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Pravastatin plus L-arginine prevents adverse pregnancy outcomes in women with uteroplacental vascular dysfunction

Aleksandar Jurisic [a,b], Zaklina Jurisic [b], Eleftheria Lefkou [c], Guillermina Girardi [d,*]

[a] University of Belgrade Medical School, Narodni Front University Hospital, Belgrade, Serbia
[b] OB/GYN Polyclinic Jurisic, Belgrade, Serbia
[c] Perigenesis, Institute of Obstetric Haematology, Thessaloniki, Greece
[d] College of Medicine, QU Health, Qatar University, Qatar

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ABSTRACT

Background: Uteroplacental vascular dysfunction, characterized by diminished uterine artery (UtA) blood flow in the second trimester is a clinically useful predictor of the further development of preeclampsia, fetal growth restriction and stillbirth. Efforts to develop effective treatments to protect pregnancies with abnormal UtA Dopplers would be of significant clinical benefit for mothers and their fetuses.

Objective: The aim of this pilot non randomized control study was to use pravastatin +L-arginine to improve uteroplacental haemodynamics and prevent adverse maternal and neonatal outcomes in women with abnormal Dopplers and high risk for developing adverse pregnancy outcomes.

Study design: This study was performed between 2015 and 2018. All women received primary care at OB/GYN Polyclinic Jurisic and Narodni Front University Hospital, University of Belgrade Medical School, Serbia. Approval for investigational drug use was obtained and all women gave informed consent.

10 pregnant women with a poor obstetric history that developed uteroplacental dysfunction (UtA pulsatility index (PI) above the 95th percentile and notching) at 20.5 weeks IQR [17.7–22] gave consent to be treated daily with pravastatin (40 mg) and L-arginine (1.5 g) to improve placental blood flow and pregnancy outcomes. 5 women remained untreated after diagnosis at 21 weeks [20−22] (control group). Due to presence of risk factors for pregnancy complications, close maternal and fetal monitoring was undertaken in all patients. Doppler examinations were performed to monitor changes in placental vascular resistance and fetal well-being and growth.

Results: PRAV+L-arginine improved uteroplacental haemodynamics, increased fetal growth and prevented early onset preeclampsia leading to delivery close to term (delivery date: median 38 weeks, IQR[36.5–39]) and appropriate weight for gestational age compared to controls, in which placental blood flow did not improve and 2 women developed severe early onset preeclampsia. Neonates from the control group were born preterm (25 weeks IQR[23.5–25]), growth restricted and spent several months at NICU. Two neonates died due to prematurity-associated complications. PRAV+L-arginine treatment prolonged pregnancies for 4.1 months, compared to 26 days in the untreated group, preventing neonatal complications associated with prematurity. The infants are now 1–3 years old and show normal growth and development.

Conclusion: This study describes the successful management with pravastatin+L-arginine of 10 pregnant patients with uteroplacental vascular dysfunction and high risk of adverse maternal and fetal outcomes. A larger study is being organized to confirm these observations.

1. Introduction

Increasing evidence suggests that most adverse pregnancy outcomes can trace their origin to the placenta [1]. In fact, placental insufficiency caused by abnormal early placental development is associated with preeclampsia (PE) and fetal growth restriction. Abnormal placentation leads to a pathological increase in placental vascular resistance, detectable by abnormal Doppler flow studies of the maternal uterine vessels. There is extensive evidence that uterine artery (UtA) Doppler ultrasound in the second trimester constitutes a useful, non-invasive

* Corresponding author.
E-mail address: guillerminagirardi@gmail.com (G. Girardi).

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method to predict the further development of PE, fetal growth restriction, placental abruption and stillbirth [2,3]. In addition, persistent bilateral notches with increased uterine arteries resistance identifies the vast majority of women who will subsequently develop complications secondary to uteroplacental insufficiency [4]. Therefore, UtA Doppler screening of high-risk women can identify those women at substantially increased risk for adverse pregnancy outcomes and to interventions that might improve clinical outcomes.

