Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 30 (2), 2023: 109–118.
www.Ejimmunology.org

Effect of 17-Hydroxyprogesterone Caproate on Interleukin-6 and Tumor necrosis factor-alpha in expectantly managed early-onset preeclampsia

Mahmoud A. Othman[1], Eman R. Badawy[2*], Ahmed M. A. Sobh[1], Asmaa S. Shaltout[3] and Sayed A. Mostafa[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Assiut, Egypt.

[2]Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.

[3]Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Corresponding author: Eman R. Badawy, Department of Clinical Pathology, Faculty of Medicine, Faculty of Medicine, Assiut University, Assiut, Egypt.
Email: e.badawy@aun.edu.eg.

Abstract

The study aimed to evaluate the effect of 17 hydroxy progesterone (17-OHPC) on interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in expectantly managed early-onset preeclampsia (PE). A randomized open-label controlled study included women who were diagnosed as early-onset PE if they assigned to expectant management according to the American College of Obstetricians and Gynecologists (ACOG) 2013 criteria for diagnosis of severity of PE. Patients were randomized into Group A (40 patients) received 17-OHPC 250 mg intra-muscular at admission and every 7 days thereafter and Group B (40 patients) was given the usual conservative measures of early-onset PE as a control group. Blood samples were obtained from all participants for measurements of TNF-α and IL-6 levels at admission and repeated at termination of pregnancy. The primary outcome was the mean difference between TNF-α and IL-6 levels before and after treatment in both groups. TNF-α and IL-6 levels at admission were not different between the two groups. However, there was a significant difference concerning these inflammatory biomarkers within the same group at admission and at termination (p<0.001), with significant decline of IL-6 and TNF-α level in the 17-OHPC treated group and significant rise of IL-6 and TNF-α in the control group. There was a strong positive correlation between systolic blood pressure (SBP) at admission and TNF-α level (r= 0.867, p=0.017), and moderately positive significant correlation between diastolic blood pressure (DBP) at admission and TNF-α (r=0.610, p<0.001). There was a mild positive significant correlation between IL-6 levels and SBP (r= 0.231, p=0.039), and DBP (r= 0.203, p= 0.041) at admission. In conclusion, 17-OHPC has no effect in improving maternal or neonatal outcomes in conservatively managed early onset PE, although it alters the inflammatory markers levels (IL-6 and TNF-α) that could improve the pathogenesis of PE.

Keywords: preeclampsia, progesterone, interleukin-6, tumor necrosis factor- alpha

Date received: 06 September 2022; **accepted:** 09 February 2023

Introduction

Preeclampsia (PE) is a disorder of widespread vascular endothelial malfunction and vasospasm that occurs after 20 weeks' gestation and can present as late as 4-6 weeks postpartum.[1] Among all cases of PE, 10% occur in pregnancies of less than 34 weeks' gestation. The global incidence of PE has been estimated at 5-14% of all pregnancies. Globally, PE is responsible for >60,000 maternal deaths annually.[2] PE is usually characterized by hypertension, abnormal amounts of protein in the urine, increased inflammatory cytokines, decreased vasodilators such as nitric oxide (NO) and other systemic disturbances.[3]

Mediators of endothelial dysfunction, such as increased production of the vasoconstrictor endothelin-1 (ET-1), antiangiogenic factor (soluble fms-like tyrosine kinase-1, sFlt-1), and decreased of vasodilators, such as NO, are thought to play a role in the development of PE. In addition, a significant increase in inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), was observed in PE and proposed to stimulate factors that play a role in vasoconstriction and hypertension during pregnancy.[4]

Animal studies have shown that normal pregnant recipient rats implanted with a pump infusing either TNF-α or IL-6 exhibited many characteristics of PE.[5] Currently there is no effective treatment for early-onset PE except for early delivery of the fetus along with the placenta. Progesterone supplementation in the form of 17-alpha-hydroxyprogesterone caproate (17-OHPC) is currently used obstetrically to prevent recurrent preterm birth in patients with pregnancies not complicated by PE.[6] Previous studies reported that patients with severe PE had significantly lowered serum progesterone concentrations than gestational age- and race-matched non-preeclamptic pregnant women.[7,8] Moreover, supplementation of placental ischemic rats with 17-OHPC decreased blood pressure, inflammatory cytokines, and ET-1 within 24 hours of treatment.[8] Additionally, there is evidence that progesterone may have vasodilatory effects and can improve NO availability.[9]

