Original Article

Endometrial Spiral Artery Doppler Parameters, Vitamin D, B2 Glycoprotien 1 Assessment in Cases with Recurrent Unexplained Pregnancy Loss

Samir M. El Sayed, Tarek M. Tappozada and Wael S El Gazayerli

Department of Obstetrics and Gynaecology, Faculty of Medicine, Alexandia University, Egypt

ABSTRACT

Background: Recurrent pregnancies loss (RPL) is physically andemotionally devasting situation for the parents, along with difficult situationfor obstetrician to handle.

Aim of the Work: The present study aimed at investigation of the significanceof various etiology in relation to pregnancy outcome in cases ofunexplained recurrent pregnancy loss.

Materials And Methods: It is a retrospective analysis of 100 patientswho presented to El-Shatby maternity university hospital, betweenMay 2017 to October 2018, with history of two or more unexplained earlytrimester abortions fullfiling the common investigations done for thesecases. all the patients during mid-luteal phase, examined by transvaginal3D pulse Doppler ultrasound to detect endometrial perfusion with measuringin the sera 25-hydroxy vitamin D3 and B2 glycoprotein 1 antibodieswhich is a marker of antiphospholipid syndrome. evaluation of uterineartery blood flow and subendometrial blood flow, detection uterine arterypulsatility index (UAPI) and subendometrial blood flow presented by the indiciesvascularisation index (VI), flow index (FI) and vascular flow index(VFI).

Results and Conclusion: All of the studied parameters namely; doppler indices, vitamin D level and B2glycoprotien were significantly related to the incidence of the pregnancy loss and should be tested in those particular category of women who are at risk of recurrent pregnancy loss..

Key Words: B2 glycoprotein 1, pulsatility index, recurrent pregnancy loss, vitamin D3.

Received: 16 October 2022, **Accepted:** 29 October 2022

Corresponding Author: Wael S. El Gazayerli, Department of Obstetrics and Gynecology, Faculty of Medicine, Alexandia University, Egypt, **E-mail:** welgazayerli@gmail.com

ISSN: 2090-7265, November 2022, Vol.12, No. 4

INTRODUCTION

Recurrent pregnancy loss is an important reproductive health issue, affecting 2%–5% of couples. Common established causes include uterine anomalies, antiphospholipid syndrome, hormonal and metabolic disorders, and cytogenetic abnormalities[1]. Other etiologies have been proposed but are still considered controversial, such as chronic endometritis, inherited thrombophilias, luteal phase deficiency, and high sperm DNA fragmentation levels.[2] Over the years, evidence-based treatments such as surgical correction of uterine anomalies or aspirin and heparin for antiphospholipid syndrome have improved the outcomes for couples with recurrent pregnancy loss. However, almost half of the cases remain unexplained and are empirically treated using progesterone supplementation, anticoagulation, and/or immunomodulatory treatments[3] Regardless of the cause, the long-term prognosis of couples with recurrent pregnancy loss is good, and most eventually achieve a healthy live birth. However, multiple pregnancy losses can have a significant psychological toll on affected couples, and many efforts are being made to improve treatments and decrease the time needed to achieve a successful pregnancy.[4]

In fertile women uterine spiral artery perfusion has been found to improve during the luteal phase, which coincides with the implantation window[5]. Endometrial receptivity is regulated by many factors including uterine perfusion[6,7]. Several studies have shown that uterine receptivity is decreased when the uterine artery impedance has been increased during the mid luteal phase[8]. Abnormal uterine perfusion may be a contributing factor to etiopathology of infertility especially in couples with unexplained infertility.

Recent studies show that autoimmune dysregulation is a probable cause of RPL, which in some cases may be overlooked.[9] Vitamin D has been shown to promote a more favorable environment for pregnancy through various mechanisms, such as enhancement of the shift toward Th2 cells and regulation of immune cell differentiation and cytokine secretion.[9] Therefore, it seems that vitamin D deficiency sways the balance toward a worse outcome and may play a part in recurrent pregnancy loss.[10]

Although it was initially thought thatantiphospholipid syndrome(aPL) reacts directly with phospholipids, subsequent studies have shown that critical epitopes also

 DOI:10.21608/ebwhj.2022.169208.1217

involve a number of phospholipids-binding proteins.[11] The most prevalent of proteins is beta2-glycoprotein I.[12] Considering that beta2-glycoprotein I (b2GPI) is a main target of aPL antibodies, anti-b2GPI antibodies may play a role in their pathogenesis and may also serve as a biological marker for APS.[13]

This study aimed to investigate of the significance of various etiologies in relation to pregnancy outcome in cases of unexplained recurrent pregnancy loss namely; endometrial spiral artery doppler parameters, vitamin D and B2 glycoprotein 1.

