Pregnancy Outcomes in Women with Recurrent Miscarriage Associated with Antiphospholipid Antibodies Treated with Low Dose Aspirin and Unfractionated Heparin

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Abstract: Objective To determine maternal and fetal outcomes in women with APS managed with aspirin or unfractionated heparin (UFH) plus aspirin during pregnancy. **Design:** prospective cohort study. **Setting**: high-risk pregnancy unit- Benha university hospital. **Methods:** Pregnant women with APS attending at high-risk pregnancy unit. Seventy seven selected patients with clinical and/or serological findings of antiphospholipid syndrome were divided into 2 groups: ***group A*** (*n* = 47) had received low-dose aspirin (81 mg once daily orally) plus heparin (5000 IU) every 12 h while group B (*n* = 30) had received aspirin (81mg once daily orally) with the first positive pregnancy test. **Main outcome measures:** Maternal outcomes included thromboembolic and haemorrhagic complications and pregnancy-induced hypertension .Prematurity, intrauterine growth restriction and neonatal death were considered as maternal and fetal complications. **Results**: There were significant differences in antenatal and maternal complications between the groups. Aspirin plus UFH was more efficacious than aspirin alone in women with antiphospholipid syndrome and recurrent miscarriage. There were significant differences between **Groups A** and **B** in the rate of miscarriages [3 miscarriages in **Group A** (6%) versus 8 miscarriage in **Group B** (27%); p = 0.03], the mean gestational age [38±1.73weeks versus 36 ±1.57; p <0.0001], the neonatal birth weight [3352.27 ± 368.2 versus 2620.45 ± 370.54 gm; p <0.0001] and pre-eclampsia [4/44 (9%) versus 8/22 (36%); p =0.03]. Although not statistically significant, women in **Group A** tended to have higher rates of number of live births [44/47(94%) versus 22/30(73%); p =0.49] but have lower rates of IUGR [5/44 (11%) versus 4/22 (18%); p =0.51] and preterm births [5/44 (11%) versus 6/22 (27%); p =0.18] than women in **Group B**. **Conclusions:** Use of low dose aspirin and heparin (5000 IU) every 12 h subcutaneously in patient with recurrent pregnancy loss due to antiphospholipid syndrome resulted in higher live birth rates compared to using low dose aspirin alone.
[Ahmed M.Abdelaziz and Khalid abd Aziz Mohammad. **Pregnancy Outcomes in Women with Recurrent Miscarriage Associated with Antiphospholipid Antibodies Treated with Low Dose Aspirin and Unfractionated.** *J Am Sci* 2014;10(1):22-29]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 6

Keywords: Anticoagulation, antiphospholipid syndrome, Aspirin, heparin, pregnancy, recurrent miscarriage

1- Introduction

Recurrent pregnancy loss (RPL) is a major problem affecting 1–2% of women of reproductive age. While Chromosomal aberrations, endocrinological dysfunction, uterine abnormalities are aetiological factors, until recently in most cases, a cause for RPL could not be identified (1, 2). Gestational outcome in women with inherited thrombophilias who present with RPL is poor with less than 25% of pregnancies resulting in live birth (3). RPL is a well-established finding in women with antiphospholipids syndrome (APS) (4). The APS was first described in 1986 by Highes Haris and Gharavi as a disorder in which antibodies are produced against a variety of phospholipids and phospholipids binding proteins. Clinical manifestations may range from no symptoms to immediately life threatening catastrophic APS (5, 6).

Antiphospholipid antibodies (APA) are a group of autoantibodies that bind to negatively charged phospholipids. These antibodies have been associated with thrombotic events which could lead to pregnancy loss (7)

A variety of mechanisms by which antiphospholipid antibodies may cause pregnancy loss and thrombosis have been suggested. Antiphospholipid antibodies may interfere with the normal *in vivo* function of phospholipids or phospholipid-binding proteins that are crucial to the regulation of coagulation. Candidate molecules or pathways that might be adversely affected include β_2-glycoprotein I (which has anticoagulant properties), prostacyclin, prothrombin, protein C, annexin V, and tissue factor. Antiphospholipid antibodies may activate endothelial cells, as indicated by increased expression of adhesion molecules, secretion of cytokines, and production of arachidonic acid metabolites. Other evidence suggests that antiphospholipid antibodies cross-react with oxidized low-density lipoprotein and bind only to oxidized cardiolipin, (8) implying that antiphospholipid antibodies may participate in oxidant-mediated injury of the vascular endothelium.

