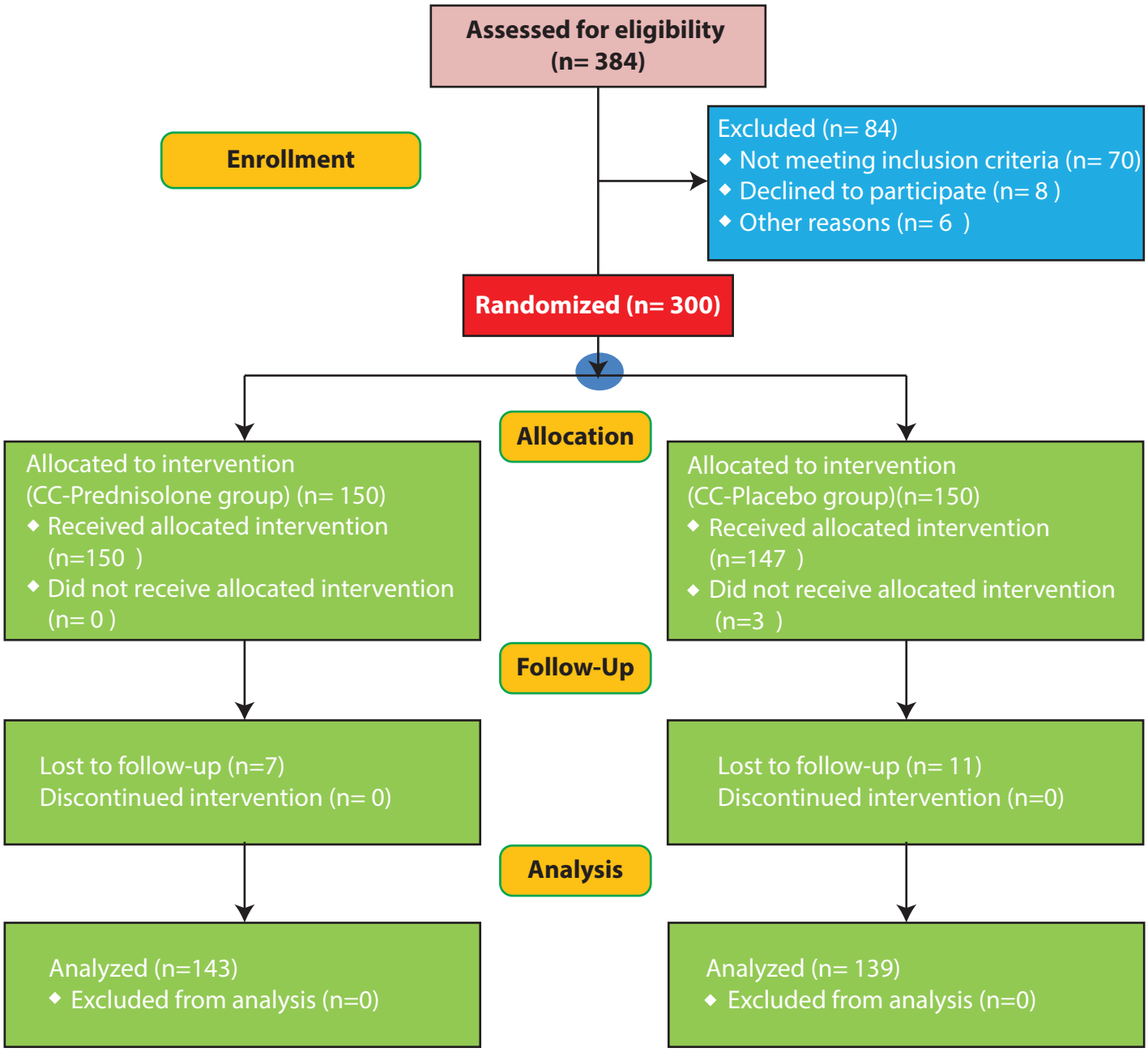

Figure 1: Study flow diagram.Abbreviation: CC, clomiphene citrate

Assessment of Maternal-Fetal outcomes by Doppler Flow Velocity Waveforms in Preterm Labor Patients Undergoing Magnesium Sulfate Tocolysis

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Abstract

Background: Preterm labor is frequency uterine contractions, progressive effacement and dilation of the cervix prior to term gestation. Prevention and treatment of preterm labor is important because it is one of the most important causes of perinatal morbidity and mortality. The etiology of preterm labor is poorly understood.

Aim of the Work: to evaluate the influence of antenatally administrated magnesium sulfate (MgSO4) given to women at risk of preterm birth on the cerebral blood flow and systemic hemodynamic in preterm infants.

Patients and Methods: This was a prospective observational interventional study conducted from October 2016 to February 2017 on (40) patients undergoing preterm labor at AL Zahraa hospital Al Azhar university and New Cairo Hospital, after informed a written consent was taken from every patient after counseling them the procedure of the study.

Results: In our study we found that the best blood level of magnesium sulfate at which contractions stopped (cut off) is (>4.18) mg/dl which equal (6.82) gm of administrated magnesium sulfate with sensitivity (94.74%) and specificity (100%), positive predictive value (PPV) was (100%) and negative predictive value (NPV) was (50%).

Conclusion: Maternal magnesium sulphate (Mgso4) decreases the cerebral perfusion pressure and blood flow, and this likely protects the germinal matrix against the development of circulatory stress in the early post natal period. Magnesium sulphate achived the primary outcome (prevention of delivery for 48 hours, with uterine quiescence) at cut-off value of (≥4.18) mg/dl which equal (6.82) gm with sensitivity (94.74%), specificity (100%), positive predictive value (100%) negative predictive value (50%).

Key words: Doppler flow velocity waveforms, preterm labor, magnesium sulfate tocolysis, maternal-fetal outcome

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Introduction

Although survival of preterm infants in intensive care has increased substantially in recent decades, the risk of their developing brain lesions with subsequent poor neurodevelopment remains high. Because of their poorly developed autoregulatory control mechanisms, cerebral blood flow may fluctuate considerably with spontaneous variations in systemic blood pressure (BP) [1].

A number of immaturity-related factors, e.g., ductus arteriosus shunting and respiratory distress syndrome (RDS), may further potentiate systemic circulatory oscillations and endanger cerebrovascular integrity in preterm infants [2]. Thus, the circulatory liability of prematurely born infants is considered to be aprimary cause of the development of both hemorrhagic and ischemic brain injuries [3].

Doppler sonography is a non-invasive procedure that detects the heartbeat of a fetus. This technology can be used to evaluate pulsations in the fetal heart and to examine blood vessels for signs of abnormalities. A medical practitioner can use Doppler sonography to see the flow of blood from the placenta to the baby, or within the baby's body. This procedure can be very helpful in determining whether the placenta is delivering a sufficient blood supply to the developing baby. It also helps medical practitioners ensure the well-being of the baby, as well as to determine whether there is a need for early delivery or for other medical procedures to ensure good fetal health [4].

Prevention of preterm delivery and preventive stabilization of both the cerebral and systemic hemodynamics in the fetus and neonate are clearly major clinical challenges for perinatal care. Tocolytic agents, such as magnesium sulfate (MgSO4) reduce slightly the incidence of preterm delivery [5], but may additionally have independent influences on the neurologic outcome of the exposed infants [6]

In fact, clinical studies indicate that antenatal MgSO4, originally used for the treatment of maternal preeclampsia and eclampsia [7] may decrease the incidence of brain injuries among very low birth weight infants [4].

The mechanism of the cerebral effects of this tocolytic is still unclear, but because this drug has potent effects on maternal systemic and cerebral circulation and readily cross the placenta, they may also exert a significant effect on the perinatal circulation during transition. Although these hemodynamic effects may prove to be important for preterm infants with immature circulatory control systems, they are still poorly understood [8].

Aim of the work

1. Evaluate the influence of antenatal administrated magnesium sulfate (MgSO4) given to women at risk of preterm birth on the cerebral blood flow and systemic hemodynamic in preterm infants.
2. Evaluation of pregnant women at risk of preterm labor by Doppler study on fetal middle cerebral, umbilical and maternal uterine arteries before and after magnesium sulfate administration.
3. Evaluation of maternal, fetal and neonatal outcomes.

Patients and Methods

This prospective observational interventional study was conducted from October 2016 to February 2017 on (40) patients undergoing preterm labor at AL Zahraa hospital Al Azhar university and New Cairo Hospital, after informed a written consent was taken from every patient after counseling them the procedure of the study.

Inclusion criteria:

Primigravida Women between (28-36) weeks depending on 1st day of last normal menstrual period (LMP) or early ultra sound U/S with singleton pregnancy.

Exclusion criteria:

1. Multi fetal gestation.
2. Not Sure of date (LMP).
3. Medical complications of pregnancy (diabetes-hypertension-kidney or liver disease - heart disease – chestdisease -Preeclampsia).
4. Evidence offetal distress.

Methods

Every patient was submitted into these data:

A-Personal history:

The patient's name, age, address, occupation and phone number.

B- Menstrual history:

1st day of last menstrual period, expected date of delivery, gestational age (28-36) wks.

C- Present history:

At least two uterine contractions were happened for 30 seconds during 10 minutes.

D-Past history:

1. Medical disorders as hypertension, diabetes, heart, chest, liver or kidney diseases.
2. Surgical history.

E- Family history of preterm labor.

Examination

1-General examination:

- Maternal vital signs (temperature, blood pressure, heart rate respiratory rate).

2-Abdominal examination:

- Uterine contractions frequency/duration/intensity are evaluated continuously using cardio-tochography (CTG).

- Ultra sound:confirm the fetal presentation, assess amniotic fluid volume and estimate fetal weight.

3-Trans Vaginal Ultra Sound (TVUS)

- To evaluate the cervical length when the diagnosis of labor is uncertain (<2.5cm).

4- **Full investigations for mother were done** (CBC-ABO-RH-FBS-urine analysis-liver and kidney function test).

5- **Pulse-wave Doppler measurements** were made from the fetal middle cerebral artery, the fetal umbilical artery, and the maternal uterine arteries before the initiation of magnesium sulfate tocolysis.

6- All patients received prophylaxis antibiotics of 1.5 gm ceftriaxone and (500) ml hydration with lactated ringers solution before magnesium sulphate infusion.