Although 17-OHPC is administered routinely for the prevention of recurrent preterm labor, its addition for the management of PE has been debated. While PE is associated with decreased circulating progesterone and increases in inflammatory cytokines, it remains unclear what is the role 17-OHPC in decreasing immune activation while improving vasodilation and hypertension in response to placental ischemia.[10]

Previous in-vitro studies concluded that the administration of 17-OHPC to preeclamptic rats decreased blood pressure, proteinuria, inflammation, TNF-α-induced hypertension, and improved vascular endothelial NO synthase expression in placenta.[11-14] This could improve the pregnancy outcomes due to placental ischemia, therefore addition of 17-OHPC should be considered for the management of early onset PE. Therefore, the present study aimed to evaluate the effect of 17-OHPC on IL-6 and TNF-α in expectantly managed early-onset PE.

Subjects and Methods

The current study was a randomized open label-controlled study (Registered at ClinicalTrials.Gov; NCT04077853), carried out in a tertiary university hospital. This study started on February 2020 and the follow-up of the patients ended in October 2021.

The study protocol was reviewed and approved by the Institutional Review Board, Faculty of Medicine, Assiut University (February 2020). The confidentiality of all patients admitted to the study was protected. Each woman participated in the study signed a written informed consent.

All patients who were diagnosed as early-onset PE (blood pressure measurement more the 139/89, with or without proteinuria, between 20- and 34-weeks pregnancy) were invited to participate in the study if they assigned to expectant management according to the American College of Obstetricians and Gynecologists (ACOG) 2013 criteria for diagnosis of severity of PE.[15]

This was a pilot, hypothesis generating study as there was no previous studies evaluating the effect of 17-OHPC on maternal and neonatal outcomes in cases of PE, therefore it was difficult to calculate a sample size. A total of 80 patients were recruited as a pilot phase divided into two groups (40 patients in each arm). Participants were randomized in a 1:1 ratio into two groups. Randomization was conducted using a computer-generated table of random numbers with allocation concealment. Allocation concealment was done using serially numbered closed opaque envelopes. Once allocation was done, it could not be changed.

Group A, included 40 women, were given 17-OHPC [Cidolut-Depot, CID Company, Egypt] 250 mg intra-muscular at admission and every 7 days thereafter in addition to other conservative measures of early-onset PE. Group B included 40 women as a control group, to whom no intervention was given apart from the usual conservative measures of early-onset PE.

Follow-up was done in the inpatient unit one week after beginning of treatment and every week till delivery or termination of pregnancy. Measurements of TNF-α and IL-6 levels were repeated at termination of pregnancy. Detailed history was obtained from each participant including risk factors for preeclampsia as: nulliparity, previous history of PE, family history of PE, diabetes mellitus (DM), chronic renal disease, chronic hypertension, and multiple pregnancies.

Blood sample (5 ml venous blood) was obtained from each participant under complete aseptic conditions, into plain tube, left to clot for 30 minutes at 37c and centrifuged at 1000 xg for 15 minutes. The separated serum was divided into aliquots for subsequent determination of serum IL6 and TNF-α.

Laboratory assays

Human Tumor necrotic factor alpha (TNF-α)

Quantitative determination of TNF-α concentration was done using commercial Enzyme-Linked Immunosorbent Assay (ELISA) Kits (Catalog No: E-EL-H0109, Elabscience Biotechnology Inc, Human TNF alpha, USA), according to the manufacturer's instructions. The optical density (OD) was measured with spectrophotometry at a wavelength of 450 nm ± 2 nm using an ELISA reader (SN:1860-2600, STAT FAX2600-MICROPLATE ELISA reader). The concentration of TNF-α in samples, was calculated by comparing the OD of the samples with a standard curve.

Human Interleukin 6 (IL-6)

Quantitative determination of IL-6 concentration was done using commercial ELISA Kits (Catalog No: E-EL-H0102, Elabscience Biotechnology Inc, Human IL6 (interleukin 6), USA), according to the manufacturer's instructions. The OD was measured with spectrophotometry at a wavelength of 450 nm ± 2 nm, as mentioned before. The concentration of IL-6 in samples was calculated by comparing the OD of the samples with a standard curve.

Statistical analysis

All data were analyzed using (Statistical Package for Social Science) SPSS software Chicago, IL, USA, version 25. Comparison between categorical variables in both groups was done by Chi-square test and continuous variables were compared using Student T-test and Mann-Whitney test. A two-sided $p < 0.05$ was considered statistically significant.