The *in vivo* target(s) of antiphospholipid antibodies remain unknown. Normal, living cells do not express phospholipids bound by antiphospholipid

antibodies on their surface. The antibodies do, however, bind to phospholipids expressed by perturbed cells, such as activated platelets or apoptotic cells. Recent work points to the complement system as having a major role in antiphospholipid syndrome–related pregnancy loss, showing that C3 activation is required for fetal loss in a mouse model(9).

Exogenous heparin has been shown to inhibit the binding of APA *in vitro* in a dose– response manner; thus, endogenous heparin produced by trophoblasts may function in a similar fashion (10). The antithromboxane effects of aspirin on inhibition of platelet aggregation are thought to work in concert with heparin to promote and enhance implantation (11, 12).While low dose aspirin may improve placental blood flow by decreasing the thromboxane to prostacyclin ratio, thus enhancing implantation (13).

A number of studies have evaluated the efficacy of treatment with low-dose aspirin, prednisolone, unfractionated low-molecular weight heparin and most recently intravenous gamma globulin, either alone or in various combinations. However, the findings have not been consistent [14, 15]. Low-dose aspirin in combination with heparin was demonstrated in 2 randomized controlled trials to lead to a significant improvement in the live birth rate [16, 17]. This study aimed to determine the pregnancy outcome in women with APS and recurrent pregnancy loss who were treated with aspirin alone or aspirin in combination with heparin during the index pregnancy.

In prospective studies, the use of s.c. heparin and aspirin has resulted in successful deliveries in >75% cases of women with RPL and APA with a low frequency of obstetric and maternal complications (16, 17).

2- Patients & Methods

This prospective comparative, cohort study was conducted at Department of Obstetrics and Gynecology, Benha University Hospital, and a private center, from June 2012 till October 2013. After approval of the study protocol by the Local Ethical Committee and obtaining written fully informed patients' consents. All patients were interviewed about their medical, personal, family, obstetrical and thrombosis history. All patients included in study met strictly with clinical and/or serological findings of antiphospholipid syndrome syndrome that is as following:

Clinical criteria

1. Vascular Thrombosis

One or more episodes of arterial, venous or small vessel thrombosis occurring in any tissue or organ.

2. Pregnancy Morbidity

a. One or more unexplained deaths of morphologically normal foetuses at or after 10 weeks of gestation with normal foetal morphology documented by ultrasound or direct foetal examination.

b. One or more premature births of morphologically normal foetuses before 34 weeks of gestation because of:-

i. Eclampsia or severe pre-eclampsia defined according to standard definitions or

ii. Recognised feature of placental insufficiency.

c. Three or more unexplained consecutive spontaneous abortions before10 weeks of gestation with maternal anatomic or hormonal abnormalities and parental chromosomal causes excluded.

Laboratory Criteria

1. Lupus anticoagulant (LA): In plasma, present on two or more occasions at least 6–12 weeks apart.

2. Anticardiolipin (aCL) of IgG and/or IgM isotype: in plasma present in medium or high titres at least 6–12 weeks apart.

(Definite antiphospholipid syndrome may be diagnosed if at least one of the clinical criteria and at least one of the laboratory criteria are met)

All patients (n=77)were offered baseline tests including aCL, LA and repeated after 12 weeks before pregnancy and findings noted. All selected patients were in good general health without previous history of Diabetes Mellitus or thyroid dysfunction or cardiac disease. Patients with Thrombocytopenia (<100000/ml), bleeding tendencies, ectopic pregnancy, past history of vascular thrombosis and multiple gestation were excluded from the study.

Baseline complete blood picture, routine urine analysis, blood sugar, blood grouping, Bleeding Time, Clotting Time, Prothrombin Time, Activated Partial Thromboplastin Time, Hepatitis B Surface Ag, Hepatitis C Virus screening were offered to all patients and findings noted as soon as they conceived. Anti Xa level were not tested. All selected patients were given routine Folic Acid, Iron and Calcium supplementation orally daily during antenatal period (whether conceived spontaneously or with treatment).

