Relation of Endothelin-1 and Malondialdehyde with Pre-eclampsia in Pregnant Women

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Abstract

A case-control study was conducted in Kirkuk city in Kirkuk general hospital from first of March 2018 and to end of September 2018 to evaluate the role of endothelin-1 and malondialdehyde in pathogenesis of pre-eclampsia in pregnant women. The study included 30 pregnant women with preeclampsia, patients were defined and divided according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. A control group of 30 healthy normotensive pregnant females was also included. All comparison groups were matched for mother's age, parity, and gestational age at the time of enrollment and blood collection. Three ml of blood was collected by vein puncture, blood samples were placed into sterile test tubes and left for 30 minutes at 37 °C then were centrifuged at 3000 rpm for 15 minutes then the clot was removed and the obtained sera were then aspirated using automatic micropipette and transferred into clean test tubes and stored in deep freeze at -20°C for determination of endothelin-1 and malondialdehyde by usingELISA technique. The study showed that the highest mean level of endothelin-1 was found in pregnant women with pre-eclampsia(13.15±2.81 pg/ml) and the lowest mean was in the control group (0.41±0.31 pg/ml). The result was highly significant (P<0.01). The study showed that the highest mean level of MDA was found in pregnant women with pre-eclampsia (6.75±2.94 nmol/ml) and the lowest mean was recorded in the control group (3.85±1.29 nmol/ml). The result was highly significant (P<0.01). The current study showed that the mean age of patients enrolled in the study was 33.5 years, the highest mean of endothelin-1 in HD patients was recorded within the age group 20-29 year of pregnant women with pre-eclampsia. Although the results was non-significant. The study showed positive correlation between endothelin-1 and MDA levels pregnant women with pre-eclampsia. It was concluded that serum endothelin-1 and MDA levels were highly correlated with pregnant women with pre-eclampsia

Keywords: *Endothelin-1; MDA; pre-eclampsia; Pregnancy.*

Introduction

One of the most common complications of pregnancy is preeclampsia. Preeclampsia occurs in ~8% of all pregnancies, and is significantly more prevalent in specific ethnic subpopulations(1). Despite an increasing awareness of the condition, and aggressive therapeutic intervention, preeclampsia remains a leading cause of both fetal and maternal perinatal morbidity and mortality, with ~15% of all preterm pregnancies attributable to preeclampsia(2). The disease is primarily seen in nulliparous women, with a significantly decreased incidence in succeeding pregnancies. Traditionally, the disorder has been defined by hypertension (systolic >140 mm Hg), proteinuria (300 mg/24 h), and edema(3). However, current guidelines omit edema as a necessary symptom for diagnosis, and there is increasing discussion as to whether the absolute values of proteinuria are the correct metrics by which to guide treatment, or whether the protein/creatine ratio would be a more diagnostic measurement (1). The molecular structure of this endothelium-derived constricting factor which produces powerful, very long-lasting constrictions of a range of mammalian blood vessels *in vitro* including human arteries and veins. It also causes long-lasting elevation of blood pressure when injected into rodents(4). Because of these observation, scientists at this time were of the opinion that this peptide - nowadays called endothelin-1 (ET-1) plays an important role in the pathogenesis of

arterial hypertension as well as pregnancy induced hypertension/preclampsia[5]. Nowadays, role of oxidative stress in PIH etiology is being researched, and acquired results show that oxidative stress could have a significant role in generation of preeclampsia since it starts damage of endothelium of placenta vascularization and immune response[6,7]. Normal pregnancy is considered to be a state where oxidative stress increases a bit, but there is no increase in, so called, free radicals[8]. Recently, a great attention is being paid to lipid peroxidation, which actually is oxidative damage of lipids and increased creation of lipid peroxides, whose final product is malondialdehyde (MDA). Nowadays, malondialdehyde (MDA) is used in many expert researches as oxidative stress marker, i.e. for assessment of lipid peroxidation[9,10]. The aim of this study was to evaluate the role of endothelin-1 and malondialdehyde in pathogenesis of pre-eclampsia in pregnant women.

Materials and Method

A case-control study was conducted in Kirkuk city in Kirkuk general hospital from first of March 2018 and to end of September 2018. The study included 30 pregnant women with preeclampsia, patients were defined and divided according to the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. A control group of 30 healthy normotensive pregnant females was also included. All comparison groups were matched for mother's age, parity, and gestational age at the time of enrollment and blood collection.

Exclusion Criteria:

1. Multiple gestations.
2. Fetal structural/genetic anomalies.
3. Maternal renal disease.
4. Metabolic disease.
5. Inflammatory disease.
6. Autoimmune disease, and other comorbidities associated with endothelial damage.