7- This protocol consisted of (4) gm bolus of magnesium sulfate infused IV over 30 min, a continuous infusion of (2) g/hr until uterine contractions stopped [9].

- During the treatment, patient were monitored for urine output, deep tendon reflexes, and respiratory rate per minute.

- Monitoring of uterine contraction and fetal heart rates by CTG.

8- All patients received intramascular corticosteroid for premature fetal lung prophylaxis: (Dexamethazone: four doses of 6 mg 12 hours apart).

9- After contractions have been stopped, the Doppler flow measurements were repeated.

10- Serum magnesium levels were measured.

11- The pre-therapy and intra-therapy Doppler flow studies were then compared to determine significant changes.

Technique

The examination was performed in a supine, slightly left lateral tilted position through the examination to avoid supine hypotension.

Ultrasonographic and Doppler flow velocity waveforms studies were done with Pulse Wave Doppler after real time colour flow localization of the umbilical and middle cerebral arteries by Samsung somoacex US (S12GM3HJ3401K) 2013.

Umbilical Artery Doppler

The patients placed in a semi-recumbent position with a left lateral tilt, and then the uterine contents are quickly scanned by the real time ultrasound in order to select an area of amniotic cavity with several loops of umbilical cord. Ideally these cord loops should not be close to the cord insertion. Using a Pulsed Wave Doppler, the characteristic sound and shape of the umbilical artery wave form was demonstrated and identified.

Three separate readings or more were averaged before the final values obtained, with three waveforms for each reading. Because of the potential effect of fetal breathing movements on waveform variability, recording were performed during periods of fetal apnea. Both the resistance index (RI) and (PI) were calculated.

Middle Cerebral Artery Doppler

The standard plane for measuring the biparietal diameter was visualized. This plane included the thalamus and the cavum septum pellucidum, the colour and flow mapping function were then superimposed and the middle cerebral artery can be seen pulsating at the level of the insula. The middle cerebral artery can be seen running from the internal carotid artery in a lateral direction into the Sylvian tissue.

Three readings were averaged; with the average of three waveforms calculated for each reading. Both the (RI) and (PI) were calculated.

Uterine Arteries Doppler: Trans vaginal Doppler.

Doppler Values:

UA, UTA and MCA (RI) and (PI) values were expressed numerically in approximation to the second decimal. Paired T test was used to compare values before and after course.

1ry outcomes of this study for mothers include: (stopped contractions- gestational age of delivery-mode of delivery)

For baby include: (Apgar score- O2 saturation-umbilical cord magnesium sulfate concentration-BP)

2ry outcomes include: ICU admission.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.(Armonk, NY: IBM Corp) Qualitative data were described using number and percent.The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

The used tests were

1- Mean value $(\bar{x}) = \frac{X}{n}$

Where X = the sum of all observations.

n = the number of observations.

2- The standard deviation S.D. = $\sqrt{\frac{\sum(x-\bar{x})^2}{n-1}}$

Where

$\sum (Xi - X)^2$ = the sum of squares of differences of observations from the mean.

3 - Paired t-test

It is used during comparison between the results before and after treatment in the same group. The "t" is calculated as follows:

$$t = \frac{X_d}{\frac{S.D_d}{\sqrt{n}}}$$

Where

X_d = Mean of the difference.

$S.D_d$ = Standard deviation of the difference.

n = Number of cases.

4- Receiver operating characteristic curve (ROC)

It is generated by plotting sensitivity (TP) on Y axis versus 1-specificity (FP) on X axis at different cut off values. The area under the ROC curve denotes the diagnostic performance of the test. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. The ROC curve allows also a comparison of performance between two tests.

5-Sensitivity

The capacity of the test to correctly identify diseased individuals in a population "TRUE POSITIVES". The greater the sensitivity, the smaller the number of unidentified case "false negatives".

6- Specificity

The capacity of the test to correctly exclude individuals who are free of the disease "TRUE NEGATIVES". The greater the specificity, the fewer "false positives" will be included

7- Positive Predictive value (PPV)

The probability of the disease being present, among those with positive diagnostic test results

8- Negative Predictive value (NPV)

The probability that the disease was absent, among those whose diagnostic test results were negative

Results

Table (1): Demographic data of the studied women (n= 40).

	Range	Mean ± SD
Age (years)	16.0 – 30.0	21.60 ± 4.17
BMI (Kg/m^2)	19.0 – 32.0	27.05 ± 3.32
Pulse (Beat/min)	65.0 – 80.0	72.72 ± 4.66
Blood pressure systolic (mmHg)	100.0 – 120.0	110.30 ± 5.91
Blood pressure diastolic (mmHg)	60.0 – 90.0	74.60 ± 9.07
Gestational age(weeks)	28.0 – 36.0	33.60 ± 2.74

Table (2): Maternal Doppler flow velocity wave form changes in studied women.

Total number=40	Magnesium sulphete administration		p-value
	Before	After	
Right uterine artery			
RI	0.44 ± 0.05	0.47 ± 0.04	0.003*(HS)
PI	0.68 ± 0.08	0.63 ± 0.09	0.007*(HS)
Left uterine artery			
RI	0.47 ± 0.05	0.46 ± 0.05	0.298(NS)
PI	0.68 ± 0.09	0.65 ± 0.11	0.695(NS)

This table shows that there is a highly significant difference as regard right uterine artery RI, PI before and after treatment with p-value (0.003), (0.007) respectively.

As regard left uterine artery RI, PI there is insignificant differences before and after treatment with p-value (0.298), (0.695) respectively.

Table (3): Fetal Doppler flow velocity wave form changes in studied women.

Total number=40	Magnesium sulfate administration		P-value
	Before mgso4	After mgso4	
Fetal middle cerebral			
RI	0.75 ± 0.05	0.69 ± 0.05	<0.001*(HS)
PI	1.65 ± 0.26	1.78 ± 0.18	0.005*(HS)
Fetal umbilical artery			
RI	0.60 ± 0.03	0.60 ± 0.03	0.323(NS)
PI	0.90 ± 0.08	0.91 ± 0.08	0.649(NS)

This table shows that there is a highly significant differences in fetal middle cerebral artery RI, PI before and after treatment with p-value (<0.001), (0.005) respectively.

As regard fetal umbilical artery there is insignificant differences.

Table (4): Side effects of magnesium sulfate on the studied women.

Total number=40	Yes	
	No.	%
NO side effects **Allergic reaction** *(itching, chest tightness, choking in the throat, nasal congestion)*	15 12	37.5 30.0
Palpitations	1	2.5
Severe drowsiness	2	5.0
Sweating	10	25.0

Table (5): Primary neonatal outcomes.

Primary fetal outcomes	Range	Mean ± SD.
Apgar score at 1 min	3.0 – 7.0	5.24 ± 1.01
Apgar score at 5 min	1.0 – 9.0	8.08 ± 1.82
Oxygen saturation %	68.0 – 92.0	84.05 ± 5.55
Fetal serum mgso4(mg/dl)	1.43 – 5.04	3.64 ± 0.86
Blood pressure systolic(mmhg)	25.0 – 56.0	46.50 ± 8.52
Blood pressure diastolic(mmhg)	15.0 – 32.0	27.90 ± 3.99
Neonatal RDS		
No	28	70.0%
Yes	12	30.0%

This table shows that the mean Apgar score at 1 min is (5.24) and at 5min is (8.05) as regard oxygen saturation the mean level is (84.05),mean systolic and diastolic neonatal blood pressure is (46.05),(27.9) respectively and the mean Fetal serum Mgso4 is (3.64). Regarding Respiratory Distress Syndrome there are 12 cases representing (30%).

Table (6): Secondary neonatal outcomes.

Secondary outcomes	No.	%
Neonatal Intensive care unit		
No	28	70.0
Yes	12	30.0

This table shows that (30%) of neonates are admitted to NICU.

Table (7): Maternal outcomes.

Maternal Outcomes	No.	%
Mode of delivary		
Cesarian Section	4	10.0
Vaginal delivary	36	90.0
Delivery <48 hours after MgSO4 infusion		
No	38	95.0
Yes	2	5.0
Stopped contractions		
No	2	5.0
Yes	38	95.0

Blood pressure systolic (mmHg)	100.0 – 120.0	110.30 ± 5.91
Blood pressure diastolic (mmHg)	60.0 – 90.0	74.60 ± 9.07
Maternal Heart Rate(beat/minute)	114.0 – 138.0	125.10 ± 5.45

This table shows that 90% of patients delivered by vaginal delivery. Delivery in less than 48 hours after treatment is only in 5% of patients as 95% of patients showed stopped contraction.

Mean systolic and diastolic blood pressure after treatment are (110.3),(74.6) mmHg respectively and mean maternal heart rate is (125.10) beat/minute.

Table (11): Cut off for Maternal serum MgSo4 to diagnosed stopped contractions and labor >48 hours

	Cut off in blood (mg/dl)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MgSO4	≥4.18	94.74	100.0	100.0	50.0

This table shows that the best blood level at which contraction stopped is (≥4.18) mg/dl which equal (6.82) gm of administrated magnesium sulfate.

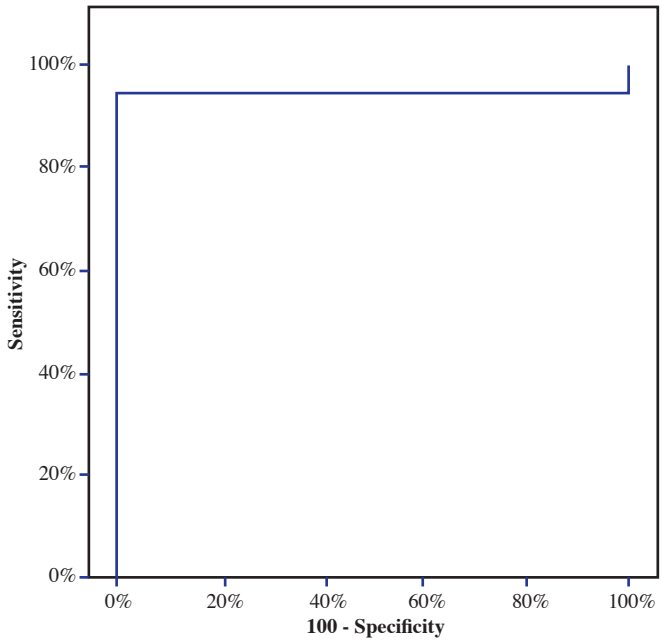

Figure (1):
Cut off for Maternal serum MgSo4 to diagnosed stopped contractions and labor >48 hours.