All were put on tab Aspirin 81 mg/day (Juspirin) daily with the first positive pregnancy test. ***Group A*** (n = 44) were put on Inj. UFH (Cal-heparin) 5000 U subcutaneous twice daily at the same time, Inj. UFH was given either into anterior abdominal wall or anterior aspect of thigh subcutaneously. While ***group B*** (n = 30) received Aspirin 81 mg/day (Juspirin) daily only.

Women with RPL and APA who desired treatment initiated treatment with s.c. heparin (5000 IU) every 12 h with the first positive pregnancy test. Platelets and PTT were obtained 6 h after heparin injection weekly for 2 weeks after the initiation of heparin therapy and 1 week following any adjustment in heparin dosage. Thereafter, platelets and PTT were checked periodically to ascertain that they were in the normal range. The heparin dosage was adjusted downward if the PTT was elevated outside the normal range or if the platelet count fell to 100000/ml. Supplementation with calcium carbonate to achieve a total daily intake of 1.5 g/day was prescribed for all patients, an approach that has been reported to counteract the osteoporotic effects of heparin (18).

Each pregnancy was documented by transvaginal ultrasonography scheduled at 7 weeks gestation for the determination of fetal heart motion. Additional ultrasonography was performed as indicated, but generally baseline sonograms were obtained at 20 weeks. Antenatal testing (fetal kick counts, non-stress tests, or biophysical profiles) was initiated at 28–30 weeks when indicated. Aspirin was discontinued 2 weeks before the estimated due date.

No woman experienced any major hemorrhagic event during pregnancy labour or post-partum. Five patients developed mild unexplained vaginal bleeding which settled by expectant management. Discontinuation of medicine was not required due to hemorrhagic problems. Patients were advised to visit fortnightly.

Foetal growth was monitored by fundal height measurement and serial ultrasounds. Doppler umbilical wave flow velocity was studied for foetuses with suspected intrauterine growth retardation. All patients were given Aspirin 81 mg daily till 37 weeks completed. Aspirin was stopped to prevent bleeding in labour. Heparin was continued until full term and was discontinued when the patient initiated spontaneous labour. The evening heparin dose was omitted prior to planned amniocentesis or a scheduled operative delivery. For some patients Aspirin and Heparin were discontinued earlier due to abortion, preterm labour, intrauterine growth retardation or pre-eclampsia leading to premature delivery.

Outcome evaluation

The primary outcome measure was the rate of live births. Secondary outcomes included rates of miscarriage, intrauterine fetal death (fetal death after 20 weeks of gestation), and obstetrical complications. Such complications included preeclampsia, small size for gestational age (birth weight below the 10^{th} percentile for gestational age and sex), placental abruption, and premature delivery.

Statistical analysis

Results were expressed as mean±SD, range, numbers and percentages. Intra-group data were statistically analyzed using paired t-test and inter-group analysis was examined using Chi-square test (X^2 test). Statistical analysis was conducted using SPSS statistical program, (Version 10, 2002). *P* value <0.05 was considered statistically significant.

3- Results

A total of 77 women with APS were included in the study: 47 women in **group A** received low-dose aspirin once plus UFH 5000 IU twice daily and 30 women in **Group B** used low-dose aspirin (juspirin) only and. ***Table 1*** shows the demographic details of the women. There were no significant differences in the patient's age at entry, weight, prior live births, prior miscarriages and prior IUFD.

As shown in ***Table 2***, there was a significant difference between **Groups A and B** in the rate of miscarriages [3 miscarriages 8 in **Group A** (6%) versus 8 in **Group B** (27%); p = 0.03]. Most miscarriages in the two groups occurred in the first trimester (3(6%) in Group A and 6 (20%) in Group B).

The number of live births was not significantly different between the two groups but the rates were higher in **Group A** than in **Group B**. the number of live births was 44/47(94%) in **Group A** versus 22/30(73%) **in Group B**; p = 0.49].

The mean gestational age at delivery and the mean birth weight were highly significant in **Group A** than in **Group B** .The mean gestational age at delivery in **Group A** was 38 ±1.73 weeks versus 36 ±1.57 in **Group B**; p <0.0001. The mean birth weight in **Group A** was 3352.27 ± 368.2 versus 2620.45 ± 370.54 grams in **Group B**; p <0.0001). There were no intrauterine or neonatal deaths in the study ***(Table 3)***.