Lipid-independent pleiotropic effects, including endothelial protection and regulation of immune, inflammatory, and procoagulant responses, have been attributed to statins [5]. Studies in animal models support the hypothesis that pravastatin may be an effective means of preventing pregnancy complications associated with placental insufficiency [6–10]. Recent studies validated these observations in women with preeclampsia and obstetric antiphospholipid syndrome [11,12].

Pravastatin have been shown not to be teratogenic as demonstrated by several studies [13–15]. In combination with L-arginine, pravastatin improved placental haemodynamics and prevented intrauterine fetal death in twin pregnancies with discordant growth through a nitric oxide dependent mechanism [16]. The development of therapies for the prevention of a number of pregnancy disorders of placental origin that include PE, intrauterine growth restriction (IUGR), preterm birth and intrauterine fetal death is of important clinical value. Therefore, in this pilot non randomized control trial, we investigated the effectiveness of pravastatin + L-arginine (PRAV+L-arg) in improving uteroplacental vascular function and preventing adverse maternal and neonatal outcomes in women with increased uteroplacental resistance and increased risk for developing adverse pregnancy outcomes.

2. Methods

2.1. Patients

Between 2015 and 2018, 15 women with abnormal uterine artery Doppler resistance indices (UtA PI >95% centile) and notching participated in this pilot study. The patients' past obstetric and medical history and characteristics of current pregnancy are presented in Table 1 and 2. All women had a poor obstetric history (miscarriages, hypertension of pregnancy (PIH), preterm birth (<37 weeks, PTB), preeclampsia, intrauterine growth restriction and stillbirth) (Table 1 and 2). 3 women were diagnosed with Hashimoto's thyroiditis. Other risks for pregnancy complications included age over 35 years old, BMI > 30 and gestational diabetes (GDM). Some women showed Factor V Leiden, methylentetrahydrofolate reductase (MTHFR) gene and plasminogen activator inhibitor type 1 (*PAI-1)* gene polymorphisms.

All 15 patients developed increased impedance to blood flow in the UtA as shown by a pulsatility index (PI) above the 95th percentile (Table 1, 2 and Fig. 1). At the time abnormal Dopplers were detected, all women were counseled about the risks for the mothers and fetuses associated with placental dysfunction and were offered to take pravastatin +L-arginine to improve placental perfusion, 10 women agreed to be treated with oral doses of pravastatin (40 mg/day) and L-arginine (0.5 g/ 3 times per day) until the end of the pregnancy. Written informed consent was obtained from all pregnant patients.

Four patients in this group showed IUGR (fetal weight below the 10th percentile for gestational age) at the time of the abnormal Doppler findings.

Five patients were included in the control group. One woman did not agree to be treated with pravastatin + L-arginine and remained untreated after diagnosis. Four cases were obtained from historic untreated controls prior to the start of this study.

Two patients (one in the treated group and one in the untreated group) showed abnormal umbilical artery (UmbA) blood flow.

Close maternal and fetal monitoring was undertaken in all patients due to presence of risk factors for pregnancy complications. Maternal monitoring included daily measurement of blood pressure, and

Table 1
Past and current obstetrical history, time of diagnosis, survival after diagnosis and pregnancy outcomes in patients with uteroplacental dysfunction (UtA PI >95%) that were treated with pravastatin +L-arginine.