Discussion

Preterm birth (PTB) is still the leading cause of perinatal morbidity and mortality. Early-term complications such as respiratory distress syndrome, necrotizing enterocolitis, and intraventricular hemorrhage are frequent problems in preterm infants. Frequent late-term complications include visual impairment,hearing loss, and cerebral palsy [10].

Although the rate of other obstetric complications has declined with the development of contemporary obstetric understanding, the treatment methods developed for preterm labor (PTL) have so far failed to reduce the number of PTBs. However, some benefits are gained through prolongation of pregnancy to enable corticosteroid administration to accelerate fetal lung maturation. The least harmful drug for the mother and fetus, and the most effective tocolytic medication should be selected and administered without any delay after diagnosing the existence of any preterm pattern"[11].

Tocolytics can be used alone and/or in combination. Each tocolytic agent, in addition to their success in stopping the premature uterine contractions, presents maternal and fetal adverse effects. The use of these drugs requires close monitoring of patients during their administration [9].

Magnesium sulfate is the most commonly used first-line tocolytic in North America although it has not been demonstrated to be superior to saline infusion, and its use has been a source of controversy. Magnesium sulfate requires intravenous administration, has potential for over medication with serious maternal adverse effects and may be associated with adverse neonatal effects [12].

In the present study we aimed to evaluate the influence of antenatal administrated magnesium sulfate (MgSO4) given to women at risk of preterm birth on the cerebral blood flow and systemic hemodynamic in preterm infants we conclude 40 patients undergoing preterm labor.

In the present study, the mean age, body mass index (BMI) and gestational age of the studied patients were (21.60±4.17) years,(27.05±3.32) kg/m2,(33.60±2.74) was respectively, All cases were primigravida.

Also pulse and blood pressure were measured to all patients at first visits. The mean and SD of pulse rate were (72.72±4.66) beat/minute. The mean and SD of systolic and diastolic blood pressure were (110.30±5.91) and (74.60±9.07) mmhg respectively as shown in table [4].

In the current study the mean systolic and diastolic blood pressure after treatment with magnesium sulphate were (110.3±5.90),(74.6±9.07) mmHg respectively and the mean maternal heart rate was (125.10±5.54) as shown in table [4]

Uterine artery Doppler flow velocity wave form changes in our study were highly significant in right uterine artery RI and PI before and after treatment with magnesium sulphate with p-value (0.003), (0.007) respectively however there were no significant changes in left utrine artery RI and PI with P value (0.298), (0.695) respectively as shown in table [5].

This is in agreement with Güden et al. [11] study that showed The PI of the right uterine and the RI were significantly high following the treatment with magnesium sulphate (p=0.001 and p=0.018) respectively. However, no changes were observed in the left uterine artery PI and RI (p=0.072 and p=0.901) respectively.

In addition to **Abd El-Hamed Sedek** [13] who found that there was a significant differences in right uterine artery Doppler parameters before and after administration of MgSo4 in the studied patients inPI and RI with p-value (0.001) and (0.002) respectively.

These changes in uterine arteries PI had been explained as vasodilation in magnesium sulphate treated uterine arteries and thus the increase in blood flow. The changes that occur in these vessels were described as physiological changes that occur with the removal of preterm labor stress on the fetus [11].

In contrary to our result, Souza et al. [14] showed that Loading dose of Magnesium Sulfate (MgSO4) causing significant reduction in the PI and S/D ratio in both uterine arteries p-value (o.005),this differences may be due to that the included patients were preclampsic

As regard fetal umbilical artery RI and PI in the current study we found that there were insignificant changes before and after treatment with magnesium sulphate with p- value (0.323) (0.649)respectively as shown in table [6].

In consistent with our result **Güden et al**. [11] found that no statistically significant difference was observed in umbilical artery PI and RI and systole to diastole (S/D) rates (p=0.358, p=0.556, and p=0.534) respectively.

Another study by **Keeley et al**. [15] showed that there is no significant changes in umbilical artery PI and RI before and after treatment with magnesium sulphate with p- value (0.235) and (0.349) respectively.

Also **Wright et al**. [16] stated that there was no effect of magnesium sulphate on umbilical artery PI and RI Doppler flow in similarity with our result with p-value (0.963) and (0.822) respectively.

This differences may explained by **Houlihan et al**. [17] showed that there is evidence that magnesium sulfate promotes vasodilatation of the umbilical artery with consequent decrease of vascular resistance.

The study had been done by **Abd El-Hamed Sedek** [13] disagree with our result as theyfound that there was a significant difference between umbilical artery Doppler parameters before and after administration of magnesium sulphate in the studied patients with p-value (0.001) and this is may be due to all patient included were pre-eclampsic also.

Also, **Souza et al**. [14] reported in their study that patients with normal blood pressure levels the vasodilator effect of magnesium is not evident; in patients with pre-eclampsia this effect is significant.

In the current study fetal middle cerebral artery Doppler flow velocity were highly significant changes in RI and PI before and after treatment with magnesium sulphate with p-value (<0.001) and (0.005) respectively as shown in table (6).

This is in agreement with the study done by **Güden et al**. [11] that showed an increase in the PI of the middle cerebral artery and a decrease in the RI with p- value (p=0.024 and p<0.001) respectively.

Another study by **Abd El-Hamed Sedek** [13] showed an increase in the PI of the middle cerebral artery and adecrease in the RI with p- value (p=0.021 and p<0.003) respectively.

This is explained by that. The alterations in fetal hemodynamics during magnesium sulfate administration suggest a physiologic normalization process related to the stressed preterm and this explained by that The increase of PI in the middle cerebral artery was attributed to the cerebral blood flow increase during preterm labor and the normalization of middle cerebral artery PI following magnesium sulphate treatment, thus the cessation of preterm labor.

In contrary tourresult **Sayin et al.** [18] study their result showed a significant decrease in middle cerebral artery PI. due to that they found in women between the 26th and 32nd weeks middle cerebral artery PI did not significantly change after 48 h. However, in women between the 32nd and 36th weeks the middle cerebral artery PI significantly differed in the treatment groups compared to controls after 48 h and concluded that these effects on blood flow are particularly significant in women between 32nd and 36th weeks this differences may be due to the increase in the blood pressure of the included patients as all cases included were also pre-eclampsic.

As regard the maternal adverse effect in our study we found that (30%,n=12) of studied patients showed allergic reactions after treatment,(25%,n=10) shows sweating,(5%,n=2) had sever drowsiness and only (2.5%,n=1) who showed palpitations rest of patients had no side effects as shown in table (7).

This result were in agreement with **Bain et al**. [19] who reported that maternal adverse effects of magnesium sulphate include flushing (22%), increased warmth (7%) and sweating (5%) due to the peripheral vasodilatory effects of magnesium.

In another study by **Lyell et al**. [12] they found that several maternal side effect occurred like Shortness of breath in (14%) of patients, Pulmonary edema and Hypotension in (3%) and Chest pain in (8%) other side effect like also occurred Nausea in (32%), Lethargy in (29%),vomiting in (26%),flushing in (22%),dizziness in (17%),blurring of vision in (13%).

Fetal primary outcomes after treatment with magnesium sulphate in the present study we found that the mean Apgar score at one and five minutes were (5.24±1.01).(8.08±1082) respectively, oxygen saturation was (84.05±5,55), Fetal serum magnesium sulfate concentration was (3.64±0.86) mg/dl as shown in table (8).

While secondary fetal outcomes as we reported (30% n=12) of neonates needed admission to neonatal intensive care unit because of neonatal respiratory distress syndrom as shown in table (9).

Lyell et al. [12] in agreement with our studythey reported that (23%) showed neonatal respiratory distress syndrome, (5%) had sepsis,(1%) died and (52%) admitted to neonatal intensive care unit due to neonatal complications.

In another studies by **Abasalizadeh et al**. [20] they reported that (42%) of neonates showed respiratory distress syndrome, (26%) showed necrotizing enterocolitis (NEC), (22%) showed Sepsis.

This explained by some investigators have speculated that magnesium sulfate may slow gastrointestinal function, leading to feeding issues, and may lead to significant respiratory suppression [21].

In disagreement with our study **Abasalizadeh et al.** [20] reported that in patients received magnesium sulfate one minute Apgar score was (2.99) and five minute Apgar score was (2.64).

After treatment and on follow up of patients during study period we found that (90.0%) delivered by normal vaginal delivery but (10%) had C.S because of contracted pelvis, (95%) of patients deliver after more than 48 hours after magnesium sulphate infusion while only (5%) deliver in less than 48 hours as (95%) of patients Stopped contractions after treatment.

This explained by **Phillippe et al**. [22] as they demonstrated that magnesium inhibited extracellular calcium entry, intracellular calcium release, cytosolic calcium oscillations, and phasic contractions of myometrial smooth muscle.

In agreement with our result **Lyell et al**. [12] reported that, patients received MgSO4 had delayed delivery, only (7%) patients out of deliver in 48 hours they showed that prevention of delivery for 48 hours with attainment of uterine quiescence occurred in (93%) in patients received magnesium sulphate.

On the other hand,the study done by **Kawagoe et al.** [23] disagree with our result as they reported that (57%) of patients received magnesium sulphate delivered by Cesarean section, (14%) Deliver in less than 48 hours.Because of massive genital bleeding of unknown causes, which occurred after

12 hours of magnesium infusion without improvement of uterine contractions.

In the current study we found that the best blood level of magnesium sulphate at which contraction stopped is (≥4.18) mg/dl which equal (6.82) gm of administrated magnesium sulphate with sensitivity (94.74%) and specificity (100%), positive predictive value (PPV) was (100%) and negative predictive value (NPV) was (50%) as shown in table (11).

In another study by **Khani et al**. [23] agree with our result as they showed that he cut off point for magnesium sulfate was (4.7) mg/dL as specificity, sensitivity, positive and negative predictive values were (95%), (50%), (66.5%) and (83.33%) respectively.