MATERIAL AND METHODS

This is retrospective study carried out on one hundred patients who presented to El Shatby Maternity university hospital, between May 2017 and october 2018. All the participants signed on informed consent. The patients were divided into two groups each includes 50 patients. Group I includes 50 patients with history of two or more unexplained early trimester abortions, and Group II includes 50 patients as a control group.

The inclusion criteria for the study group Group I were the followings:Not being pregnant, having had regular menstrual cycles in the 3 months preceding enrollment using neither hormonal contraception nor an intrauterine device. All the patients have a history of two or more unexplained miscarriages in the first trimester of pregnancy fathered by the same partner, and having no living child. Autoimmune and endocrine disorders were ruled out in women with unexplained RPL. Results of glucose tolerance test were also normal. Hysterosalpingography revealed no congenital anomalies, masses adhesions within the uterine cavity and patent fallopian tube, and TVUS revealed adequate secretory endometrium within the luted phase. Both parents had normal karyotyping.

The inclusion criteria for the control group were as following: Not being pregnant, having had regular menstrual cycles in the 3 months preceding enrollment, using neither hormonal contraception nor an intrauterine device, having normal obstetric history with no miscarriages, and at least one child born at term.

Exclusion criteria for both groups were having uterine alterations on TVUS, systemic diseases that may affect haemodynamic indices, and a history of oophorectomy or tubal ligation.

Methods

All the patients in both groups were fulfilling the following investigations:

1. Examination by 3D transvaginal pulsed Doppler ultrasound to detect endometrial perfusion.
2. Measurement of 25 hydroxy vitamin D3.
3. Measurement of B2 glycoprotein 1 antibodies.

Three dimensional pulsed Doppler ultrasound:

The ultrasonographic examination was done on the 21st menstrual day by using GE logic P6 (GE Healthcare, Buckingomshare) ultrasound machine with a 6–10MHz endovaginal transducer. After placing the transducer in to the vagina, transverse and sagittal images at the uterus and ovaries were obtained. Color Doppler examination of the endometrium was performed with a 1.1 kHz pulse repetition frequency (PRF) to evaluate a minimum flow velocity at 5 cm/sec. in the spiral arteries. Triplex mode examination included gray scale image combined with color frame and a flow spectrum on the spectral wave. Doppler indices: UA-PI (uterine artery pulsatility index), VI (vascularization index), FI (flow index), and VFI (vascularization flow index) were evaluated.

Vitamin D

Blood samples from fasting women were collected. After centrifugation for 10 min at 3000 r.p.m. at room temperature, the serum specimens were stored at −80 °C. Before assaying, all samples were thawed to room temperature and assayed on the same day to avoid inter-assay variation. Quantitation of serum 25(OH)D, CYP27B1 was performed using commercial ELISA kits (Catalog numbers CSB-E08097h, CSB-EL006406HU and CSB-E06934h, CUSABIO BIOTECH, Wuhan, China). The intra-assay and inter-assay precision for the ELISA were less than 10% and 15% for 25(OH)D, 8% and 10% for CYP27B1 and 8% , respectively. The optical density of each sample was determined using a microplate reader (SynergyMx, BioTek, Winooski, VT, USA) set to 450 nm. The normal range for serum Vitamin D (25OHD) was 20-80 ng/ml. Thus, 8-20 ng/ml and less than 8 ng/ml levels were considered as severe vit.D inadequacy and vit.D deficiency, respectively.

B2 glycoprotein 1

B2 glycoprotein 1 antibody tests are used along with cardiolipin antibody and lupus anticoagulant testing to help diagnose the cause of an unexplained blood clot (thrombotic episode) or recurrent miscarriages, to help diagnose antiphospholipid syndrome (APS). Strongly positive results for Beta 2 glycoprotein 1 (Beta2 GP1) andibodies "(>40 u/ml) for IgG and/or IgM" are diagnostic criterion for antiphospholipid syndrome. Beta2 GP1 antibodies must be detected on 2 or more. occasions at least 12 weeks aport to fulfill the labaratory diagnostic criteria for APS.To measure anti-b2GPI antibody, we used an antib2GPI antibody kit supplied for DLD Diagnostika GMBH, Hamburg (Germany). Reagents were all stored at