Results

The study included 80 patients divided in a 17-OHPC group (n=40) and a control group (n=40). The personal and demographic data of women in both groups are presented in Table 1.

Table 1. Personal and demographic data of the study groups.

Demographic data	17-OHPC group (n= 40)		Control group (n= 40)		*p*-value
Age: (years)					
Mean ± SD	27.93 ± 6.50		27.67 ± 6.70		NS
Residence: (n, %)					
Rural	15	37.5%	18	45.0%	NS
Urban	25	62.5%	22	55.0%	
BMI					
Mean ± SD	30.95 ± 6.19		31.75 ± 6.67		NS
GA by LMP: (weeks)					
Mean ± SD	31.20 ± 2.45		31.50 ± 2.22		NS
Mean ± SD	30.00 ± 3.75		30.48 ± 2.28		NS

BMI: Body mass index, GA: gestational age, LMP: last menstrual period. Values are presented as mean ± SD or number (%)
P > 0.05 is not significant (NS).

Table 2 shows that there was no difference in blood pressure in both groups at admission and at termination of pregnancy. There was a statistically significant difference within the same group regarding systolic blood pressure (SBP) and diastolic blood pressure (DBP) between admission and termination (p<0.05).

Table 2. Blood pressure in the 17-OHPC vs control groups in patients with early onset preeclampsia.

Blood pressure	17-OHPC group (n= 40)	Control group (n= 40)	*p*-value[1]
	Mean ± SD	Mean ± SD	
SBP			
At admission	152.00 ± 19.45	152.12 ± 23.47	NS
At termination	138.50 ± 14.92	137.12 ± 23.15	NS
p-value[2]	0.001*	0.001*	
DBP:			
At admission	98.00 ± 10.01	100.00 ± 6.72	NS
At termination	82.73 ± 8.74	89.38 ± 11.18	0.049
p-value[2]	0.001*	0.007*	

SBP: systolic blood pressure; DBP: diastolic blood pressure. Values are presented as mean ± SD.
* *P* > 0.05 is not significant (NS). *P*[1]: p value between both groups. *P*[2]: p value inside the same group.

Table 3 shows the levels of inflammatory biomarkers in the 17-OHPC treated and control groups. TNF-α and IL-6 levels were not different between the two study groups. However, the concentrations of the inflammatory biomarkers (IL-6 and TNF-α), within the same group at admission and at termination, were significantly different (p<0.001). As there was a significant decline of IL-6 and TNF-α level in the 17-OHPC treated group and significant rise of IL-6 and TNF-α in the control group.

Table 3. Inflammatory biomarkers in the 17-OHPC vs control groups in patients with early onset preeclampsia.

Inflammatory biomarkers	17-OHPC group (n= 40)	Control group (n= 40)	*p*-value[1]
	Median (Range)		
TNF -α (pg/dl)			
At admission	60.00 (23.00-156.00)	56.56 (16.00-201.00)	NS
At termination	47.00 (26.90-114.00)	100.00 (50.00-171.00)	0.049
p-value[2]	<0.001*	<0.001*	
IL-6 (pg/dl)			
At admission	49.00 (10.40-67.00)	58.00 (27.00-94.00)	NS
At termination	21.50 (10.00-40.00)	89.00 (78.00-148.00)	0.046
p-value[2]	<0.001*	<0.001*	

TNF-α: tumor necrosis factor-α; IL-6: interleukin-6. *P* > 0.05 is not significant (NS).
Values are presented as mean ± SD. *P*1: P Value between both groups. *P*2: p value inside the same groups.

Table 4 shows the maternal outcomes in the two study groups. There was no difference between both groups regarding eclampsia, renal failure, disseminated intravascular coagulation (DIC), placental abruption and rate of admission to intensive care unit (ICU). Additionally, there was no difference between both groups regarding the rate of low 5 mins Apgar score at birth, respiratory distress, birth weight, and admission to neonatal ICU.

Table 4. Maternal complications in the 17-OHPC vs control groups in patients with early onset preeclampsia.

Maternal complications	17-OHPC group (n= 40)		Control group (n= 40)		*p*-value
	No.	%	No.	%	
Eclampsia	5	12.5%	4	10.0%	NS
Renal failure	5	12.5%	2	5.0%	NS
DIC	1	2.5%	2	5.0%	NS
Placental abruption	2	5.0%	4	10.0%	NS
ICU admission	10	25.0%	9	22.5%	NS
Days of ICU					
Median (Range)	1.5 (1.0-3.0)		2.0 (1.0-4.0)		NS

DIC: disseminated intravascular coagulation; ICU: intensive care unit.
Values are presented as number (%).*P* > 0.05 is not significant (NS).