Patient	Past obstetric history/Risk factors	Current pregnancy/ time of diagnosis (weeks)	Survival after Diagnosis (days)	Delivery/ cause Neonatal weight
1.	1 stillbirth (24 weeks) Endometriosis, GDM, FVL	20 weeks: ↑UtA PI, (mean: 2.03, R notching) IUGR (20 weeks)	119	37 weeks C-section 2900 g
2.	1 miscarriage (12 weeks) Hashimoto, Age > 35	18 weeks: ↑UtA PI (mean: 2.52, bilateral notching)	147	39 weeks C-section 3950 g
3.	2 PIH (30, 32 weeks) Hashimoto, Age > 35	16 weeks: ↑UtA PI (mean: 1.90, bilateral notching)	154	38 weeks vaginal 2850 g
4.	1 miscarriages (14 weeks) *MTHFR* polymorphism	19 weeks: ↑UtA PI (mean: 2.6, bilateral notching) ↑Umb Art PI, IUGR (19 weeks) 34 weeks: ↑UtA PI PE (BP 150/ 100)	112	35 weeks, C-section: fetal distress 2300 g
5.	1 PTB BMI > 30, GDM	22 weeks: ↑Ut art PI (mean: 1.6, R notching)	126	40 weeks vaginal 3500 g
6.	1 miscarriage (12 weeks) *PAI-1* polymorphisms	22 weeks: ↑UtA PI (mean: 2.48, bilateral notching) 32 weeks: ↑UtA PI, PE (BP 150/ 95)	77	33 weeks C-section: fetal distress 1850 g
7.	3 miscarriages (8, 12, 12 weeks) Fibroids	17 weeks: ↑UtA PI (mean: 2.1, bilateral notching) IUGR (17 weeks)	147	38 weeks, C-section 3100 g
8.	1 miscarriage (8 weeks), 1 IUGR BMI > 30, age > 35	21 weeks: ↑UtA PI (mean: 1.89, bilateral notching)	119	38 weeks, C-section Breech presentation 3150 g
9.	1 PE, IUGR (28 weeks) *MTHFR* polymorphism Age > 35	21 weeks: ↑UtA PI (mean: 1.5, bilateral notching) IUGR (21 weeks)	119	38 weeks C-section 3200 g
10.	2 PE + IUGR (26, 28 weeks) Age > 35	22 weeks: ↑UtA PI (mean: 2.24, bilateral notching)	119	39 weeks C-section 3000 g
Median, [IQR]	Age: 36 [33.7–37] Previous adverse	Time of diagnosis: median: 20.5	119 days [117.25–147]	38 weeks [36.5–39]

(*continued on next page*)

Table 1 (*continued*)

Patient	Past obstetric history/Risk factors	Current pregnancy/ time of diagnosis (weeks)	Survival after Diagnosis (days)	Delivery/ cause Neonatal weight
	pregnancy outcomes: 100%	weeks [17.7–22] notching: 20% unilateral, 80% bilateral		3050 g [2712–3275]

GDM: gestational diabetes mellitus, PIH: pregnancy induced hypertension, BMI: body mass index, IUGR: intrauterine growth restriction, UtA PI: uterine artery pulsatility index (R: right, L: left), Umb art PI: umbilical artery pulsatility index, PTB: preterm birth, PE: preeclampsia.
FVL: Factor V Leiden, *MTHFR:* methylentetrahydrofolate reductase gene, *PAI-1:* plasminogen activator inhibitor type 1 gene, IQR: interquartile range.

proteinuria. Patients were scanned every 2–3 weeks to monitor changes in uteroplacental and fetal circulation. Fetal biophysical profile was performed after 28 weeks, and cardiotocography was performed in the third trimester to assess fetal wellbeing. Intrauterine growth restriction (IUGR) was defined as estimated weight below the 10th percentile for its gestational age.

2.2. Ultrasonography

Doppler examinations were performed by two examiners (AJ and ZJ) using RM6C matrix 4D convex probe (Voluson E10, GE Healthcare) and V4-8 4D convex probe (Medison V20 Prestige, Korea) with the high-pass filter at 60 Hz. Spectral Doppler analysis of flow velocity waveforms of UtA and UmbA were performed. EFW percentile was derived from sonographic measurements of fetal biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL), (Hadlock 4 formula) and birth weight percentile according to previous published studies [16,17].

2.3. Statistics

Statistical analysis to compare pregnancy and neonatal outcomes between treated and untreated patients was conducted using a paired *t*-test. $P < 0.05$ was considered statistically significant. Medians and IQRs are reported for all variables measured. All analysis was conducted with GraphPad Prism statistical software (GraphPad Software Inc.).

2.4. Study approval

Ethical Approval for this study and informed consent was obtained from all pregnant patients.