2–6C. All reagents were allowed to reach room temperature before its use. Purified human b2-GPI was coated onto the surface of the microwells. Diluted serum specimens (1:51) were incubated for 30 min to permit to b2-GPI to bind to the plastic surface. After washing away unbound antibodies and serum constituents, the specific antibodies were detected by anti-IgG peroxidase. The TMB/peroxidase reaction is monitored at 450 nm optical density in a microplate photometer within 10 min. As indicated by the manufacturer, the positive results must be considered above 10 U/mL. We tested it in our laboratory, obtaining the same observations, as well. The statistical basis for significant differences between values is established by the criterion (cut-off) of mean plus 2S.D.

RESULTS

The age in the two studied groups was 28.02±2.95 and 29.0±3.01 years in the two groups respectively, there was no significant difference between the two groups regarding age. The body mass index was matched in the two studied groups no significant difference between the two groups (p >0.05). (Table 1)

Table 1: Comparison between the two studied groups regarding demographic data

	Group I "n=50"	Group II "n=50"	Test *P*
Age (years)			
Range	23.0-33.5	24.0-34.0	T=0.48
Mean±S.D.	28.02±2.95	29.0±3.01	P=0.652
Body mass index (BMI)			
Range	20.5-29.0	21.1-29.1	T=0.97
Mean±S.D.	24.5±2.65	24.6±2.74	P=0.212

LIA-PI three dimensional show a significant increase in LIA-PI in group I more than group II. VI show a significant increase in group II more than group I. FI show a significant increase in group II more than group I. VFI show a significant increase in group II more than group I. (Table 2)

Table 3: LIA-PI three dimensional subendeometrial indices in the two groups

	Group I "n=50"	Group II "n=50"	t-Test *P*
LIA-PI	2.7±0.125	1.8±0.201	2.64
Mean±S.D.			0.011*
VI	0.41±0.020	1.02±0.113	4.25
Mean±S.D.			0.001*
FI	9.30±0.82	16.9±2.11	.65
Mean±S.D.			0.002*
VFI	0.10±0.011	0.26±0.016	2.65
Mean±S.D.			0.015*

The LIA – PI was significantly increased in group I 2.7+0.125 than in group 2 1.8+0.201 whereas the VI,FI,VFI were significantly higher in group 2.

The level of vit D was significantly highen in the control group with significant decrease with increase the number of abortions. Further more ,there was significant increase of B2 – glycoprotein 1 level in the abortion group.

The level of vitamin D was significantly higher in group 1 than in the control group, furthermore, In comparing the two groups regarding the level of vitamin D in each subgroup of number of abortion, it was found that there was a significant increase of vitamin D level in group II more than group I in each abortion groups. On the other hand the level of B2 glycoprotein 1was significantly high in group 1, in comparison to the control group (Table 3).

Table 3: Relation between number of abortion in each group and both vitamin D and B2 glycoprotein

	Number of abortion			*P1*
	2	3	>3	
I. Vitamin D				
Group I	12.5±1.06	10.8±1.15	6.5±0.71	0.001*
Group II	24.0±2.36	22.5±3.11	21.3±2.06	0.032*
P2	0.001*	0.003*	0.005*	
II. B2 glycoprotein				
Group I	42.8±5.01	45.2±5.04	60.2±5.82	0.0025*
Group II	5.9±0.61	8.2±0.97	12.5±1.36	0.001*
P2	0.001*	0.001*	0.0001*	

P1 comparison between different subgroup regarding number of abortion in each group I, II.

P2 comparison between group I and II in each subgroup of number of abortion.

DISCUSSION

The first observation of the current study was that all doppler parameters were abnormal this includes UA-PI, VI, FI and VFIwhich were 2.8 ± 0.27, 0.39 ± 0.1=18, 11.10 ± 0.95 and 0.14 ± 0.05 respectively.Moreover, using a regression analysis model, we found that all of these parameters were significantly related the number of pregnancy losses.

Garhy *et al*[14] which was conducted in 2018 aiming to to find out any difference in endometrial spiral artery pulsitility index (PI) between women with history of recurrent unexplained first trimestric abortion and women without this history.For that purpose, they included 100 women in the study classified into two groups namely Recurrent pregnancy loss (RPL) group which included 50 cases with history of recurrent unexplained abortion and a Control group which included 50 cases with no history of abortion and one full term child at least.They found that dopller parameters were statistically significant between the two groups and concluded that assessment of uterine

perfusion through measurement of endometrial spiral artery Doppler could be of value in cases with recurrent unexplained first trimestric abortion.