Hypertensive disorders were defined according to classification of The National High Blood presure Education program Working group on High Blood pressure in pregnancy . Twenty-four-hour autamatic blood pressure monitoring was performed Blood pressure readings were taken at 30minute intervals. For patients who was admitted to the hospital,and two readings of blood pressure was taken for patients who was not admitted to the hospital and diagnosed as hypertensive disorders depend on history and examination and blood pressure readings that record on antenatal card in each antenatal visit. Measurement of Bp done by sphygmomanometer in sitting position,with cuff size appropriate to patients arm circumference was used, and to eliminate apossible stressor for the patients,the visual preview of blood pressure measurement was removed. Three ml of blood was collected by vein puncture, blood samples were placed into sterile test tubes and left for 30 minutes at 37 °C then were centrifuged at 3000 rpm for 15 minutes then the clot was removed and the obtained sera were then aspirated using automatic micropipette and transferred into clean test tubes and stored in deep freeze at -20°C for determination of endothelin-1 and malondialdehyde by usingELISA technique.

Finding: As shown in Table 1. There was no significant difference between studied cases and the control group regarding patient age and gestational age at sampling and parity (P>0.05) while there was a significant difference between studied cases and the control group regarding systolic and diastolic blood pressure (P <0.05).

Table 1: Clinical characteristics of studied women

Parameters (Mean±SD)	Pre-eclampsia	Control Group
No.	30	30
Maternal age (yeas)	32.2±5.9	32.2±6.2
Gestational age	34.1±3.2	35.4±6.6
Parity, median (Range)	1 (1–6)	2 (1–6)
Mean 24 h SBP, mm Hg	148.6±14.3*	110.8±7.4
Mean 24 h DBP, mm Hg	96.6±12.9*	69.4±8.3
Maximal SBP, mm Hg	177.9±24.1*	119.0±10.2
Maximal DBP, mm Hg	118.8±12.8*	74.7±13.3

* P<0.01.

The study showed that the highest mean level of endothelin-1 was found in pregnant women with pre-eclampsia(13.15±2.81 pg/ml) and the lowest mean was in the control group (0.41±0.31 pg/ml). The result was highly significant (P<0.01), Table 2.

Table 2: Level of endothilin-1 in pregnant women with pre-eclampsia and the control group.

Endothelin 1 (pg/ml)	Pregnant women with pre-eclampsia	Control group
No.	30	30
Mean±SD.	1.78±1.02	0.41±0.31

(P<0.01)

The study showed that the highest mean level of MDA was found in pregnant women with pre-eclampsia (6.75±2.94 nmol/ml) and the lowest mean was recorded in the control group (3.85±1.29 nmol/ml). The result was highly significant (P<0.01), Table 3.

Table 3: Level of Malondialdehyde in pregnant women with pre-eclampsia and the control group.

MDA (nmol/ml)	Pregnant women with pre-eclampsia	Control group
No.	30	30
Mean±SD.	6.75±2.94	3.85±1.29

(P<0.01)

The current study showed that the mean age of patients enrolled in the study was 33.5 years, the highest mean of endothelin-1 in HD patients was recorded within the age group 20-29 year of pregnant women with pre-eclampsia. Although the results was non-significant, Table 4.

Table 4: Distribution of patients in the study according to age and endothelin-1 level.

Age groups (years)	No.	Pregnant women with pre-eclampsia
20-29	10	2.10±1.8
30-39	15	1.11±1.01
40-42	5	1.46±1.13

(P.value 0.08)

The study showed positive correlation between endothelin-1 and MDA levels pregnant women with pre-eclampsia (Figure 1).