3. Results

A significant improvement in the uteroplacental haemodynamics was observed after PRAV+L-arg treatment. UtA PI diminished reaching normal values within 3 weeks (median:3 weeks IQR[2–3.5]) (Table 3, Fig. 1A) of treatment. The improvement in UtA vascular resistance was associated with an increase in expected fetal weight (EFW) (Fig. 1B).

On the other hand, resistance in the uteroplacental flow remained high throughout gestation in the women that did not receive PRAV+L-arg (Fig. 1A). Fetal weight remained below the 10%centile in the 2 women that developed IUGR in the untreated groups (Fig. 1B) Two women in the untreated group developed early severe PE shortly after abnormal Dopplers were detected (Table 2) and were treated with methyl Dopa (MDP). Uncontrolled hypertension and/or fetal distress prompted emergency deliveries in the control untreated patients. All neonates from untreated mothers were born preterm (25 weeks IQR [23.5–26]) and all neonates were admitted at neonatal intensive care (NICU). Two neonates died shortly after birth. The other 3 neonates spent a significant time at NICU (10, 11 and 16 weeks) and presented health complications associated with prematurity such as intraventricular haemorrhage (IVH) and rethinopathy of prematurity (ROP).

In the PRAV+L-arg group, 2 patients developed late onset hypertension and abnormal Dopplers at 32 and 34 weeks. The patients responded to MDP but C-sections were performed 1 week later due to fetal distress. Overall, pregnancies continued for 4.1 months (median:

Table 2
Past and current obstetrical history, time of diagnosis, survival after diagnosis and pregnancy outcomes in patients with uteroplacental dysfunction (UtA PI >95%) [1–5] who did not receive pravastatin +L-arginine.

Patient	Past obstetric history/Risk factors	Current pregnancy/ time of diagnosis (weeks)	Survival after diagnosis	Delivery/cause Neonatal weight Admission to NICU
1	Age > 35 1 miscarriages Hashimoto	22 weeks: ↑UtA PI (mean: 1.8), bilateral notching IUGR (EFW:438 g, 22 weeks) 25 weeks: PE (BP 160/100)	26 days	26 weeks C-section: Uncontrolled hypertension, fetal distress Bw: 805 g NICU (10 weeks) - IVH
2	1 miscarriage Early onset PE age > 35	20 weeks: ↑UtA PI (mean 2.1, bilateral notching) ↑Umb Art PI IUGR (EFW 267 g, 20 weeks)	17 days	23 weeks C-section: fetal distress Bw: 325 g NICU – died of infection 4 days after birth
3	1 Miscarriage *PTB* *MTHFR* polymorphism	21 weeks: ↑UtA PI (mean 2.5, bilateral notching) 22 weeks: PE (BP 155/98) IUGR (EFW:380 g, 22 weeks)	21 days	24 weeks: C-section: fetal distress, uncontrolled hypertension Bw: 575 g NICU: died after 24 h
4	Age > 35, BMI > 30 1 miscarriage IVF	20 weeks: ↑UtA PI (mean 1.80, unilateral notching, R)	35 days	25 weeks: C-section: fetal distress Bw: 634 g NICU: 16 weeks,
5	2 early onset PE 2 miscarriages	22 weeks: ↑UtA PI (mean 1.9, unilateral notching, L)	29 days	26 weeks maternal C-section: fetal distress Bw: 720 g NICU: 11 weeks, ROP
Median, [IQR]	Age: 36 [30, 37.5] Previous adverse pregnancy outcomes:100%	Median: 21 weeks IQR [20–22] Notching: bilateral: 60%, Unilateral 40%	26 days IQR [19–32]	Delivery: 25 weeks IQR[23.5–26] Birth weight: 644 g [400–750] Admission to NICU: 100%

Patient 3 was given methydopa (MDP) (1 g/d in 2 divided doses) to treat hypertension.
IVF: *in vitro* fertilization, UtA: uterine arteries, PI: pulsatility index, BP: blood pressure, bw: birth weight, NICU: neonatal intensive care unit, PE: preeclampsia, aEDV: absent end diastiolic velocity, rEDV: reverse end diastolic velocity, BMI: body mass index, IVH: intraventricular haemorrhage, Retinopathy of prematurity (ROP).
MTHFR: methylentetrahydrofolate reductase gene, *PAI-1:* plasminogen activator inhibitor type 1 gene.