However, there was a strong positive correlation between SBP at admission and TNF-α level (r= 0.867, *p*=0.017), and moderately significant positive correlation between DBP at admission and TNF-α (r=0.610, *p*<0.001). On the other hand, there was a mild significant positive correlation between IL-6 levels and SBP (r= 0.231, *p*=0.039) and DBP (r= 0.203, *p*= 0.041) at admission (Figures 1 – 4).

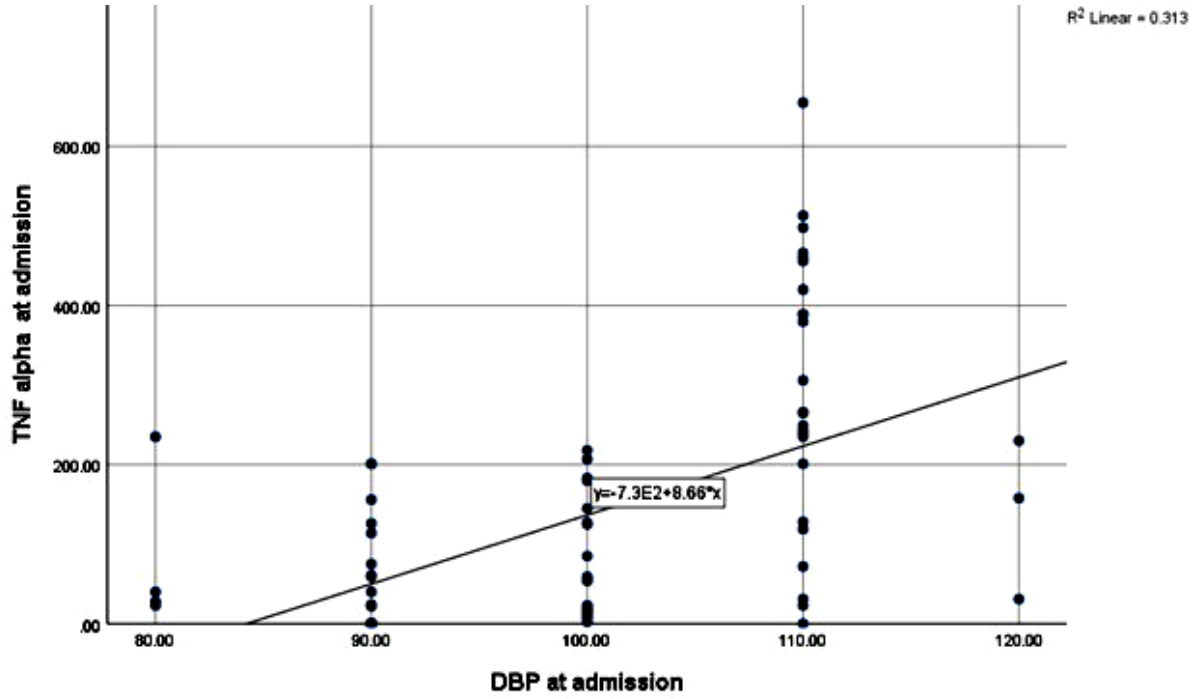

Figure 1. Correlation between TNF alpha and systolic blood pressure (SBP) at admission.