As shown in ***Table 4***, there was a significant difference between **groups A** and **B** in the rate of pre-eclampsia [4/44 (9%) versus 8/22 (36%); p =0.03].

Although not statistically significant, women in **Group A** tended to have lower rates of IUGR [5/44 (11%) versus 4/22 (18%); p =0.51] and preterm births [5/44 (11%) versus 6/22 (27%); p =0.18] than in **Group B** ***(Table 4).***Also there was no statistically significant difference in the mode of delivery.

Sixteen of the 67 women with successful pregnancies (24%) delivered prematurely (<37 weeks' gestation). five of them was in Group **A** (17%) and due to preterm labor while the remaining 11 was in **Group B** (50%) [6(27%) was due to preterm labor, 4 (18%) due to severe preeclampsia and 1(5%) due to IUGR)].the difference was significant between the two groups (p = 0.01).

Intrauterine growth retardation (estimated fetal weight below the 10th centile for gestational age) was the indication for delivery in 5 women in **Group A** (11%) and for 4 women in **Group B** (18%). No woman developed a thromboembolic complication during pregnancy or the puerperium ***(Table 4)***.

All babies were examined by a paediatrician shortly after delivery. No congenital abnormalities were detected.

Ten Babies (15%) were admitted to the neonatal unit because of prematurity. Five babies (in **Group B**), delivered by caesarean section for IUGR and preeclampsia, required ventilator support for a week. The other five babies (3 in **Group A** and 2 in **Group B**) were admitted to the neonatal unit for help with feeding.

Both low dose aspirin and UFH were well tolerated. Of those taking heparin, none developed thrombocytopenia or had symptomatic complications apart from mild bruising localised to the injection site.

Table 1: Age and pregnancy characteristics of women treated with aspirin only or aspirin plus UFH:

Variables	Aspirin plus UFH Group A (n=47)	Aspirin alone Group B (n=30)	Test of significance	*p*-value
Maternal age (years)	30.34±4.51	30.1±4.397	T=0.23	= 0.81
Weight	71.72 ± 7.33	70.17 ± 7.41	T=0.90	= 0.37
Previous miscarriages	4.12±1.52	4.03±1.47	T=0.26	= 0.798
Prior live births (%)	20/47(42.5%)	13/30(43%)	X^2=0.001	= 0.97
prior IUFD (No.)	11/47(23%)	9/30(30%)	X^2=0.24	= 0.62

Data are presented as percentage or mean ±standard deviation.
T =t-test. X^2= Chi-square test

Table 2: Outcome data from patients who had miscarriage:

Variables	Aspirin plus UFH Group A (n=47)	Aspirin Group B (n=30)	Test of significance	*p*-value
Miscarriages (%)	3/47(6%)	8/30(27%)	X^2= 4.47	= 0.03
EGA at loss (weeks)	10±1	12.25 ±3.45	T= -1.08	= 0.3

Data are presented as percentage or mean ±standard deviation.
T =t-test. X^2= Chi-square test

Table 3: Outcome data from patients who had live births:

Variables	Aspirin plus UFH Group A (n=47)	Aspirin Group B (n=30)	Test of significance	*p*-value
Live births (%)	44/47(94%)	22/30(73%)	X^2= 0.49	= 0.49
EGA at birth (weeks)	38 ±1.73	36 ±1.57	T=4.56	< 0.0001
Birth weight (gm)	3352.27 ± 368.2	2620.45 ± 370.54	T=7.6	< 0.0001
Mode of Delivery	(n=44)	(n=22)	X^2=0.005	= 0.94
1. Caesarean Section	35(74%)	19(86%)		
2. Vaginal delivery	9(27%)	3(14%)		

Data are presented as percentage or mean ±standard deviation.
T =t-test. X^2= Chi-square test

Table 4: Obstetric and maternal complications of patients who delivered a live born:

Variables	Aspirin plus UFH Group A (n=44)	Aspirin Group B (n=22)	Test of significance	*p*-value
Pre-eclampsia (No.)	4(9%)	8(36%)	X^2=4.77	= 0.03
Preterm delivery (No.)	5(11%)	6(27%)	X^2= 1.83	= 0.18
Prematurity (N0)	5(11%)	11(50%)	X^2= 6.72	= 0.01
Intrauterine growth retardation (No.)	5(11%)	4(18%)	X^2= 0.43	= 0.51

Data are presented as percentage or mean ±standard deviation.
T =t-test. X^2= Chi-square test

4- Discussion

APS is widely recognized as a risk factor for numerous obstetric complications, including recurrent miscarriage, IUGR, pre-eclampsia, fetal death and preterm labour. Since its original description, APS has emerged as the most important treatable cause of recurrent miscarriage [19].