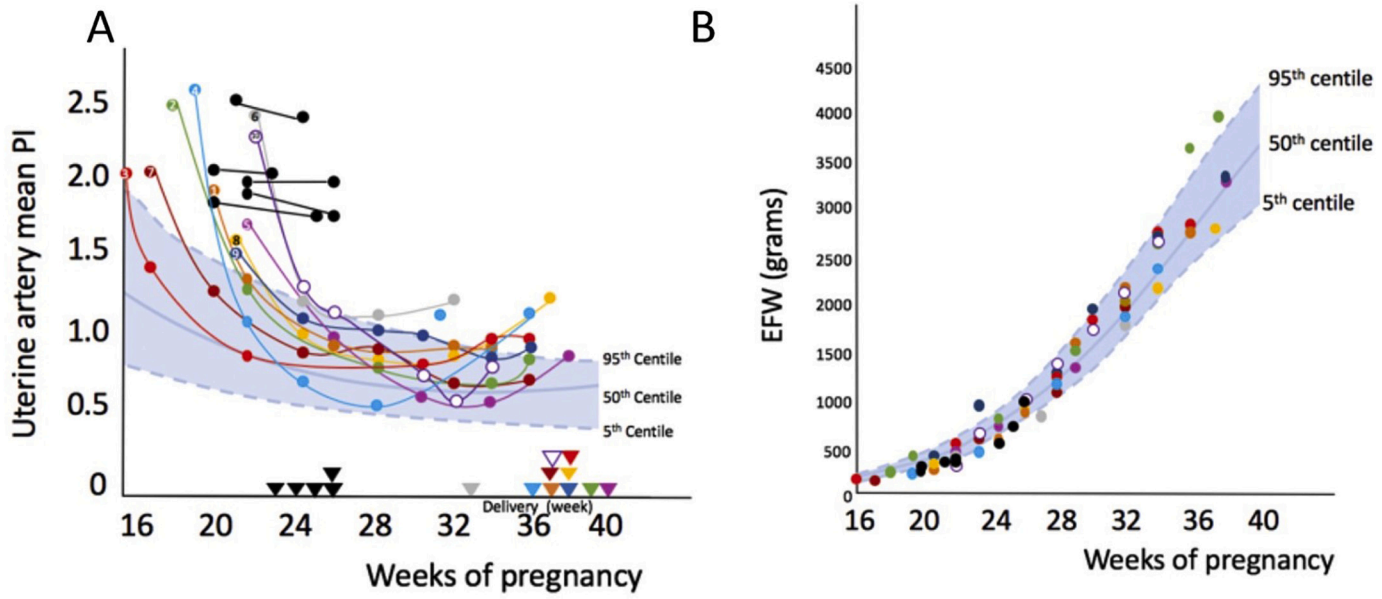

Fig. 1. Mean uterine artery pulsatility index (A) and expected fetal weight (EFW, grams) during the course of pregnancy in patients treated with PRAV+L-arg and untreated.
The area in light blue represents the normal values (upper and lower lines correspond to 95 and 5 percentile respectively). The numbers in the colored dots indicate the patient number in the PRAV+L-arg-treated group. Black circles correspond to untreated patients.
A- *Mean Uterine artery pulsatility index (UtA PI)*. All 15 patients showed mean Ut Art PI above the 95th centile. 3 weeks after PRAV+L-Arg treatment (median 3, IQR [2–3.23]) mean Ut art PI diminished to values within normal values (colored circles). Ut Art PI in the control group (black circles) remained above the 95th centile until the end of pregnancy. Colored and black triangles correspond to the date of delivery of treated and untreated patients respectively. All patients treated with PRAV+L-arg gave birth close to term (median 38 weeks IQR [36.5–39]). Untreated patients gave birth preterm (27 weeks IQR[26–27.5]).
B- *Expected fetal weight (EFW)*. 4 Patients (1, 4, 7 and 10) showed IUGR at the time abnormal placental perfusion was observed and treatment was initiated. EFW increased and reached normal values after PRAV+L-arg treatment (colored circles). Patient 6 developed IUGR at week 27 after treatment. EFW in the control group (remained below 10th centile until the end of pregnancy.