The current results were also in agreement with the study of Lazzarin *et al*[15] who aimed to compare the impedance to endometrial spiral artery blood flow, during the mid-luteal phase, in women with recurrent spontaneous abortion (RSA) as compared to normal fertile controls. They concluded from their results that increased resistance to uterine blood flow may be an important contributing factor to some causes of RSA and may represent an independent indication of the risk of pregnancy loss.

Another study that was consistent with our results is Abdelwahab *et al*[16] as they found that the presence of good uterine and endometrial blood flow is an important prerequisite for successful implantation and continuation of pregnancy as shown by higher uterine artery blood flow resistance and lower endometrial blood flow in recurrent miscarriage cases and those patients with unexplained RPL may have abnormalities in the uterine and endometrial blood flow. Nevertheless, they differed from our results in that theyfound no doppler parameters was significant in regression analysis.

The second finding of the current results was the observation that the level of vitamin D is significantly related to the number of pregnancy losses. This was in complete accordance with the recent study of Hou *et al*[17] who aimed to analyze the relationship between vitamin D deficiency in childbearing aged women and pregnancy loss (PL) in the first trimester. They had a conclusion similar to our conclusion which was Vitamin D deficiency associated with PL in the first trimester of pregnancy. Decreased serum vitamin D levels among childbearing aged women with the failed clinical pregnancies history may predispose to increased risk for PL.

Li and his colleagues[18] also agreed to our results. They tried to investigate 25-hydroxyvitamin D [25(OH) D] concentration and vitamin D receptor (VDR) expression in the decidual tissues of RSA patients. Their results indicated that vitamin D concentrations in the decidua are associated with inflammatory cytokine production, suggesting that vitamin D and VDR may play a role in the etiology of RSA.

The final observation of the present study was the B2 glycoprotein 1 level was significantly related to the incidence of pregnancy loss. Reig *et al*[19] matched our results in that concern. They aimed to study not only B2 glycoprotein 1 but also lupus anti-coagulants in relation to recurrent pregnancy loss. They found that B2 glycoprotein 1 were significantly higher in patients with recurrent pregnancy loss and concluded that it may be considered a biological marker for obstetric APS.

Sigh and his colleagues[20] also matched our results in that B2 glycoproteins 1 level was significantly higher in patients with recurrent pregnancy loss and recommended that women with recurrent pregnancy losses should be tested for anti beta-2 Glycoprotein I antibodies & anti prothrombin antibodies in addition to conventional lupus anticoagulant and anticardiolipin antibodies.

CONCLUSION

All the studied parameters manely Doppler indices, vit D level and B2 glycoprotien 1 were significantly related to the incidence of recurrent pregnancy

Loss should be tested in women with unexplained recurrent pregnancy loss.

CONFLICT OF INTERESTS

There are no Conflicts of interest.

REFERENCES

1. Practice Committee of the American Society for Reproductive Medicine Evaluation and treatment of recurrent pregnancy loss: a committee opinion. FertilSteril. 2012;98(5):1103–1111.

2. Kolte AM, Bernardi LA, Christiansen OB, *et al*. ESHRE Special Interest Group, Early Pregnancy Terminology for pregnancy loss prior to viability: a consensus statement from the ESHRE early pregnancy special interest group. Hum Reprod. 2015;30(3):495–498.

3. El Hachem H, Crepaux V, May-Panloup P, Descamps P, Legendre G, Bouet PE. Recurrent pregnancy loss: current perspectives. Int J Womens Health. 2017;9:331-345. Published 2017 May 17. doi:10.2147/IJWH.S100817

4. Royal College of Obstetricians and Gynaecologists, Scientific Advisory Committee, Guideline No. 17. The Investigation and treatment of couples with recurrent miscarriage. 2011.

5. Isakson R, Tiitinen A, Reinikainen LM, Cacciatore B. Comparison of uterine and spiral artery blood flow in women with unexplained and tubal infertility. Ultrasound Obstet and Gynceol. 2003;21:174–80.

6. Abulafia O, Sherer DM. Angiogenesis of the ovary. Am J Obstet Gynecol. 2012;182:240–6.

7. Yılmaz N, Kılıç S, Madendağ Y, Madendağ İ, Özgün A, Özakşit G, *et al*. Endometrial parameters in IVF and IUI administration on elderly women. Turk J Med Sci. 2013;40:343–8.