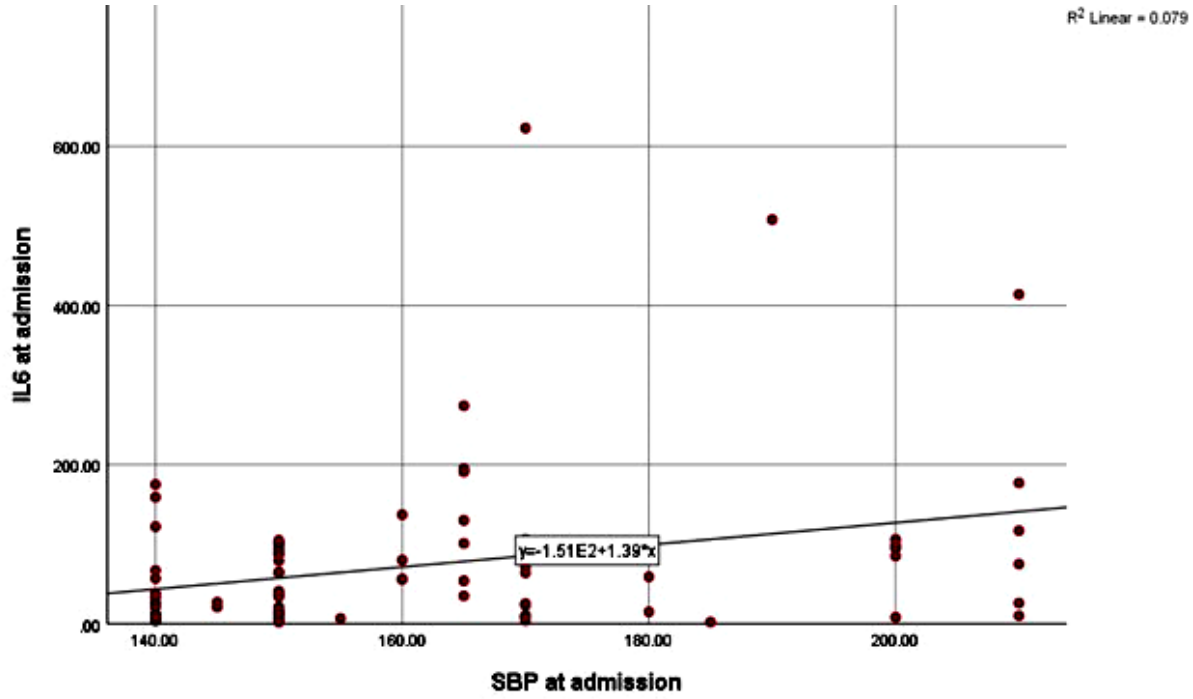

Figure 2. Correlation between TNF alpha and diastolic blood pressure (DBP) at admission

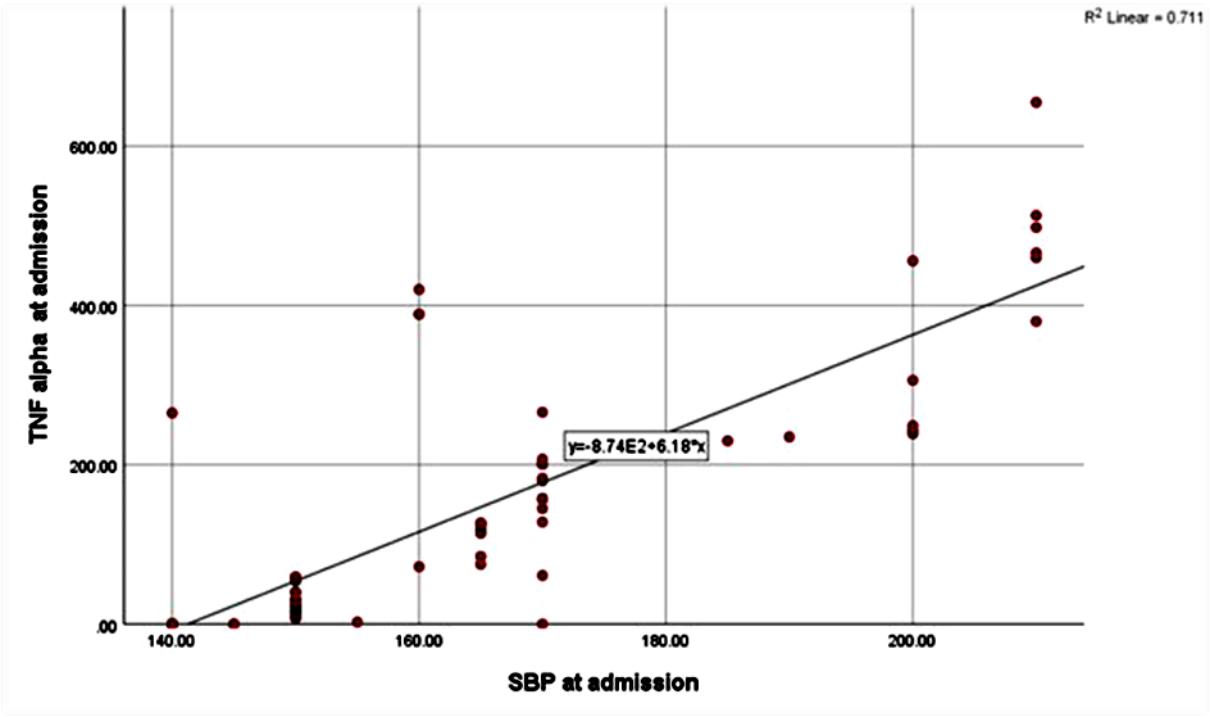

Figure 3. Correlation between IL-6 and systolic blood pressure (SBP) at admission