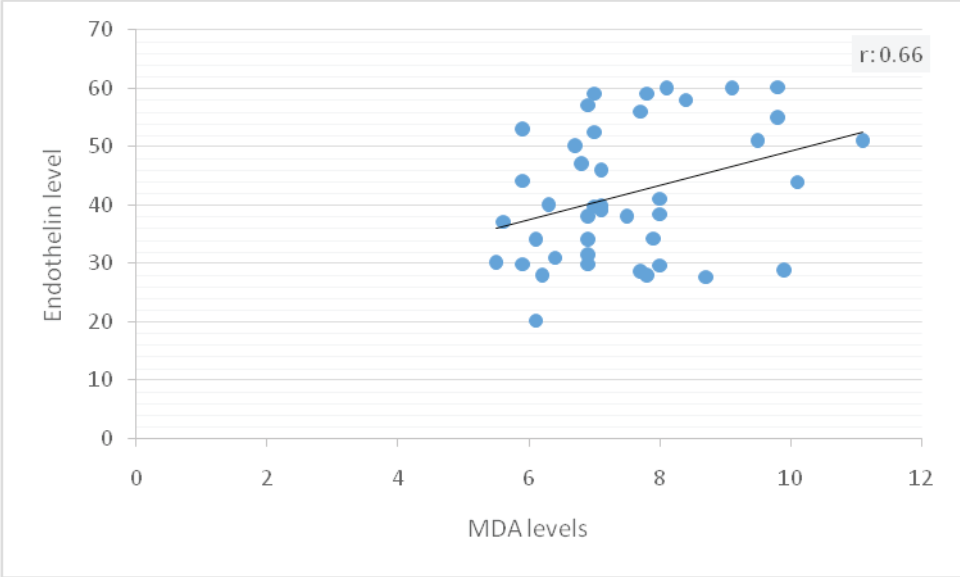

Figure 1: Correlation between endothelin-1 and MDA levels pregnant women with pre-eclampsia

Discussion

The result of the current study was in consistence with past study,showed that, plasma ET-1 concentrations are markedly increased in pregnant women with pre-eclampsia as compared with healthy individuals[11]. Xu, *et al*[12] also found that ET-1 was more likely to be elevated in pregnant women with pre-eclampsia. Li, *et al*[13] indicated that ET-1 was activated in several diseases, including renal failure and arterial hypertension. Additionally, Cin, *et al*[14] found that level

of ET-1 was significantly higher in pregnant women with pre-eclampsia compared to the controls ($P < 0.001$).

Similar results were also achieved by Hocher, *et al* [15] who found that MDA concentrations were significantly higher in pregnant women with pre-eclampsia than in controls. An other study demonstrated that Plasma level of MDA was significantly elevated in pregnant women with pre-eclampsia group than in healthy controls[16]. Additional reports demonstrated mean serum MDA levels was significantly elevated in pregnant women with pre-eclampsiacompared with normal level in healthy control group[17,18]. Formar report also showed that MDA was elevated in pregnant women with pre-eclampsia in comparison to the control groups. Several other studies also agreed with our findings, they found high serum MDA levels in pregnant women with pre-eclampsia compared with healthy individuals [19,20].

Former researches dealing with problem of MDA indicate that pregnancy is a state of physiological, slightly increased oxidative stress in comparison to healthy non-pregnant women. Oxidative stress could have a significant role in generation of pregnancy induced hypertension (PIH), since it starts damage of endothelium of placenta vascularization and immune response [21,22]. Others also concluded in their study that pregnancy induced hypertension (PIH) is a state of extreme increase of oxidative stress and lipid peroxidation [22]. In our study, we proved the increased MDA level as oxidative stress marker in pregnant women with PIH, too. Based on our acquired results, that means that pregnant women of test group had extremely increased MDA values in comparison to control group. Our results in this research show that pregnancy induced hypertension is a state of extremely increased oxidative stress which corresponds to the other studies, too [23-25].

Conclusion

Serum endothelin-1 and MDA levels were highly correlated with pregnant women with pre-eclampsia

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Obstetric and Gynecology Kirkuk health directorate and all experiments were carried out in accordance with approved guidelines.

References

1. Roberts JM, Pearson GD, Cutler JA, Lindheimer MD National Heart Lung and Blood Institute. Summary of the NHLBI Working Group on Research on Hypertension During Pregnancy. Hypertens Pregnancy. 2003;22:109–127.
2. Meis PJ, Goldenberg RL, Mercer BM, Iams JD, Moawad AH, Miodovnik M, Menard MK, Caritis SN, Thurnau GR, Bottoms SF, Das A, Roberts JM, McNellis D. The preterm prediction study: risk factors for indicated preterm births. Maternal-Fetal Medicine Units Network of the National Institute of Child Health and Human Development. Am J Obstet Gynecol. 1998;178:562–567.
3. Caritis S, Sibai B, Hauth J, Lindheimer M, VanDorsten P, Klebanoff M, Thom E, Landon M, Paul R, Miodovnik M, Meis P, Thurnau G, Dombrowski M, McNellis D, Roberts J. Predictors of pre-eclampsia in women at high risk. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Am J Obstet Gynecol. 1998;179:946–951.
4. Turner JA. Diagnosis and management of pre-eclampsia: an update. Int J Womens Health. 2010;2:327–337.
5. Lindheimer MD, Kanter D. Interpreting abnormal proteinuria in pregnancy: the need for a more pathophysiological approach. Obstet Gynecol. 2010;115:365–375. [
6. Hladunewich M, Karumanchi SA, Lafayette R. Pathophysiology of the clinical manifestations of preeclampsia. Clin J Am SocNephrol. 2007;2:543–549.
7. Khong Y, Brosens I. Defective deep placentation. Best Pract Res ClinObstetGynaecol. 2011;25:301–311. [PubMed] [Google Scholar]
8. Brosens IA, Robertson WB, Dixon HG. The role of the spiral arteries in the pathogenesis of preeclampsia. ObstetGynecolAnnu. 1972;1:177–191.
9. Rodesch F, Simon P, Donner C, Jauniaux E. Oxygen measurements in endometrial and trophoblastic tissues during early pregnancy. Obstet Gynecol. 1992;80:283–285.
10. Rajakumar A, Doty K, Daftary A, Harger G, Conrad KP. Impaired oxygen-dependent reduction of HIF-1alpha and-2alpha proteins in pre-eclamptic placentae. Placenta. 2003;24:199–208.