Table 3
Maternal and fetal outcomes in women with uteroplacental insufficiency (UtA PI>95%centile) untreated and treated with Pravastatin+L-arginine (PRAV+L-arg).

	Time of diagnosis UtA > 95%centile (weeks)	PRAV + L-arg	Time of Improvement (weeks)	Survival (days)	End of pregnancy (week)	Birth weight (g)/Neonatal outcomes
N = 10	20.5 weeks[a] IQR [17.7–22]	YES	3 weeks IQR[2–3.23]	119 days[a] IQR [117.25–147]	38 weeks[a] IQR [36.5–39])	3050 g[a] IQR[2712.5–3275] Admission to NICU: no Complications: none
N = 5	24 weeks IQR [23–25]	NO	No improvement	26 days IQR[19–32] 2 neonatal deaths	25 weeks IQR[23.5–26]	644 g IQR[400–750] Admission to NICU: 100% Complications: IVH, ROP 2 neonatal deaths

Values are expressed as median and IQR: interquartile range.
NICU = neonatal intensive care unit.
[a] Different from untreated women, $p < 0.01$.

119 days IQR[117.25–147]) in women treated with PRAV+L-arg, compared to 26 days IQR[19–32] in the untreated group (Table 3). All patients treated with PRAV+L-arg delivered close to term (median 38 weeks, IQR[36.5–39] and neonates showed appropriate for gestational age weights (median 3100 g IQR[2575–3350]) and no neonatal complications (Table 3, Fig. 1A). The infants are now 1–3 years old and show normal growth and development.

4. Discussion

This pilot non randomized controlled trial evaluated the effects of pravastatin + L-arginine in preventing adverse maternal and neonatal outcomes in pregnant women with uteroplacental vascular insufficiency and high risk for developing preeclampsia and intrauterine growth restriction.

Pregnant women with increased uteroplacental vascular resistance evidenced by uterine artery pulsatility index over the 95th centile and additional risk factors for developing adverse pregnancy outcomes (gestational diabetes, increased age, increased BMI and thrombophilia [18–23]) responded favorably to pravastatin + L-arginine given at the time of abnormal Doppler detection. A significant diminution in placental vascular resistance was observed after PRAV+L-arg treatment. The beneficial effects of PRAV+L-arg on placental hemodynamics is in agreement with our prior studies in twin pregnancies with discordant fetal growth [16]. By improving placental haemodynamics, PRAV+L-arg prolonged pregnancies for 4 months, compared to less than a month in the untreated group. Placental haemodynamics, maternal signs of preeclampsia and fetal weight did not improve in patients that did not receive PRAV+L-arg treatment, leading to emergency Caesarean deliveries associated with neonatal complications due to prematurity. Two of the neonates in this group died due to prematurity associated complications. After spending several months at NICU, the surviving 3 infants show normal growth and development.

In contrast, women that received pravastatin+L-arginine treatment

gave birth close to term, 38 weeks, and no complications were observed in the neonates. The infants are now 1–3 years old and show normal growth and development.

In agreement with other studies [13–15] this study also showed that pravastatin is safe to use during pregnancy.

Although causality has not been demonstrated, endothelial dysfunction and reduced nitric oxide (NO) bioavailability have been associated with the maternal and fetal pathophysiology of preeclampsia. Therefore, it has been suggested that drugs that increase NO bioavailability would be a good therapeutic modality in the context of preeclampsia [24]. Among pravastatin many pleiotropic effects, endothelial protection and anti-inflammatory effects showed to be important in the protective effects of pravastatin in improving pregnancy outcomes in animal models and women [6–12]. In addition, pravastatin might improve uteroplacental haemodynamics by diminishing lipid deposition and the influx of inflammatory cells in the walls of the uterine arteries [25]. Atherosis of uteroplacental spiral arteries, leading to increased placental resistance is observed in 20% to 40% of cases of preeclampsia.