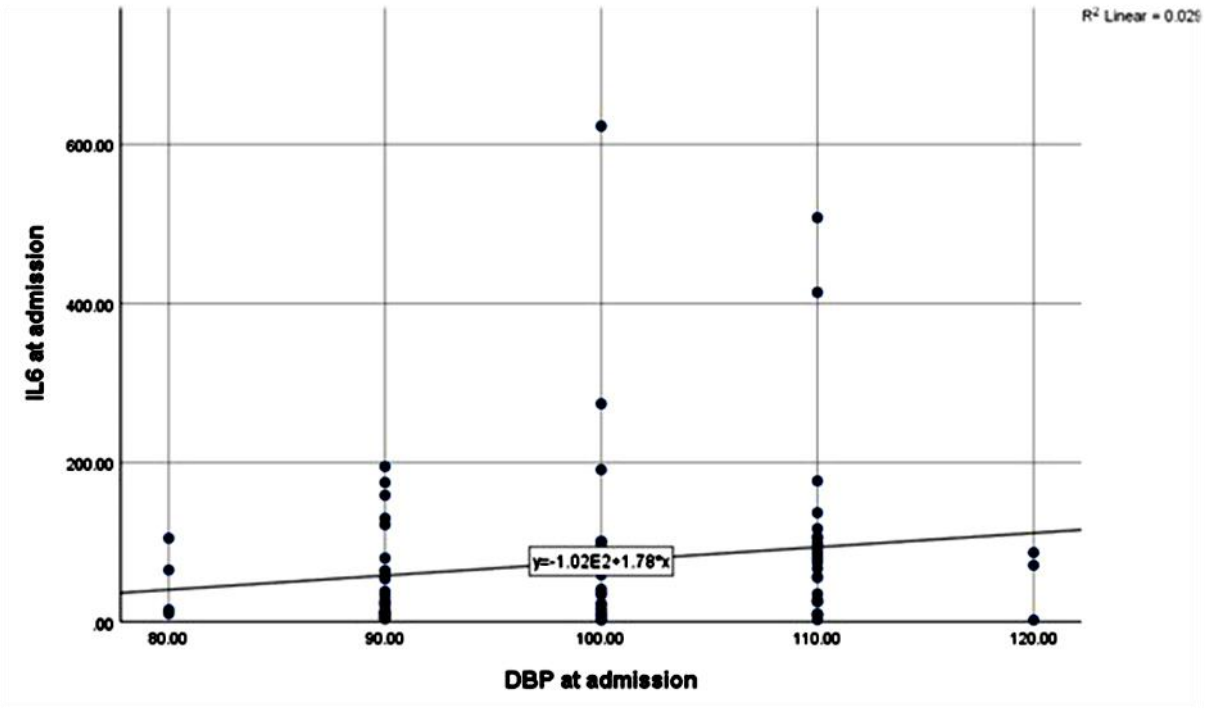

Figure 4. Correlation between IL-6 and diastolic blood pressure (DBP) at admission

Discussion

The present study was designed to evaluate the effect of 17-OHPC treatment on IL-6 and TNF-α in expectantly managed early-onset PE. It demonstrated that treatment with 17-OHPC can alter the level of the inflammatory biomarkers in patients with expectantly managed early-onset PE. However, it did not improve the maternal or the neonatal outcomes in these patients.

Several factors are known to define PE, end-organ damage can be assessed using a variety of laboratory procedures, none of which are particular to PE, such as liver function tests, urine protein measurement, or serum creatinine. In underdeveloped countries, PE has been shown to have detrimental impacts on maternal and neonatal health.[16] It is one of the most serious complications of pregnancy.[17] Women diagnosed with PE or eclampsia during pregnancy are more likely to have renal disease and hypertension later in life. Therefore, it is important to perform laboratory tests at admission and termination.

In the present study, there was no significant differences regarding, TNF-α and IL-6 levels between the study groups. However, intergroup comparison showed a significant difference between their levels at admission and at termination. In addition, the TNF-α concentration at admission was nearly equal in both groups. There was a statistically significant decrease regarding the TNF-α levels at termination in the 17-OHPC treated group, but the TNF-α level at termination was significantly higher in the control group.