11. Lu YP, Tsuprykov O, Vignon-Zellweger N, Heiden S, Hocher B: Global Overexpression of ET-1 Decreases Blood Pressure - A Systematic Review and Meta-Analysis of ET-1 Transgenic Mice. Kidney Blood Press Res 2016; 41: 770-780.
12. Xu M, Lu YP, Hasan AA, Hocher B: Plasma ET-1 Concentrations are Elevated in Patients with Hypertension -Meta-Analysis of Clinical Studies. Kidney Blood Press Res 2017; 42: 304-313.
13. Li J, Chen YP, Dong YP, Yu CH, Lu YP, Xiao XM, Hocher B: The impact of umbilical blood flow regulation on fetal development differs in diabetic and non-diabetic pregnancy. Kidney Blood Press Res 2014; 39: 369-377.
14. Cen YP, Lu YP, Li J, Liu ZW, Chen WJ, Liang XJ, Chen X, Wen WR, Xiao XM, Reichetzeder C, Hocher B: Fetal and maternal angiotensin (1-7) are associated with preterm birth. J Hypertens 2014; 32: 1833-1841.
15. Hocher B, Chen YP, Schlemm L, Burdack A, Li J, Halle H, Pfab T, Kalk P, Lang F, Godes M: Fetal sex determines the impact of maternal PROGINS progesterone receptor polymorphism on maternal physiology during pregnancy. Pharmacogenet Genomics 2009; 19: 710-718.
15. American College of Obstetricians and Gynecologists, Task Force on Hypertension in Pregnancy. Hypertension in pregnancy: Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol 2013; 122: 1122-1131.
16. Sibai BM: Diagnosis and management of gestational hypertension and preeclampsia. ObstetGynecol 2003; 102: 181-19.
17. Hauth JC, Ewell MG, Levine RJ, Esterlitz JR, Sibai B, Curet LB, Catalano PM, Morris CD: Pregnancy outcomes in healthy nulliparas who developed hypertension. Calcium for Preeclampsia Prevention Study Group. Obstet Gynecol 2000; 95: 24-28.
18. Suhail M, Suhail MF. Koncentracijemalondial-dehidaiantioksidativnihvitaminapupkovinekod-majkioboljelihodpreeklampsijeizdravihmajki. Biochemia Medica. 2009;19(2):182–91. [Google Scholar]
19. Huber C, Robertson J, Taylor R, et al. Lipid peroxidation in pregnancy. New perspectives on preeclampsia. Am J Obstet Gynecol. 1989;161:1025–34. [PubMed] [Google Scholar]
20. Sutherland MR, Bertagnolli M, Lukaszewski MA, et al. Preterm birth and hypertension risk: the oxidative stress paradigm. Hypertension. 2014;6:12–8. [PubMed] [Google Scholar]
21. Bhale DV, Hivre MD, Mahat RK, Bujurge AA. Comparative study of serum malondialdehyde levels as marker of oxidative stress in patients of pregnancy–induced hypertension and controls. Journal of Medical Sciencis. 2014;1(2):53–5. [Google Scholar]
22. Leidiane L, Francisco M, Pancich G, Thissiane LG. Oxidative Stress Markers in Pregnant Women with Preeclampsia. American Journal of Medical and Biological Research. 2015;3(3):68–73.
23. Watanabe K, Mori T, Iwasaki A, et al. Increased oxygen free radical production during pregnancy may impair vascular reactivity in preeclamptic women. Hypertens. Res. 2013;36:356–60.
24. Egić A. Predikcijapreeklampsije u prvomtrimestru. Imaging biomarkeri -perspektiveiočekivanja. Udruženjezaperinatalnu medicinu Srbije; 2013. Tara. Zbornikradova. 2013:101–4.
25. Kurlak LO, Green A, Loughna P, Broughton Pipkin F. Oxidative stress markers in hypertensive states of pregnancy: preterm and term disease. Frontiers in Physiology. 2014;5(310):1–8.