8. Battaglia C, Sgarbi L, Salvatori M, Maxia N, Gaillinelli A, Volpe A. Increased anticardiolipin antibodies are positively related to the uterine artery pulsatility index in unexplained infertility. Human reproduction. 2008;13:3487–91

9. Christesen, HT, Falkenberg, T, Lamont, RF, Jorgensen, JS. The impact of vitamin D on pregnancy: a systematic review. ActaObstetGynecol Scand. 2012; 91: 1357- 1367.

10. Giulia Bivona, Luisa Agnello, Bruna Lo Sasso, ConcettaScazzone, Daniela Butera, Caterina Maria Gambino, GiorgiaIacolino, Chiara Bellia and Marcello Ciaccio, Vitamin D in malaria: more hypotheses than clues, Heliyon, 10.1016/j.heliyon.2019.e01183, 5, 2, (e01183), (2019).

11. 15 Hirose N, Williams R, Alberts AR, Furie RA, Chartash EK, Jain RI, Sison C, Lahita RG, Merill JT, Cucurull E, Gharavi AE, Sammaritano LR, Salmon JE, Hashimoto S, Sawada T, Chu CC, Gregersen PK, Chiorizzi N: A role for the polymorphism at position 247 of the B2-Glicoprotein I gene in the generation of anti-B2-Glicoprotein I antibodies in the antiphiospholipid syndrome. Arthritis Rheum 1999; 12:1555–1561.

12. Ossting JD, Derksen RHWM, Entjes HT, Bouman BN, de Groot PG: Lupus anticoagulant activity is frequently dependent on the presence of B2Glicoprotein I. ThrombHaemost 1992; 67:499–502.

13. Di Simone N, Raschio E, Testoni C, Castellani R, D'asta M, Shi T, Krillis SA, Caruso A, Meroni PL: Pathogenic role of anti-B2-glicoprotein I antibodies in antiphospholipid associated fetal loss: characterization of B2-glycoprotein I binding to trophoblast cells and functional effects of anti-Beta2-glycoprotein I antibodies in *vitro*. Ann Rheum Dis 2005;64:462–467. doi: 10.1136/ard.2004.021444

14. Garhy, I. T. E., *et al*. (2018). "Uterine and Subendometrial Arteries Doppler in Patients with Recurrent First Trimestric Abortion %J The Egyptian Journal of Hospital Medicine." 73(5): 6683-6690.

15. Lazzarin N, Vaquero E, Exacoustos C, Romanini E, Amadio A, Arduini D.Midluteal phase Doppler assessment of uterine artery blood flow in nonpregnant women having a history of recurrent spontaneous abortions: correlation to different etiologies. FertilSteril. 2017 Jun;87(6):1383-7.

16. Hala Abdel Wahab, Doaa Salah El-Din, EmanZain, MohamedAbdelgany, Mohamed A.F.M. Youssef (2011): Uterine artery Doppler and subendometrial blood flow in patients with unexplained recurrent miscarriage. Middle East Fertility Society Journal, 16 (3): 209-214.

17. Hou W, Yan XT, Bai CM, Zhang XW, Hui LY, Yu XW. Decreased serum vitamin D levels in early spontaneous pregnancy loss. Eur J ClinNutr. 2016;70(9):1004-8.

18. Li N, Wu HM, Hang F, Zhang YS, Li MJ. Women with recurrent spontaneous abortion have decreased 25(OH) vitamin D and VDR at the fetal-maternal interface. Braz J Med Biol Res. 2017;50(11):e6527. Published 2017 Sep 12. doi:10.1590/1414-431X20176527

19. Alijotas-Reig J, Casellas-Caro M, Ferrer-Oliveras R, Llurba-Olive E,Hermosilla E, Vilardell-Tarres M, Cabero-Roura L. Are anti-beta-glycoprotein-Iantibodies markers for recurrent pregnancy loss in lupusanticoagulant/anticardiolipinseronegative women? Am J ReprodImmunol. 2008 Sep;60(3):229-37.

20. Singh A, Nangia A, Sharma S, Puri M. A Study of Anti Beta-2 Glycoprotein I and Anti-Prothrombin Antibodies in Patients with Unexplained Recurrent Pregnancy Losses. Indian J Hematol Blood Transfus. 2015;32(2):198-201.