In our study we reached to that magnesium sulphate is agood tocolytic agent (as 95% of preterm women stopped contractions),if given in asuitable dose (4) gm bolus of magnesium sulfate infused IV over 30 min,a continuous infusion of 2 g/hr), to allow enough time for pregnant women to receive intramascular dexamethazone for premature fetal lung prophylaxis and provide time for safe transport of the mother, if indicated, to afacility that has an appropriate level of neonatal care if the patient delivered preterm,with specificity, sensitivity, positive and negative predictive values were (95%), (50%), (66.5%) and (83.33%) respectively.

Conclusion

1. Maternal magnesium sulphate (Mgso4) decreases the cerebral perfusion pressure and blood flow,and this likely protects the germinal matrix against the development of circulatory stress in the early post natal period.
2. The decreased maternal uterine artery (PI) values indicated arelative increase in uterine blood flow velocity and this may be one explanation for the success of magnesium sulphate as atocolytic agent.
3. No significant changes in umbilical artery (PI).
4. Magnesium sulphate achived the primary outcome (prevention of delivary for 48 hours, with uterine quiescence) at cut-off value of (≥4.18) mg/dl which equal (6.82) gm with sensitivity (94.74%),specificity (100%), positive predictive value (100%) negative predictive value (50%).

Recommendations

Our study recommended that:

1. Administration of magnesium sulphate at cut off value (≥4.18) mg/dl which equal (6.82) gm to women at risk of preterm birth with close monitoring of patient would achieve the tocolytic effect with the least morbidity for both mother and fetus.
2. Other further studies for more evaluation of magnesium sulphate as atocolytic agent in preterm labor.
3. Other further studies to assess fetal neuroprotection of magnesium sulphate.

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Role of Maternal Serum Ferritin in the Prediction of Asymmetric Intrauterine Growth Restriction. Short running title: Ferritin predicts asymmetric IUGR

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Disclosure statement

The authors guarantee that they:

(1) have stated explicitly, in the cover letter and the main text, that there are no conflicts of interest in connection with this article.

(2) have participated sufficiently in the work to take responsibility for it;

(3) have self-funded this research;

(4) have reviewed the final version of the manuscript and approve it for publication; and

(5) have neither published nor submitted this manuscript or one with substantially similar content elsewhere.

All of the included women gave their **informed consent** prior to their inclusion in the study and their anonymity was maintained.

Abstract

Background and aim: Asymmetric intrauterine growth restriction (IUGR) carries increased intrauterine and neonatal risks. Since most cases are idiopathic, identifying a predictive test remains an ideal prevention management. Maternal serum ferritin level is a potential predictor of later developing asymmetric IUGR.

Methods: Blood samples were drawn, at 30-32 gestational weeks, from 450 women who were then followed-up, resulting in 32 term pregnancies with asymmetric IUGR. The control group included the first recruited 32 women delivering adequate for gestational age neonates at term. Serum ferritin was then measured in the stored serum samples. Ultrasound scanning was performed at 30-32 weeks then at 37 weeks. Umbilical and middle cerebral artery Doppler scans were added at 37 weeks.

Results: Serum ferritin, at 30-32 weeks, was higher in women delivering IUGR babies (19.3±6.83 vs 14±5.18, $p<0.01$). At 37 weeks, pregnancies with asymmetric IUGR had significantly ($p<0.01$) higher umbilical artery resistance and pulsitility indices (0.71±0.06 vs 0.59±0.07 and 1.24±0.17 vs 0.86±0.09, respectively). Their middle cerebral artery RI and PI were significantly ($p<0.01$) lower than

controls (0.74±0.09 vs 0.81±0.05 and 1.64±0.07 vs 1.9±0.15, respectively). Ferritin level correlated significantly with neonatal birthweight. A cut-off level >18.2 ng/mL had a sensitivity of 59.4% and a specificity of 90.6%. The area under curve showed an accuracy of 76.8%. Women with ferritin >18.2 ng/ml, were 10.23 times more likely to get asymmetrically growth restricted neonates (CI 2.89–36.17, p<0.001).

Conclusion: This study presents further evidence that maternal serum ferritin may be a useful test in the prediction of asymmetric IUGR.

Key words:
Intrauterine Growth Restriction,
Doppler,
Ferritin,
Neonatal Birthweight,
Small for gestational age.

Synopsis

Maternal serum ferritin may be a useful test in the prediction of asymmetric IUGR.

Introduction

Asymmetric, late-onset (type II), intrauterine growth restriction (IUGR) may be defined as pathological slow fetal growth, beginning in late pregnancy, due to uteroplacental insufficiency (1,2). This definition differentiates it from the term "Small for Gestational Age" (SGA) which implies a birthweight less than the 10th centile for gestational age even if it is a healthy but simply small baby (2). The incidence of IUGR is 3.3–10% in the developed countries and 6.7–17% in developing ones (3,4,5).

A fetus with IUGR is exposed to increased intrauterine risks of fetal distress and death, neurologic developmental disorders as well as meconium aspiration at birth. Neonatal risks include hypoglycemia, long admission to intensive care units, hypothermia, polycythemia, jaundice, feeding difficulties, necrotizing enterocolitis, late-onset sepsis, hypoxic-ischemic encephalopathy and pulmonary hemorrhage. These infants also have increased risks of type 2 diabetes, obesity, autoimmune diseases, cardiovascular diseases and hypertension in adult life (2,6).

Asymmetric IUGR, accounting for 70-80% of cases of IUGR (2), is associated with reduced umbilical blood flow, brain sparing effect, oligohydramnios and a low Ponderal index (7). In the absence of any direct causal therapy, and in view that most cases of IUGR are idiopathic, the identification of adequate predictive tests for IUGR remains one of the top priorities in obstetrics. Several studies suggested different biomolecules as early markers for IUGR, such as lactate dehydrogenase, leptin, metastin, adiponectin, s-endoglin, endothelin-1 and pregnancy associated plasma protein (8-10). Most of these tests have a low sensitivity, or are expensive, not widely available and/or invasive tests requiring amniocentesis (9,10).

Ferritin, the main iron storage protein, was suggested to be an adequate alternative as a screening test, being a relatively cheap and easily available blood test. Its level is known to rise in response to hypoxia or as an acute phase reactant in infections (11). Ferritin blood level normally drops, in correlation with the progressive depletion of iron reserves, by 32% during the first trimester, 39% in the second and up to 53% during the third trimester (12). This level reaches a nadir at 30-32 weeks (13), after which it stays constant.

The theory behind the rise in maternal serum level of ferritin during pregnancies destined to develop late-onset (asymmetric) IUGR relies on decreased placental extraction of ferritin from the systemic circulation (13). Its predictive value was previously investigated, in a few small-sized studies, on a small number of cases (9,13-16).

This study aimed to assess the accuracy of maternal serum ferritin level at 30 weeks to 32 weeks in predicting the development of IUGR, and to identify the ferritin cutoff level with the best predictive value, in a well-selected, adequate-sized group of pregnant women.

Material and Methods

This was a prospective nested longitudinal study conducted in the outpatient clinics of Ain Shams University Maternity Hospital, Cairo, Egypt. The study protocol was approved by the Faculty of Medicine Research Review Board on 15/03/2016. The study protocol was registered on 14/04/2016 on www.clinicaltrials.com website as NCT02738463.

All women were informed about the procedures of the study. Confidentiality of the collected data and anonymity of the participants were ensured. Written informed consent was obtained from the women before participation. The study protocol complied with the Declaration of Helsinki regarding ethical conduct of research involving human subjects.

All included women had singleton 30-32 weeks pregnancies (with sure and reliable dates of the last menstrual periods and ultrasonographic estimation of the gestational age during the first trimester). Exclusion criteria included history of anemia, iron supplementation, recent blood transfusion, age <20 years, BMI <18, smoking, diabetes mellitus during pregnancy, hepatic, renal, hypertensive or cardiovascular abnormalities, detected congenital fetal anomalies, antepartum hemorrhage, preterm delivery, and cases developing acute infections during pregnancy with raised leukocytic count or C-reactive protein. This was meant to rule out any confounding effect on the level of serum ferritin.

Sample size calculation:

Data from a previous study showed that maternal serum ferritin levels in pregnancies resulting in neonates with IUGR were on average 6.43 mg/L higher than in those with adequate sized neonates (15). Using the equation of sample size calculation from Fox et al (17), with a power of 80% (β=0.2) and a confidence level of 95% (α=0.05), the sample size calculation for the study group is as follows:

$N = D \times K \times 2 \times [\partial / (f1-f2)]^2$

$= 133.3\% \times 7.9 \times 2 \times [7.84 / (17.54 - 11.11]^2$

= 32 cases per group

Where N= Sample size (in a single arm), K= Constant, which varies according to α and β values, here K= 7.9, ∂= standard deviation in ferritin level, and f1 and f2= the ferritin level among cases versus controls in a previously published study (15). D= percentage increase to compensate for the drop-out rate, here D=133.3%.

Assuming an incidence of IUGR of 8% (15), 400 patients were to be recruited for follow up to obtain the desired 32 cases with IUGR. Another 10% rate was added to that number of women to ascertain obtaining the desired number of IUGR cases during patient follow-up. Thus, a total of 450 patients were assessed and followed-up. The study group included patients delivering at ≥37 weeks, giving birth to neonates with type II IUGR (32 pregnancies). The control group included the first recruited 32 women delivering adequate for gestational age neonates at term.

Morning fasting blood samples were drawn from the cubital vein at 30-32 weeks. The specimens were centrifuged immediately for 5 minutes at 4000 rpm, and the supernatant serum was transferred into another Eppendorf tube and immediately frozen and stored at -20C in the biochemistry laboratory of Ain Shams University Hospitals.

Obstetric ultrasound scanning was performed on all included women at 30-32 weeks and was repeated at 37 weeks. Umbilical and middle cerebral artery Doppler scans were added at 37 weeks in IUGR cases (the first 32 women diagnosed with IUGR) as well as controls (the first 32 normal pregnancies who reached maturity).