Our study found that IL-6 levels at admission was lower in the 17-OHPC treated group than in the control group. Its level at termination was markedly decreased in 17-OHPC treated group but increased in the control group. Furthermore, IL-6 levels were significantly correlated with SBP and DBP (p=0.041). Additionally, there was a strong positive correlation between TNF-α level and SBP (p=0.017) in addition to a moderate positive correlation with DBP (p <0.001).

These results agreed with those reported in a study by Amaral, et al., 2017, that 17-OHPC injection to pregnant rats treated with IL-6 reduced the blood pressure during pregnancy.[12] The mean arterial blood pressure (MAP) in normal pregnant (NP) rats was 100 (3) mm Hg, which increased to 112 (4) mm Hg in the presence of IL-6 (p= 0.05). Pregnant rats administered 17-OHPC alone had a MAP of 99 (3) mm Hg, whereas pregnant rats with high IL-6 treated by 17-OHPC had a MAP of 103 (2) mm Hg (p= 0.05). Another study by Amaral, et al., 2015 noted that 17-OHPC decreased inflammatory cytokines. TNF-α was 65.84±17.7 pg/mL in reduced uterine perfusion pressure (RUPP) rats (n=5) but was decreased to 17.24±3.9 in rats treated with17-OHPC.[18] Similarly, another recent study reported that, 17-OHPC improved hypertension in response to elevated sFlt-1 in pregnant rats.[18] Also, our study results agreed with those of a study by Elfarra, et al., 2020, reported that MAP in NP rats (n =9) was 100±2, 104±6 in Sham rats (n =8), 128±2 in RUPP (n =11) and 115±3 mmHg in RUPP + 17-OHPC (n =10), p < 0.05. Pup weight and Uterine artery Resistance index were improved after administration of 17-OHPC.[19]

In 2003, Meis, et al., published a double-blind, placebo-controlled trial study which showed that there was a numerically higher rate of hypertension (HTN)/PE reported in the 17-OHPC group [27 (8.8%)] than in the placebo [7 (4.6%)].[20] However, in another multicenter study, Progestin's Role in Optimizing Neonatal Gestation (PROLONG) study, the rate of HTN/PE was higher in the placebo group than in the 17-OHPC group. The combined data from the two studies showed that the two groups had the same rate of success.[21]

In contrast, a multicenter prospective observational cohort study revealed that 62 pregnant women were admitted to the hospital for delivery. The cervical fluid concentrations of IL-6, IL-10, and TNF-α at baseline were estimated. The cervical fluid inflammatory cytokines level, of women characterized by inflammation, was unaffected by 17-OHPC treatment.[22] This outcome is contrary to that reported by Facchinetti, et al., 2008 who founded that there was no significant change in IL-6, IL-8, TNF-α, and NOx in the 17-OHPC and control groups.[23]

In conclusion, 17-OHPC has no effect in improving maternal or neonatal outcomes in conservatively managed early onset PE, although it alters the inflammatory markers levels (IL-6 and TNF- α) that could improve the pathogenesis of PE.

Author Contributions

AMAS and SAM contributed to the study conception and design. MAO, AMAS and SAM, contributed to data collection and analysis. ERB and ASS contributed to lab methodology. AMAS and MAO wrote the manuscript draft. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors acknowledge receipt of financial support for the research from Assiut Faculty of Medicine Research Grant Office.

Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board, Faculty of Medicine, Assiut University (February 2020).

Informed consent

An informed consent form was signed by each study participant before included in the study.