A variety of treatment regimens have been used, both single agents and combinations, to improve the poor live birth rates among these women, with live births reported from 30% to 100% of pregnancies [20]. However, treatment of pregnant aPL-positive women to improve pregnancy outcome remains completely empirical.

The present standard of care for women with aPL and RPL is treatment with heparin and low dose aspirin [21]. There have been a number of randomized control trials for patients with RPL due to aPL evaluating either unfractionated heparin (UFH) or LMWH over the past 15 years. Each trial determined its inclusion criteria; however, regardless of differences in aPL status among the various trials, the live birth rates were similar ranging from 71.1 to 84%. The only significant differences among trial outcomes were in the LDA-only treatment arms: the live birth rates in those varied from a low of 42.2% to a high of 80% [21].

There were no major adverse events associated with both treatment arms and this result was in agreement with an RCT done by Carla *et al.* to compare live birth rates in women with RPL and either autoantibodies or a coagulation abnormality, treated with heparin plus aspirin (LMWH/ASA) or ASA alone [22].

The therapeutic benefit of heparin is thought to arise from its ability to bind aPL. By doing so, the pathological interaction between aPL and the trophoblast and maternal decidual vessels is inhibited, and placentation is more likely to be successful. Later in pregnancy the anticoagulant properties of heparin are likely to be beneficial in reducing the risk of placental thrombosis and infarction. Aspirin, by inhibiting platelet aggregation, also has a favourable thromboprophylactic effect. Unfractionated and low molecular weight heparins have been shown to be equally beneficial in the treatment of women with PAPS, the latter having the advantage of being a once daily injection (23).

Studies on human tissue and in mice suggest that phospholipid antibodies cause pregnancy loss by binding to phospholipids expressed on the invading trophoblast (24, 25) thereby inhibiting successful embryonic implantation into the endometrium. Once placentation is established their thrombogenic action leads to decreased placental perfusion and subsequent infarction (26, 27).

Low dose aspirin may improve pregnancy outcome in women with phospholipid antibodies by irreversibly blocking the action of cyclo-oxygenase in platelets, thereby inhibiting platelet thromboxane synthesis and preventing thrombosis of the placental vasculature.(28) Heparin may act to reduce fetal loss by binding to phospholipid antibodies, thereby protecting the trophoblast phospholipids from attack(29) and promoting successful implantation in early pregnancy, in addition to its anticoagulant action. This is supported by the finding that there was no difference in pregnancy outcome between the two treatment arms in the pregnancies that survived beyond 13 weeks' gestation. By this time the first wave of trophoblast invasion is complete and placentation established.

Heparin is a polymer of acidic, sulphated disaccharides, derived from porcine or bovine mucosa. The length of the polysaccharide chain determines the properties of the molecule—shorter chains are low molecular weight heparins and longer chains are high molecular weight (or unfractionated) heparins. The length of the chain also determines the properties of the molecule. Heparin binds to and potentiates the action of antithrombin III, inducing a conformational change. Unfractionated heparin binds thrombin and antithrombin III simultaneously, facilitating the inactivation of thrombin. The conformational change in antithrombin III induced by heparin allows the molecule to bind to, and inactivate, factors involved in the clotting cascade (30)

Our study investigated women with APS in the index pregnancy treated with both aspirin and UFH together (**Group A**) or aspirin alone **(Group B)**. Analysis of the data reveal led statistically significant difference between the treatment groups in terms of the mean gestational age at delivery [38± 1.73 versus 36±1.57 weeks; p <0.0001], the mean birth weight [3352.27±368.2 versus 2620±370.54 grams ; p <0.0001] and pre-eclampsia [4/44 (9%) versus 8/22 (36%); p =0.03]. The number of live births was not significantly different between the two groups but the rates were higher in **Group A** than in **Group B** 44/47(94%) in **Group A** versus 22/30(73%) **in Group B**; p = 0.49] Preterm deliveries were experienced by 11% of women in group A compared with 27% in group B, while 11% and 18% of women suffered IUGR in groups **A** and **B** respectively. These results are comparable to international data [*17, 31-34*].