Pregnant women were followed until delivery to identify those with asymmetric IUGR. Serum ferritin levels were then measured in their stored samples of cases and controls using Enzyme-Linked Fluorescent Assay. The result of their complete blood count at delivery was also recorded.

Neonates were then evaluated at birth for vitality using the Apgar score of 0-10 which checks the heart and respiratory rates, the muscle tone, reactivity and skin color. Neonatal weight and body length were also measured and documented, as well as any clinical evidence of IUGR, such as raised head to abdomen circumferences ratio, loose and dry skin, absent buccal fat (old man look), small or scaphoid abdomen, thin umbilical cord often stained with meconium, excessive skin folds with decreased underlying skeletal muscle mass and loss of subcutaneous fat.

Data were collected, revised, coded and entered to the Statistical Package for Social Science program (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24. Armonk, NY: IBM Corp). Qualitative data were presented as number, and percentage, and were compared using Chi-square x^2 test or Fisher exact test. Quantitative parametric data were presented as mean ±standard deviation (±SD), and were compared using Inde-

pendent t-test. Non-parametric data were presented as median, with interquartile ranges (IQR), and were compared using Mann-Whitney test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. Logistic regression analysis was used to assess predictors of IUGR. Receiver operating characteristic curve (ROC) was used to assess the best cut-off point for ferritin in predicting IUGR with its area under curve (AUC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Results

A total of 450 women were recruited during the course of this study. Four pregnancies ended with intrauterine death, ten women developed gestational diabetes mellitus, 28 developed preeclampsia, 32 delivered prematurely and 48 were lost to follow up. Out of the remaining 328 pregnancies, the first 32 cases of IUGR and 32 adequate for gestational age (AGA) controls were included in data analysis. The mean age of the included women were statistically similar (28±4.1 and 28.4±4, in controls and cases, respectively, p=0.7). Both groups had comparable rates of previous IUGR deliveries (6.3 and 17% respectively, p=0.17).

Women with asymmetric IUGR had similar hemoglobin level, hematocrit value erythrocytic and leukocytic counts as controls (table 1). Serum ferritin at 30-32 weeks was significantly higher in women who ended up delivering IUGR babies (table 1).At the 37 weeks' Doppler scan, pregnancies with asymmetric IUGR had significantly higher umbilical artery resistance indices (RI) and pulsitility indices (PI). Their middle cerebral artery RI and PI were significantly lower than their controls (table 1).

Neonates with asymmetric IUGR had a similar mode of delivery and gestational age at birth as their AGA controls. On the other hand, the birthweight, Apgar scores and rate of admission to neonatal intensive care units were all significantly different between IUGR and AGA neonates (table 2).

Maternal serum ferritin level was the only variable which correlated significantly with neonatal birthweight. Maternal age and hematology variables, neonatal gestational age, Apgar scores or body length were all not correlated to ferritin level (table 3).

There was a non-significant correlation between the history of IUGR in previous pregnancies and the development of asymmetric IUGR in the current pregnancy. A highly significant correlation was noted between serum ferritin level at 30-32 weeks as well as Doppler indices at 37 weeks and present history of asymmetric IUGR (table 4).

The receiver operating characteristic (ROC) curve (table 5, figure 1) showed that the best ferritin cutoff level, between mothers with asymmetric IUGR neonates and those with AGA, was >18.2 ng/mL. This cutoff had a sensitivity of 59.38% and a specificity of 90.62%. The area under curve (AUC) showed an accuracy of 76.8%, thus serum ferritin was found a good predictor for predicting babies with IUGR. The data above depict that women with mean serum ferritin above 18.2 ng/ml, were 10.23 times more likely to get asymmetrically growth restricted babies (CI 2.89–36.17, p<0.001).

Discussion

Fetal growth restriction is a serious condition deserving early diagnosis or good predictive tests to help reduce the impact of its serious short and long term complications. The current study found a significantly higher mean maternal serum ferritin level in the group of pregnant women who ended up being diagnosed with IUGR, as opposed to that in control pregnancies (19.3±6.83 vs. 14±5.18 mg/L, respectively; p<0.001). This is, roughly, similar to the so far recorded ferritin levels in similar studies. The value of maternal serum ferritin in predicting asymmetric IUGR was previously investigated, for few times, on small numbers of cases, i.e. 7 cases in Ozgu-Erdinc et al (9), 18 cases in Milasinovic et al (13), 10 cases in Hubel et al (14), 17 cases in Visnjevac et al (15), and 31 cases in Bindal et al (16), or with limitations in the research methodology such as measuring ferritin at a gestational age that was to early (20 weeks) or to late (36 weeks) (1,4), or including cases with maternal iron deficiency anemia (15).

Lower expression of placental transferritin is known to be associated with IUGR (18). This may cause less iron extraction by the placenta from maternal serum, thus raising the level of serum fer-

ritin (16). Furthermore, other studies suggest that placental damage, in cases of asymmetric IUGR, releases some of the placental ferritin into the maternal circulation, thus further raising its serum level (1,14,19). Ferritin is also a known acute phase reactant released by leukocytes into the systemic circulation in response to immune activation. High serum ferritin level may reflect a non-infectious vascular inflammatory response causing oxidative damage to cells and tissues (14,20). The association of IUGR with inflammation has been previously proven (21). This is the reason why this study set acute infections during pregnancy, leukocytosis or raised C-reactive protein titer among its exclusion criteria to rule out any misleading rise in the level of ferritin.

As opposed to previous studies (3,15,16), we set anemia among our exclusion criteria. Our results showed non-significant differences between controls and IUGR cases as regards erythrocytic counts, hematocrit and hemoglobin levels, compared to a rate of anemia of 47.4% in the control group of one study (15). This is probably why the current study found a higher degree of correlation between maternal serum ferritin and neonatal birthweight (-0.453) which is higher than those found by Višnjevac et al (-0.24) (15), and Bindal et al (-0.36) (16). On other grounds, Akkurt et al reported a higher hematocrit level in pregnancies with IUGR than controls (1). They hypothesized that this was related to a degree of hemoconcentration related to IUGR development. The current study did not show such difference in hematocrit.

We showed maternal serum ferritin to have a good predictive power when used at 30-32 weeks for prediction of IUGR. The results of the current study suggest that a ferritin level of >18.2ng/mL can predict asymmetric IUGR with a sensitivity of 59.38%, a specificity of 90.62%, a positive predictive value of 86.4%, and a negative predictive value of 69%. The very good overall accuracy of 76.8% drawn from the ROC analysis and the associated odds ratio of 10.23 are promising, denoting that serum ferritin measurement may be considered an adequate predictive test for IUGR. Uberos et al, Višnjevac et al and Bindal et al also came to similar conclusions but with a lower odds ratio (3,15,16), probably due to the inclusion of cases with anemia in the study population (3,16). Uberos et al concluded that ferritin levels >13 ng/ml had 4.5 times more risk to result in IUGR at 38 weeks of gestation (3). Hubel et al showed no significant difference in ferritin between AGA and IUGR cases, but their study only included 10 asymmetric IUGR cases (14). Soubasi et al found high maternal ferritin levels to correlate significantly with higher rates of gestational diabetes mellitus and IUGR, but did not define a cutoff to differentiate IUGR from AGA neonates (22). Hou et al, Milasinovic et al and Višnjevac et al identified the ferritin threshold to predict IUGR or SGA as >13.6 ng/ml (4,13,15). Ozgu et al reported maternal serum ferritin levels >60 ng/ml to increase the risk for IUGR (9). Bindal et al concluded that maternal serum ferritin >20 ng/ml predicted IUGR with an odds ratio of 6.6 (16). Akkurt et al took one step further and showed a role for maternal serum ferritin measurements in distinguishing pregnancies with asymmetric IUGR from those with simple small for gestational age babies and no signs of placental insufficiency (1).

In conclusion, this study presents further evidence, on a relatively larger group of patients, that maternal serum ferritin might serve as a useful marker to identify pregnancies at risk of resulting in asymmetric IUGR infants. It found a significantly higher maternal serum ferritin level, at 30-32 weeks, in pregnancies destined to develop asymmetric IUGR at a later gestational age than in controls. A cutoff of >18.2 ng/mL had an accuracy of 76.8% to predict IUGR with a PPV of 86.4%. Pregnancies with ferritin level higher than such cutoff were 10.23 times more likely to deliver asymmetrically growth restricted babies when compared to women with serum ferritin value less then <18.2 ng/ml. Large scale studies are recommended to further establish this theory and to test related points regarding the effect of associated anemia or the gestational age at which the test is best to be done. The role of maternal serum ferritin in distinguishing pregnancies with asymmetric IUGR and signs of placental insufficiency from those with simple small for gestational age babies also still needs further research.

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Conflicts of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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Figures Legends:

Figure (1) Receiver operating characteristic curve showing the best cutoff point for serum ferritin level between mothers with asymmetric IUGR neonates and those with AGA, was >18.2 ng/mL.

Tables Legends:

Table (1) Comparison between cases and controls regarding laboratory and Doppler variables.

Table (2) Comparison between cases and controls regarding neonatal outcome at delivery.

Table (3) Correlation between serum ferritin level and maternal and neonatal data in cases and controls.

Table (4) Correlation between the development of IUGR in the current pregnancy and the past history of previous IUGR deliveries, current pregnancy serum ferritin level or umbilical and middle cerebral artery Doppler indices.

Table (5) Data showing sensitivity, specificity, positive predictive value, negative predictive value of various serum cutoffs to predict asymmetrical IUGR.

Table (1) Comparison between cases and controls regarding laboratory and Doppler variables.