Nitric oxide (NO), an important modulator of the vascular tone, is synthetized from amino acid L-arginine by the NO synthase enzyme (NOS). Importantly, NO is a crucial mediator of the vascular function in the placenta [26]. We previously demonstrated that pravastatin increased placental NO production and prevented pregnancy complications in a mouse model of PE [27]. Pravastatin has vasorelaxant properties by activating endothelial nitric oxide synthase (eNOS) [28]. Wire myography studies demonstrated that pravastatin relaxes mouse aortic rings preconstricted with norepinephrine through a mechanism that is NO-dependent [16,28].

The amino acid L-arginine is converted to L-citrulline and NO by NOS and is a limiting factor in NO availability [29]. A recent study demonstrated that pravastatin induces NO synthesis by enhancing microsomal arginine uptake in healthy and preeclamptic placentas [30].

L-arginine has been tested in combination with antioxidants in a randomized controlled trial (RCT) with promising results. L-arginine combined with antioxidant vitamins reduced the incidence of preeclampsia in a high-risk population [31]. However, the relative contributions of L-arginine and antioxidant vitamins to the observed effects of the combined treatment were not determined. A randomized, double-blind, placebo-controlled, clinical trial demonstrated that L-argininie (3 g/day) is effective in preventing preeclampsia. In this RCT, the placebo group had a higher number of cases of preeclampsia compared with the L-arginine treated-group [32]. In addition, higher birth weights and a smaller number of preterm births was observed in the L-arginine-treated group [33].

The recently published stAmP trial [15] reported no improvement in angiogenic imbalance and preeclamptic symptoms after pravastatin treatment in preeclamptic women. However, lower mortality rate was observed in the treatment group [15]. In the stAMP trial, pravastatin was given after the onset of preeclampsia. On the other hand, some studies demonstrated favorable effects of pravastatin in treating and/or preventing preeclampsia (13,33). A study performed in Australia, showed that pravastatin decreased antiangiogenic factors and stabilized clinical and biochemical features of preterm preeclampsia (<30 weeks) [33]. In addition, another study showed that pravastatin was associated with a more favorable pregnancy angiogenic profile and prevented the development of preeclampsia when given at 12–16 weeks of pregnancy [13]. This study, in addition to the present study, suggests that better results might be obtained if treatment is started as soon as uteroplacental vascular abnormalities are detected instead of the time of onset of maternal and fetal complications. L-arginine addition to pravastatin might also explain the improved outcomes of our study.

Interestingly, women with antiphospholipid syndrome and placental malperfusion characterized by UtA PI above the 95thcentile, same inclusion criteria than in this study, showed a significant improvement in placental blood flow and maternal and neonatal outcomes after pravastatin was added to standard of care low molecular weight heparin plus low dose aspirin [11]. In this study [11], pravastatin was started as soon as abnormal uteroplacental vascular function was detected.

In conclusion, the combination of pravastatin with L-arginine improved uteroplacental vascular function and increased fetal weight allowing progression of pregnancy close to term with favorable maternal and neonatal outcomes in women at high risk of developing PE and IUGR.

Limitations of this pilot study include the small number of patients that prevented a clear generalized statement about the beneficial effects of the combined therapy pravastatin and L-arginine. Larger studies are now being organized to confirm these observations.

Authors contributions

AJ, EL and GG planned the studies. AJ, EL and ZJ performed the human studies. GG wrote the manuscript text and prepared the figures. All authors analysed the data and reviewed the manuscript.

Role of funding source

Narodni Front University Hospital, University of Belgrade Medical School in Serbia provided the patients and consumables used for the clinical care and routine exams of the patients. The study was designed, conducted, analysed, and reported entirely by the authors. Open Access funding provided by the Qatar National Library.

Credit author statement

Aleksandar Jurisic (AJ), Eleftheria Lefkou (EL) and Guillermina Girardi (GG) planned the studies. AJ, EL and Zaklina Jurisic performed the human studies. GG wrote the manuscript text and prepared the figures. All authors analysed the data and reviewed the manuscript.

Declaration of Competing Interest

None.

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