References

1. Jim B, Karumanchi SA. (2017). Preeclampsia: pathogenesis, prevention, and long-term complications. In *Seminars in nephrology* 37 (4), 386-397.
2. Webster K, Fishburn S, Maresh M, et al. (2019). Diagnosis and management of hypertension in pregnancy: summary of updated NICE guidance. *BMJ.* 9, 366.
3. Wisner K. (2019). Gestational hypertension and preeclampsia. *MCN Am J Matern Child Nurs*. 44(3), 170.
4. El-Sayed AA. (2017). Preeclampsia: A review of the pathogenesis and possible management strategies based on its pathophysiological derangements. *Taiwan J Obstet Gynecol.* 56(5), 593-598.
5. Murphy SR, LaMarca BB, Parrish M, et al (2013). Control of soluble fms-like tyrosine-1 (sflt-1) production response to placental ischemia/hypoxia: Role of tumor necrosis factor-alpha. Am J Physiol. 304, R130–135
6. Merlob P, Stahl B, Klinger G. (2012). 17α Hydroxyprogesterone caproate for prevention of recurrent spontaneous preterm birth. *Reprod Toxicol*. 33(1), 15-19.
7. Zhang Q, Huang Y, Zhang K, et al. (2018). Progesterone attenuates hypertension and auto-antibody levels to the angiotensin II type 1 receptor in response to elevated cadmium during pregnancy. *Placenta* 62, 16-24
8. Kiprono LV, Wallace K, Moseley J, et al. (2013). Progesterone blunts vascular endothelial cell secretion of endothelin-1 in response to placental ischemia. *Am J Obstet Gynecol*. 1, 209(1), 44-e1.
9. Veillon Jr EW, Keiser SD, Parrish MR, et al. (2009). 17-Hydroxyprogesterone blunts the hypertensive response associated with reductions in uterine perfusion pressure in pregnant rats. *Am J Obstet Gynecol.* 201(3), 324-e1.
10. Simoncini T, Fu XD, Caruso A, et al. (2007). Drospirenone increases endothelial nitric oxide synthesis via a combined action on progesterone and mineralocorticoid receptors. *Human Reprod.* 22(8), 2325-2334.
11. Amaral LM, Kiprono L, Cornelius DC, et al. (2014). Progesterone supplementation attenuates hypertension and the autoantibody to the angiotensin II type I receptor in response to elevated interleukin-6 during pregnancy. *Am J Obstet Gynecol.* 211(2), 158-e1.
12. Amaral LM, Faulkner JL, Elfarra J, et al. (2017). Continued investigation into 17-OHPC: results from the preclinical RUPP rat model of preeclampsia. *Hypertension.* 70(6), 1250-1255.
13. Amaral LM, Cottrell JN, Comley KM, et al. (2020). 17-Hydroxyprogesterone caproate improves hypertension and renal endothelin-1 in response to sFlt-1 induced hypertension in pregnant rats. *Pregnancy Hypertens.* 22, 151-155.
14. Mohammed NA, Ali AH, Gamal El-Tahawy NF, et al. (2018). Effect of Hydroxyprogesterone (17-OHPC) on Placenta in a Rat Model of Preeclampsia: Histological and Immunohistochemical Study. *J Cytol Histol.* 9, 496.
15. American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy (2013). *Obstet Gynecol.* 122,1122–31.

16. Lin S, Leonard D, Co MA, et al. (2015). Pre-eclampsia has an adverse impact on maternal and fetal health. *Translational Res*. 165(4), 449-63.
17. Ekun OA, Olawumi OM, Makwe CC, et al. (2018). Biochemical assessment of renal and liver function among Preeclamptics in Lagos Metropolis. *Int J Reprod Med.* 2018.
18. Amaral LM, Cornelius DC, Harmon A, et al. (2015). 17-hydroxyprogesterone caproate significantly improves clinical characteristics of preeclampsia in the reduced uterine perfusion pressure rat model. *Hypertension.* 65(1), 225-231.
19. Elfarra JT, Cottrell JN, Cornelius DC, et al. (2020). 17-Hydroxyprogesterone caproate improves T cells and NK cells in response to placental ischemia, new mechanisms of action for an old drug. *Pregnancy Hypertens.* 19, 226-232.
20. Meis PJ, Klebanoff M, Thom E, et al. (2003). Prevention of recurrent preterm delivery by 17 alpha-hydroxyprogesterone caproate. *New Eng J Med.* 348(24), 2379-2385.
21. Blackwell SC, Gyamfi-Bannerman C, Biggio Jr JR, et al. (2020). 17-OHPC to prevent recurrent preterm birth in singleton gestations (PROLONG study): a multicenter, international, randomized double-blind trial. *Am J Perinatol.* 37(02), 127-136.
22. Caritis SN, Hankins G, Hebert M, et al. (2018). Impact of Pregnancy History and 17-Hydroxyprogesterone Caproate on Cervical Cytokines and Matrix Metalloproteinases. *Am J Perinatol.* 35(05), 470-480.
23. Facchinetti F, Dante G, Venturini P, et al. (2008).17α-hydroxy-progesterone effects on cervical proinflammatory agents in women at risk for preterm delivery. *Am J Perinatol*. 25(08), 503-506.