			Cases group n=32	Control group n=32	p value
Erythrocytes (10^6/µl)		Mean (±SD)	4.12 (±0.58)	3.88 (±0.66)	0.13
		Range	3.2 – 5.23	3.04 – 5.04	
Hemoglobin (gm/dl)		Mean (±SD)	11.5 (±0.43)	11.4 (±0.36)	0.12
		Range	11 – 12.5	11 – 12.3	
Hematocrit (%)		Mean (±SD)	34.6 (±1.84)	33.9 (±1.59)	0.12
		Range	31.3 – 38.6	30.8 – 37.8	
Leucocytes (10^3/cc)		Mean (±SD)	8.6 (±2.13)	8.5 (±1.76)	0.84
		Range	5.4 – 12.3	5.4 – 12.2	
Ferritin (ng/ml)		Mean (±SD)	19.3 (±6.83)	14 (±5.18)	<0.01
		Range	8.5 – 40	5 – 26.3	
Umbilical artery Doppler	RI	Mean (±SD)	0.71 (±0.06)	0.59 (±0.07)	<0.01
		Range	0.6 – 0.81	0.49 – 0.69	
	PI	Mean (±SD)	1.24 (±0.17)	0.86 (±0.09)	<0.01
		Range	1.03 – 1.94	0.72 – 0.94	
Middle cerebral artery Doppler	RI	Mean (±SD)	0.74 (±0.09)	0.81 (±0.05)	<0.01
		Range	0.61 – 0.92	0.72 – 0.89	
	PI	Mean (±SD)	1.64 (±0.07)	1.9 (±0.15)	<0.01
		Range	1.36 – 1.88	1.82 – 2.11	

N=number of women, SD=standard deviation, RI=resistance index, PI=pulsitility index.

Table (2) Comparison between cases and controls regarding neonatal outcome at delivery.

		Cases group n=32	Control group n=32	p value
Gestational age at delivery (days)	Mean (±SD)	272.9 (±7.25)	274.3 (±6.79)	0.14
	Range	259 – 282	260 – 285	
Mode of delivery (n, %)	Cesarean	19, 59.4%	16, 50%	0.45
	Vaginal	13, 40.6%	16, 50%	
Birth Weight (gm)	Mean (±SD)	2134 (±143)	3419 (±352)	<0.001
	Range	1750 – 2390	2800 – 4100	
Apgar score (1st minute)	Median (IQR)	7 (7–8)	8 (7–9)	0.04
	Range	6 – 9	6 – 10	
Apgar score (5th minute)	Median (IQR)	8.5 (8–9.5)	9 (9–10)	0.03
	Range	7 – 10	8 – 10	
Admission to NICU (n, %)	No	19, 59.4%	28, 87.5%	0.01
	Yes	13, 40.6%	4, 12.5%	

N=number of women, SD=standard deviation, IQR=interquartile range, NICU=neonatal intensive care unit.

Table (3) Correlation between serum ferritin level and maternal and neonatal data in cases and controls.

	Ferritin level (ng/ml)			
	Cases		Controls	
	r value	p value	r value	p value
Maternal Age (years)	-0.195	0.28	-0.112	0.54
Erythrocytes (10^6/µl)	0.111	0.55	-0.073	0.69
Hemoglobin (gm/dl)	0.080	0.66	0.148	0.42
Hematocrit (%)	0.225	0.22	0.044	0.81
Leucocytes (10^3/ cc)	0.250	0.17	-0.109	0.56
Gestational age at delivery (days)	-0.261	0.15	0.103	0.57
Birth Weight (gm)	-0.453	0.009	0.174	0.34
Apgar score (1st minute)	-0.268	0.14	0.063	0.73
Apgar score (5th minute)	-0.090	0.63	0.083	0.65

r value = Spearman coefficient of correlation.

Table (4) Correlation between the development of IUGR in the current pregnancy and the past history of previous IUGR deliveries, current pregnancy serum ferritin level or umbilical and middle cerebral artery Doppler indices.

	Development of IUGR in the current pregnancy	
	r value	p value
Previous IUGR	1.435	0.09
Ferritin (ng/ml)	0.183	0.001
Umbilical artery RI	16.866	<0.001
Umbilical artery PI	6.596	<0.001
Middle cerebral artery RI	-12.308	0.002
Middle cerebral artery PI	-17.688	0.03

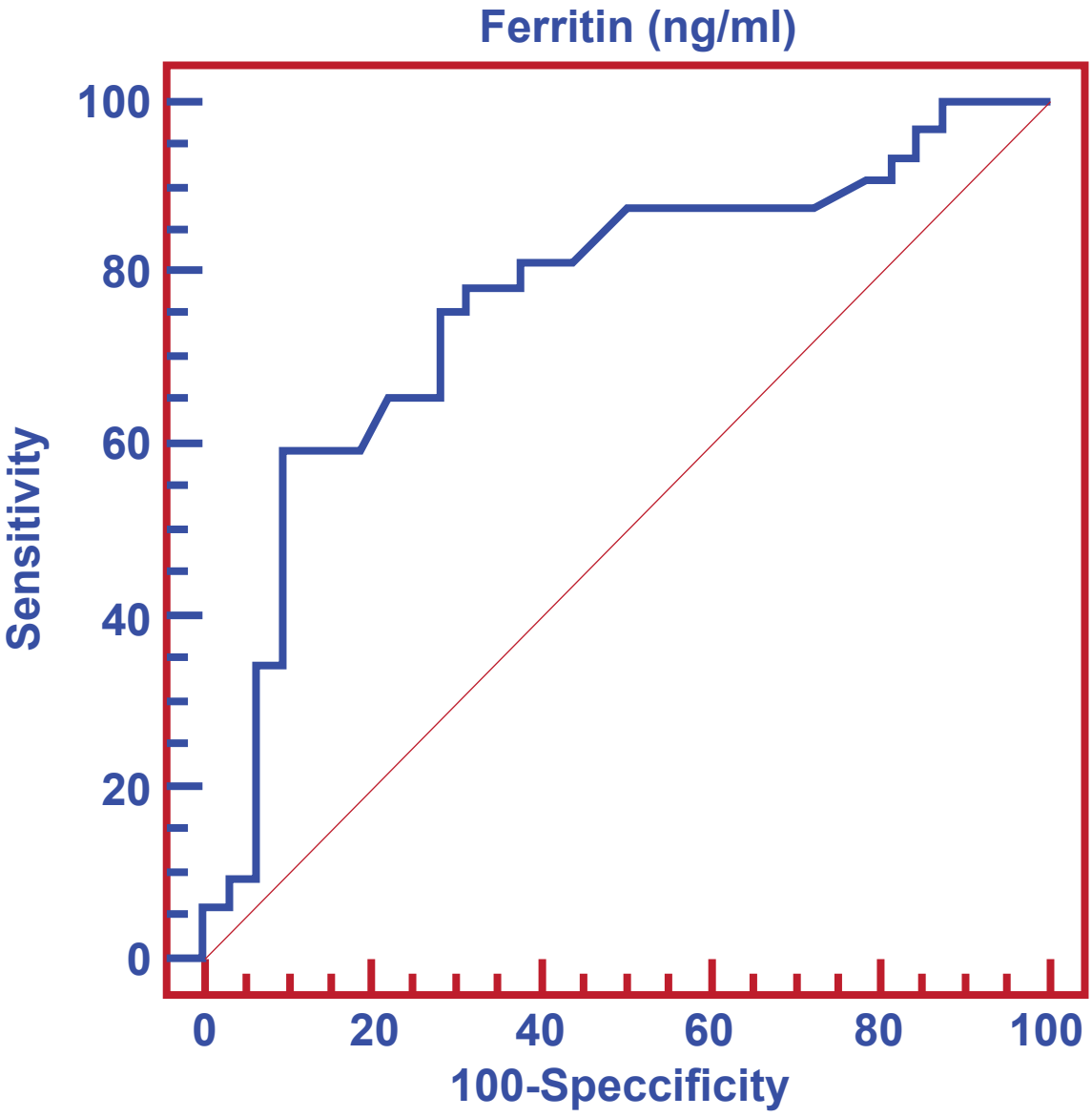
r value = Spearman coefficient of correlation.
RI=resistance index, PI=pulsitility index.

Table (5) Data showing sensitivity, specificity, positive predictive value, negative predictive value of various serum cutoffs to predict asymmetrical IUGR.

Cutoff point	AUC	Sensitivity	Specificity	PPV	NPV
>14.7	0.734	75.00	71.87	72.7	74.2
>15.6	0.687	65.62	71.87	70.0	67.6
>16.4	0.719	65.62	78.12	75.0	69.4
>17	0.703	59.38	81.25	76.0	66.7
>18.2	**0.768**	**59.38**	**90.62**	**86.4**	**69.0**
>20.1	0.625	34.38	90.62	78.6	58.0
>22	0.641	34.38	93.75	84.6	58.8
>25	0.516	9.38	93.75	60.0	50.8
>26	0.531	9.38	96.87	75.0	51.7

AUC=area under the curve, PPV=positive predictive value, NPV=negative predictive value.

Figure (1)
Receiver operating characteristic curve showing the best cutoff point for serum ferritin level between mothers with asymmetric IUGR neonates and those with AGA, was >18.2 ng/mL.



Hematological inflammatory biomarkers affecting the success rate of in vitro fertilization among cases of unexplained infertility
Short running title: Hematological biomarkers and IVF outcome.

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Disclosure statement

The authors guarantee that he:

(1) has stated explicitly, in the cover letter and the main text, that there are no conflicts of interest in connection with this article.

(2) has self-funded this research;

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All of the included women gave their informed consent prior to their inclusion in the study and their anonymity was maintained.

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Abstract

Objective: This study aimed to evaluate the possible relation between the outcome of in-vitro fertilization (IVF) in non-obese females with unexplained infertility, and inflammatory indices obtained from complete blood count (CBC), including white blood cell and platelet counts (WCC and PC), neutrophil-to-lymphocyte-ratio (NLR) and platelet-to-lymphocyte-ratio (PLR).

Methodology: Forty lean cases (BMI <25 kg/m^2) undergoing IVF for unexplained infertility were involved. This study evaluated the effects of CBC inflammatory markers, measured at the outset of ovarian stimulation protocol, on IVF outcomes.

Results: The mean values of CBC parameters were normal, except for 30% leukocytosis and 4% thrombocytosis. Despite similar embryological findings, the women who eventually got pregnant had significantly lower WCC, neutrophil counts (NC), platelet counts (PC) and NLR (all $p<0.001$). On the other hand, thrombocytosis, lymphocyte counts and PLR were similar between women with positive and negative IVF outcomes. A significant correlation was found between platelet count and the number of oocytes and embryos, as well as the number of day-3 and grade I embryos.