The live birth rate in our present study was 94% for **group A** (Aspirin only) and 73% for **group B** (Aspirin plus heparin). These data are in agreement with prospective reports that have shown a successful obstetric outcome with heparin and aspirin therapy and/or aspirin alone therapy in women with positive AP antibodies (15- 17).

A prospective study by ***Kutteh,1997*** revealed that viable infants were delivered 20/25 (80%) women treated with heparin and aspirin and of 11/25 (44%) women treated with aspirin (p= 0.2)and this agreed with our present study[44/47(94%) versus 22/30(73%); p =0.49].And there were no significant differences between the heparin plus low-dose aspirin and the low-dose aspirin groups with respect to gestational age at delivery (37.8 ± 2.1 weeks vs 37.2 ±3.4) these disagreed with our results[37.91±1.2 versus 36.54± 2.99 weeks; p =0.0001], while agreed with ours in difference in number of cesarean sections, or complications) [*16*]. A comparable trial also found aspirin alone inferior to aspirin plus heparin (42% versus 71% live births) [*17*]. But, ***Farquharson et al., 2002*** found the birth rate to be similar in both groups (72% with aspirin alone compared with 78% when heparin was added to the regimen) [*35*].

Backos et al., 1999 agreed with our present study in that combination treatment with aspirin and heparin leads to a high live birth rate among women with recurrent miscarriage and antiphospholipid antibodies (71%) resulted in a live birth and (27%) miscarried, the majority in the first trimester. Gestational hypertension complicated 17% (18/108) of ongoing pregnancies and antepartum haemorrhage 7% (8/108). Twenty-six babies (24%) were delivered before 37 weeks of gestation. Fifty women (46%) were delivered by caesarean section. The median birth weight of all live born infants was 3069 g (range 531-4300); however 15% (16/108) of the infants were small for gestational age (33).

Rai et al., 1997 Treatment with aspirin and heparin leads to a significantly higher rate of live births in women with a history of recurrent miscarriage associated with phospholipid antibodies than that achieved with aspirin alone. While in our study the rate of live births was higher in heparin plus low dose aspirin than in low dose heparin group but this did not reach a statistically significant level. Twelve of the 51 women with successful pregnancies (24%) delivered prematurely (<37 weeks' gestation) and this was comparable to our results 16/64 (24%). This was due to preterm labor in 2/19 women treated with low dose aspirin (11%) and in 5/32 women treated with low dose aspirin and heparin (16%; P=0.70). Intrauterine growth retardation (estimated fetal weight below the 10th centile for gestational age) was the indication for delivery in one woman treated with low dose aspirin (5%) and for three women treated with low dose aspirin and heparin (9%; P=0.99). One woman treated with low dose aspirin alone was delivered at 36 weeks' gestation for pre-eclampsia. No woman developed a thromboembolic complication during pregnancy or the puerperium (17).

Our study suggests that the women with APS and in pregnancy can be treated effectively with low-dose aspirin plus heparin. As this study did not include untreated controls, we cannot exclude the possibility that aspects of obstetric care other than the treatment influenced pregnancy outcome. There have been a number of randomized control trials for patients with APS evaluating either unfractionated heparin (UFH) over the past 15 years. Each trial determined its inclusion criteria the live birth rates were similar ranging from 71.1 to 84%. The only significant differences among trial outcomes were in the low dose aspirin only treatment arms: the live birth rates in those varied from a low of 42.2% to a high of 80% [26, 38].

Conclusion:

Combination treatment with aspirin and heparin leads to a high live birth rate among women with recurrent miscarriage and antiphospholipid antibodies. This combination may promote successful embryonic implantation in the early stages of pregnancy and protect against thrombosis of the uteroplacental vasculature after successful placentation. Future studies should be aimed at refining the protocol used in this trial to determine the benefits of preconceptional administration of heparin and whether it can be stopped after 13 weeks' gestation without adversely affecting the rate of live births.

Acknowledgement:

We would like to thank our staff of Obs./Gyn. Department, Benha Faculty of Medicine for supporting and their advises to complete this work.

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1/8/2014