The WCC, NC, PC as well as the NLR all had a significant diagnostic performance in predicting clinical pregnancy. The NLR showed the highest AUC (0.89), with a cutoff of ≤2.4, a sensitivity of 0.78 and a specificity of 0.9. On logistic regression analysis, the NC and PC were the most significantly affecting clinical pregnancy rate (p=0.011 and 0.049, odds ratio =0.25 and 0.95, respectively).

Conclusion: Poorer IVF clinical outcomes may be expected if unexplained infertility is associated with elevated WCC, NC, PC or NLR

Keywords: In-Vitro Fertilization, Complete Blood Count, Leukocytosis, Thrombocytosis.

Introduction

Accounting for almost 20% of infertile couples, unexplained infertility is defined whenever the usual cornerstones of fertility, such as ovulation, sperm function and deposition as well as tubal patency and endometrial receptivity, are all apparently normal [1,2].

The previously recorded higher failure rates of IVF among unexplained couples may imply that there may be other factors, such as a hostile inflammatory milieu, immune problems or functional abnormalities in the gametes and the endometrium, having a crucial role [3-9].

Embryonic implantation is a complex molecular and cellular process relying on various factors, most of which have not been clarified completely until now. Implantation needs coordination between various cellular events, such as the trophoblastic evolution and timing of some molecular action processes, which play a crucial role in embryonic apposition, penetration and invasion to the endometrial lining. In spite of good quality embryo transfer, failure of implantation is a relatively common issue that has been attributed to various factors, including a low-grade chronic inflammatory status [10-14]. The later may be defined as raised inflammatory biomarkers, including complete blood count (CBC) indices, such as total leukocytic count (TLC), neutrophil count (NC), and neutrophil-to-lymphocyte ratio (NLR) [5-9]. Furthermore, NLR is a recognised marker of systemic inflammation [15], PLR was recently introduced as a marker for inflammation and thrombosis [16]. The effect of raised inflammatory biomarkers on the outcome of IVF/ICSI remains a poorly studied topic [17,18].

This study will examine the theory whether IVF success rates may be correlated with the level of such inflammatory markers.

Methodology

This was a retrospective study conducted in the IVF unit of Ain Shams University Maternity Hospital on 40 cases with unexplained infertility undergoing long protocol of stimulation for IVF. All results and research data were obtained from medical records between January and August 2018. Inclusion criteria involved cases of unexplained infertility having ≥2 previous IVF failures, with adequate ovarian reserve (FSH >12 mIU/ml, AMH <1 ng/ml, or AFC <5) and no apparent causes of infertility (e.g. male factor, tubal factor) and with BMI ≤25 kg/m^2.

This study also excluded women with endocrine disturbances, chronic renal, hepatic, hypertensive or diabetic women. It excluded those with pelvic inflammatory disease or other chronic inflammatory conditions, hematologic diseases and splenectomy.

All cases had been evaluated by full clinical history and full infertility workup, including Day 3 FSH, LH, estradiol, TSH and prolactin, as well as antral follicle count (AFC). All cases had a CBC test and all indices of interest, particularly CBC inflammatory parameters, were recorded for comparative analysis as regards IVF outcomes.

All cases underwent down regulation using long agonist protocol. All cases have had ovarian stimulation using human menopausal gonadotropins (hMG). Human chorionic gonadotropins (hCG, 10,000 iu) were used to trigger ovulation. Transvaginal oocyte retrieval under sonographic guidance was conducted for all women. All cases underwent intracytoplasmic sperm injection (ICSI) as per the routine protocol in our unit. Recorded embryologic data were obtained for determining oocyte and embryonic features. The embryologic data were correlated to CBC inflammatory markers. The recorded oocyte features included the total number of retrieved oocytes, metaphase II (MII),

metaphase I, germinal vesicles, oocyte anomalies, degeneration and empty zona pellucida. The embryonic features included the total number of embryos, grades 1 to 3 embryos, day 3 embryos with 7-8 cells, day 5 embryos with blastocyst features, transferred embryos. Oocyte die-off ratios (DOR) was calculated from the ratio between MII's and day-3 embryos. The embryo DOR (EDOR) was calculated from the ratio between pronucleate to day-3 embryos [19]. Pregnancy was defined as beta HCG result >25 iu on day 15 post egg collection. Clinical pregnancy was defined as cardiac pulsations detected at 6 weeks of pregnancy. Implantation was defined as visualization of an intrauterine gestational sac by transvaginal ultrasonography. Biochemical pregnancy was defined as one ending before detection of a gestational sac on ultrasonography. Livebirths were defined as living births at a gestational age ≥ 26 weeks. The implantation rate per embryos transferred was defined as the number of gestational sacs detected by ultrasonography divided by the number of embryos transferred. The implantation, biochemical pregnancy, clinical pregnancy and take-home baby rates per embryo transfer were calculated to compensate for different numbers of embryos transferred.

Statistical methods

The collected data were coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 18.0, IBM Corp, Chicago, USA, 2009. The collected variables were compared between the IVF successful and failed cycles. Descriptive statistics was conducted for quantitative research data as minimum and maximum of the range as well as mean ± standard deviation (SD) for quantitative normally distributed research data, while it was done for qualitative research data as number and percentage. Data assumed to be normal for their size, independent t-test in cases of two independent groups. In qualitative data, inferential analyses for independent variables were done using Chi square test for differences between proportions and Fisher's exact test for variables with small expected numbers. Correlations were done using Pearson correlation. Receiver operating characteristic curve (ROC) curve was used to evaluate the performance of different tests differentiating between certain groups. The level of significance was taken at p value <0.05.

Results

The forty patients included were young (<35 years old), normoweight cases of unexplained infertility with adequate ovarian reserve (normal gonodotropin levels and antral follicle counts). They had a mean duration of infertility of 3.5±1.1 years and a mean of 2.9±1 previous IVF failures. Their mean WCC and PC were normal, but twelve women (30%) had leukocytosis while 12.9% (n=4) had thrombocytosis (table 1).

The included patients had a mean of 9.6±2.2 eggs retrieved, with an MII rate of 68.3% and a fertilization rate of 67.7 %. They had a mean of 4.5±1.8 embryos. They had a mean Biochemical pregnancy rate was 5% (n=2), their clinical pregnancy rate was 22.5% (n=9) and the take home baby rate was 20% (n=8). Two women developed twin pregnancies (5%) while one miscarried (2.5%). Out of the 92 embryos which were transferred, the biochemical pregnancy rate per embryo transferred was 12% (n=11), the implantation rate per embryo transferred was 12% (n=11), the clinical pregnancy rate per embryo transferred was 9.8% (n=9) and the take home baby rate per embryo transferred was 8.7% (n=8). Their mean oocyte DOR was 1.8±0.4, while their EDOR was 1.2±0.2 (Table 2).

Despite similar embryological findings (Table 2), the women who eventually got pregnant had significantly lower WCC, NC and platelet counts (PC) and NLR (p<0.001) than those who failed to conceive. The former had fewer cases with absolsute leukocytosis (p=0.04) (Table 1). On the other hand, thrombocytosis, lymphocyte counts (LC) and PLR were similar between both groups (Table 1). A significant correlation was found between platelet count and the number of oocytes and embryos, as well as the number of day-3 and grade I embryos. (Table 3). The WCC, NC and PC as well as the NLR all had a significant diagnostic performance in predicting clinical pregnancy (Table 4) The NLR showed the highest AUC (0.89), with a cutoff of ≤2.4, a sensitivity of 0.78 and a specificity of 0.9. (Table 4)

On Logistic regression analysis (Table 5), the NC and PC were the ones most significantly affecting the clinical pregnancy rate (p=0.011 and 0.049, odds ratio =0.25 and 0.95, respectively).

Discussion

This study recruited relatively young cases with adequate ovarian reserve in an attempt to minimize the effects of embryo number and quality. Furthermore, only cases with a BMI of <25 kg/m2 were enrolled to eliminate any alleged effect of obesity on the development of an inflammatory process [20-22]. It showed a statistically significant difference between women who had a successful IVF cycle versus a failed one in terms of most of their CBC inflammatory variables. Namely, the number of retrieved oocytes as well as the number and quality of the resulting embryos were all significantly correlated to the platelet count. The WCC, NC, PC and the NLR all were predicting clinical pregnancy.

This study disagrees with that of Cakiroglu et al [18], who found that MPV is correlated to the clinical pregnancy rate among PCOS women while the women's age was the only factor affecting success rate among women with unexplained infertility. This may be due to the difference in the study design since the latter did not really examine the differences in CBC between successful and failed IVF cycles, they rather compared obese to normal weight women. On the other hand, TOLA et al found no correlation between the clinical pregnancy rate and any of the CBC inflammatory markers [17]. This may be due to the fact that their study included women of an older age group (up to 40 years old) which may be more affected by problems related to embryo quality or endometrial receptivity [17]. Furthermore TOLA et al did not discuss the details of their differences between the successful and failed cycles in terms of embryo quality [17].

Increasing research evidence implies that infertility is correlated with a chronic low-grade inflammatory process at cellular and molecular levels. In harmony with the current research results, prior studies have revealed higher levels of IFN-γ, TNF-α, IL-2, IL-6 and IL-21, and reduced TGF-β among cases with unexplained infertility during the luteal phase of the menstrual cycle [4,8,23-27].

Previous studies have shown lower IVF success rates among cases of unexplained infertility in comparison to other infertile women [9,11,20]. There was no statistical correlation between the CBC inflammation markers and FSH levels [17]. Few research studies investigated the role of CBC indices on ICSI clinical outcomes [17]. It is hypothesized that implantation failure in unexplained infertility may result from reduced endometrial receptivity and abnormal immune responses. It has been suggested that increased platelet counts, and reduced lymphocytic counts are suggestive of chronic low-grade inflammation [21,23]. Already NLR, PLR and MPV (mean platelet volume) are being suggested as markers of chronic inflammation which may be significantly higher among women with PCOS [28,29].

The chief restrictions in the current study are the small cohort size, mainly related to infertile women in a retrospective design, and using data from a single IVF center. Although this may potentially undermine the value of the observed differences, yet since the clinical, embryological and endocrine parameters were similar in the pregnant and non-pregnant groups; this suggests that the results of the current study are most probably significant. Yet still, the current findings merit more attention and call upon further larger and prospective studies in this field.

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Table (1): Demographic characteristics and laboratory findings of the studied cases; and comparison of clinical pregnancy according to demographic characteristics and laboratory findings

Findings (Total N=40)	Mean±SD	Range	Positive (N=9)	Negative (N=31)	p value
Age (years)	28.5±2.5	25–34	28.3±2.3	28.5±2.6	^0.88
BMI (kg/m^2)	23.1±0.9	21.1–24.5	23.1±0.7	23±0.9	^0.86
Infertility duration (years)	3.5±1.1	2–6	3.4±0.9	3.5±1.1	^0.86
Previous IVF cycles	2.9±1	2-6	4.1±1.2	3.8±1	^0.39
FSH (mIU/L)	7.1±1.5	3.9–12	7.1±1	7.1±1.6	^0.95
LH (mIU/L)	5.1±1.1	3.4–7.2	4.9±0.9	5.1±1.1	^0.59
Prolactin (ng/mL)	13.1±2.4	9.5–19.5	13.4±2.7	13.0±2.4	^0.71
Estradiol (pg/mL)	54.6±7.7	34–67.5	51.8±8.9	55.4±7.3	^0.22
TSH (mIU/L)	2.2±1.3	0.5–5.4	1.9±1.5	2.3±1.3	^0.46
Antral follicle count (AFC)	7.9±1.8	5–11	7.4±1.6	8±1.9	^0.41
White cell count (WCC) (x10^3/mL)	8.5±1.7	5.1–13.9	7.7±1.5	10.1±1.4	^<0.001*
Neutrophil count (NC) (x10^3/mL)	6.1±1.3	3.2–9.7	4.6±1.3	6.5±1	^<0.001*
Lymphocyte count (LC) (x10^3/mL)	1.6±0.6	0.9–4.2	2.3±1.2	2.2±0.6	^0.74
Platelet count (PC) (x10^3/mL)	364.1±49.7	166.0–452.0	316.7±29.7	377.9±45.9	^<0.001*
Neutrophil-to-lymphocyte ratio (NLR)	4.1±1.5	1.2–7.9	2.1±0.5	3.1±0.7	^0.001*
Platelet-to-lymphocyte ratio (PLR)	178.4±59.5	58.5–337.1	159.5±57.4	183.9±59.9	^0.28
	Number	**Percentage (%)**	**Number and Percentage (%)**		**p value**
Leukocytosis (>11.0x10^3/mL)	12	30	0 (0%)	12 (38.7%)	#0.04*
Thrombocytosis (>450x10^3/mL)	4	12.9	0 (0%)	4 (12.9%)	#0.56

Data are presented as mean ±SD or number and percentage
^Independent t-test, #Chi-square test, *statistically significant.

Table (2): Embryological findings of the studied cases; and comparison of clinical pregnancy according to embryological findings.

	Findings	Mean±SD	Range	Positive (n=9)	Negative (n =31)	p value
Oocytes	**Total**	9.6±2.2	6–14	10.6±2.5	9.4±2.1	^0.15
	MII	6.6±1.7	4–9	7.2±1.9	6.4±1.7	^0.21
	MI	1.6±0.7	1–3	2.1±1.4	1.5±0.7	^0.22
	Germinal vesicles	1.2±0.9	0–3	0.8±0.8	1.2±1	^0.29
	Anomaly	0.2±0.4	0–2	0.1±0.3	0.1±0.3	^0.9
	Degenerated	0.1±0.3	0–1	0.3±0.5	0.1±0.4	^0.23
	Empty zona pellucida	0.1±0.3	0–1	0±0	0.1±0.3	^0.08
		Number	**Percentage (%)**	**Number and Percentage (%)**		**p value**
	MII Rate (MII/total)	263/385	68.3%	65/95 (68.4%)	198/290 (68.3%)	#0.98
	Fertilization Rate (Fertilized Oocytes/MII)	178/263	67.7%	65/95 (68.4%)	198/290 (68.3%)	#0.98
		Mean±SD	**Range**			
Embryos	**Total**	4.5±1.8	2–8	5.1±2	4.3±1.7	^0.21
	Grade I	4±1.8	1–8	4.9±2.2	3.7±1.6	^0.08
	Grade II	0.3±0.6	0–2	0.1±0.3	0.4±0.7	^0.24
	Grade III	0.1±0.3	0–1	0.1±0.3	0.1±0.3	^0.89
	Day-3	3.9±1.6	2–8	4.9±2.1	3.6±1.4	^0.14
	Day-5	3.1±1.8	1–7	4.1±2.4	2.8±1.6	^0.17
	Oocyte die-off ratio (DOR; MII/Day-3 embryos)	1.8±0.4	1–3	1.7±0.7	1.9±0.4	^0.19
	Embryo die-off ratios (EDOR; Day-3/Day-5 embryos)	1.2±0.2	1–2	1.1±0.2	1.2±0.2	^0.23
	Embryos Transferred	2.3±0.9	1–3	2.6±0.5	2.2±0.9	^0.19

Data are presented as mean ±SD or number and percentage.
^Independent t-test, #Chi-square test.

Table (3): Correlations of CBC with the different patients's characteristics.

Findings	WCC		NC		LC		PC		NLR		PLR	
	r	p	r	p	r	p	r	p	r	p	r	p
Woman's age	0.28	0.09	0.18	0.26	0.46	0.1	-0.13	0.44	-0.26	0.11	-0.36	0.12
Woman's BMI	0.001	0.99	0.01	0.93	-0.03	0.88	0.07	0.68	0.05	0.78	0.04	0.79
Infertility duration	-0.15	0.36	-0.21	0.2	-0.2	0.21	0.06	0.7	-0.07	0.68	0.11	0.49
Previous IVF	-0.1	0.54	-0.04	0.81	0.11	0.49	-0.004	0.98	0.02	0.92	-0.11	0.49
FSH	0.06	0.73	0.09	0.6	0.16	0.34	-0.06	0.73	-0.05	0.74	-0.19	0.24
LH	0.17	0.29	0.18	0.28	0.21	0.19	0.09	0.59	-0.04	0.83	-0.05	0.74
Prolactin	-0.05	0.77	-0.03	0.88	0.16	0.32	-0.22	0.17	-0.12	0.45	-0.39	0.11
Estradiol (pg/mL)	0.31	0.06	0.27	0.09	0.29	0.07	0.12	0.46	-0.12	0.46	0.2	0.22
TSH (mIU/L)	-0.08	0.63	-0.02	0.93	-0.2	0.21	-0.21	0.2	0.24	0.14	-0.25	0.11
Antral follicle count (AFC)	0.18	0.27	0.22	0.18	0.07	0.69	-0.03	0.86	0.03	0.84	-0.15	0.37
Total oocytes	0.07	0.69	0.01	0.95	0.04	0.81	-0.36	0.02*	-0.14	0.39	-0.11	0.49
MII	-0.19	0.23	-0.18	0.28	0.02	0.89	-0.26	0.1	-0.09	0.59	-0.06	0.72
MI	-0.17	0.3	-0.14	0.38	0.02	0.91	-0.38	0.02*	-0.07	0.69	-0.09	0.6
Germinal vesicles	-0.27	0.09	-0.31	0.06	-0.16	0.31	0.06	0.71	-0.14	0.4	-0.12	0.47
Anomaly	0.21	0.21	0.24	0.14	0.2	0.21	-0.18	0.26	0.03	0.85	-0.13	0.41
Degenerated	-0.001	0.99	-0.03	0.85	0.05	0.75	-0.05	0.75	-0.13	0.43	0.1	0.55
Empty zona pellucida	-0.22	0.17	-0.21	0.21	-0.01	0.94	0.09	0.6	-0.03	0.88	0.17	0.31
MII Rate	0.07	0.68	0.08	0.63	-0.12	0.46	0.07	0.69	0.26	0.11	0.08	0.61
Fertilization Rate	-0.02	0.89	0.004	0.98	-0.02	0.88	-0.38	0.02*	0.04	0.82	-0.18	0.28
Total Embryo	-0.16	0.32	-0.16	0.34	0.07	0.67	-0.35	0.03*	-0.13	0.44	-0.13	0.44
Grade I	-0.21	0.19	-0.19	0.24	0.08	0.63	-0.45	0.01*	-0.13	0.42	-0.14	0.4
Grade II	-0.26	0.11	-0.22	0.18	0.07	0.66	0.11	0.51	-0.13	0.42	-0.01	0.98
Grade III	0.04	0.8	0.03	0.84	-0.02	0.92	0.27	0.1	0.01	0.97	0.06	0.73
Day-3	0.15	0.35	0.09	0.58	0.07	0.65	-0.35	0.03*	-0.02	0.9	-0.09	0.58
Day-5	-0.24	0.15	-0.2	0.21	0.07	0.65	-0.28	0.08	-0.11	0.51	-0.05	0.77
DOR	-0.29	0.07	-0.27	0.1	0.03	0.86	0.27	0.09	-0.12	0.46	0.14	0.37
EDOR	0.14	0.38	0.12	0.47	-0.07	0.67	-0.08	0.61	0.07	0.68	-0.11	0.51

r: Pearson correlation, p: p-value, *: statistically significant.

Table (4): Diagnostic performance of CBC in predicting clinical pregnancy

CBC	AUC	SE	p-value	95% CI	Cut off	Sensitivity	Specificity
White cell count	0.88	0.071	0.001*	0.71–1	≤8.2	0.78	0.9
Neutrophil count	0.87	0.07	0.001*	0.71–1	≤5.5	0.78	0.87
Lymphocyte count	0.57	0.12	0.56	0.34–0.79	≤1.9	0.56	0.65
Platelet count	0.86	0.06	0.001*	0.74–0.98	≤345	0.89	0.74
Neutrophil-to-lymphocyte ratio	0.89	0.06	<0.001*	0.74–1	≤2.4	0.78	0.9
Platelet-to-lymphocyte ratio	0.58	0.11	0.51	0.36–0.78	≤209	0.89	0.36

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

Table (5): Logistic regression for factors affecting clinical pregnancy rate

Factor	β	SE	p-value	OR (95% CI)
Neutrophil count	-1.39	0.55	0.011*	0.25 (0.09–0.73)
Platelet count	-0.05	0.03	0.049*	0.95 (0.9–1)
Constant	24.19	10.02	0.016*	--

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