Open Access Maced J Med Sci **electronic publication ahead of print,**
published on June 16, 2018 as https://doi.org/10.3889/oamjms.2018.252

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences.
https://doi.org/10.3889/oamjms.2018.252
eISSN: 1857-9655
Clinical Science

Nitric Oxide and Pre-Eclampsia: A Comparative Study in Ghana

Ebenezer Owusu Darkwa[1*], Robert Djagbletey[1], Raymond Essuman[1], Daniel Sottie[2], Gifty Boatemaa Dankwah[3], George Aryee[1]

[1]School of Medicine and Dentistry, University of Ghana, Accra, Ghana; [2]Department of Anaesthesia, Korle-Bu Teaching Hospital, Accra, Ghana; [3]School of Biomedical and Allied Health Sciences, University Of Ghana, Accra, Ghana

Citation: Darkwa EO, Djagbletey R, Essuman R, Sottie D, Dankwah GB, Aryee G. Nitric Oxide and Pre-Eclampsia: A Comparative Study in Ghana. Open Access Maced J Med Sci. https://doi.org/10.3889/oamjms.2018.252

Keywords: Pre-eclampsia; Healthy pregnant women; Nitric oxide; Endothelial function; Griess Reagent

***Correspondence:** Ebenezer Owusu Darkwa. School of Medicine and Dentistry, University of Ghana, Accra, Ghana. E-mail: eoddarquah@yahoo.co.uk

Received: 22-Feb-2018; **Revised:** 23-May-2018; **Accepted:** 25-May-2018; **Online first:** 16-Jun-2018

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: Preeclampsia is one of the commonest aetiologies of foetal and maternal mortality and morbidity. Though common, the aetiology of preeclampsia has remained unknown with several inconclusive theories surrounding the disease. Recent studies have implicated vascular endothelial dysfunction and possibly nitric oxide in preeclampsia.

AIM: To compare plasma nitric oxide levels in pre-eclampsia and healthy pregnant women in a large tertiary hospital in Ghana.

METHODS: This was a case-control study conducted among pre-eclampsia and healthy pregnant women in Korle-Bu Teaching Hospital over a four-month period. Thirty (30) pre-eclamptic and 30 healthy pregnant women aged 18-35 years with over 30 weeks' gestation were consecutively recruited into the study after obtaining informed consent. Plasma nitric oxide levels were determined using the Griess Reagent system. Data were analysed using Statistical Package for the Social Sciences (SPSS) software version 20.0 and results were compared using the independent t-test. A P-value of ≤ 0.05 was considered statistically significant.

RESULTS: The parity and body mass index (BMI) of the participants were similar. There was a significant difference in the blood pressure of the pre-eclamptic compared to healthy pregnant women. There was no statistically significant difference (P-value = 0.160) in the plasma levels of nitric oxide in pre-eclamptic (Mean = 1178.78; SD = 89.70 nM) compared to healthy pregnant women (Mean = 1365.43; SD = 95.46 nM).

CONCLUSION: Plasma nitric oxide levels may not play a significant role in the aetiology of pre-eclampsia.

Introduction

Two to eight per cent (2-8%) of all pregnancies worldwide are complicated by pre-eclampsia causing over 63,000 maternal deaths annually [1]. The maternal mortality rate of pre-eclampsia is highest in low and middle income countries. However, pre-eclampsia is still a life-threatening disorder even in developed countries [2]. There is a five-fold increase in perinatal deaths from intrauterine growth restriction and prematurity as a result of pre-eclampsia [3]. Preterm birth in itself is responsible for the majority of neonatal deaths and nearly one half of all cases of congenital neurologic disability [4]. Fifteen percent (15%) of all premature deliveries in the United States (US) are as a result of pre-eclampsia [3]. The aetiology of pre-eclampsia lies in the placenta though it remains unknown [5]. Pre-eclampsia is known to occur only in the presence of a placenta as in for example molar pregnancy and resolves after its delivery. Placental growth is a regulated process and it is vital for normal foetal development and for maintenance of successful pregnancy. Normal placental development requires that cytotrophoblast invades the maternal spiral arterioles [5]. There is an impairment of cytotrophoblastic invasion of the myometrial portion of the spiral arteries in pre-eclampsia leading to narrowing of the spiral arteries with limited blood supply to the foetus [6]. This eventually causes placental ischaemia and microinfarction with subsequent release of placental factors leading to an imbalance in angiogenic factors and therefore widespread endothelial dysfunction that is seen in pre-eclampsia [6]. The ability of the maternal system to handle the deficits in placentation and subsequent challenge to the maternal cardiovascular system

partly depend on the immune system, as systemic inflammatory stress plays a key role in endothelial cell activation [6].

Nitric oxide (NO), a vascular endothelial relaxant may be involved in the development of pre-eclampsia. An endothelial form of NO synthase has been localised to the syncytiotrophoblast and villous endometrium in term pregnancies [5] [6]. The placenta is, therefore, an important source of NO during pregnancy. Various animal models in which NO synthesis has been inhibited have been associated with symptoms such as hypertension, proteinuria, thrombocytopenia and restricted foetal growth [5] [6]. The main placental vasodilator is nitric oxide, and it regulates placental vascular resistance and reactivity, apoptosis and invasion by trophoblast, and aggregation and adhesion of platelets in the placental bed [7]. Numerous studies hold the view that pre-eclampsia is a multisystem disorder with vascular endothelial dysfunction, however, as to whether the change in the function of the endothelium noted in pre-eclampsia results in a decrease, an increase or an unchanged endothelial NO synthesis is still debatable [8].

Literature has reported inconsistent results as far as serum nitric oxide levels in pre-eclampsia compared to healthy pregnant women is concerned. Various studies have reported raised serum nitric oxide levels [9] while others have reported non-significant change [8] [10], and others too, a reduced serum level of nitric oxide [5] [6] [11] in pre-eclampsia compared to normal pregnant women. There is also conflicting literature as to whether the alteration in the function of the endothelium seen in pre-eclampsia results in a pathophysiologic decrease in NO synthesis [8] [10]. Notwithstanding the above controversies, systematic reviews and meta-analyses have shown that pre-eclamptic have a significant increased risk of incidence of cardiovascular diseases, obesity, diabetes and insulin resistance later in life [12]. Thus pre-eclampsia has a huge global and economic burden. There is, therefore, the need to carry out this study to find out the role NO play in the pathophysiology of pre-eclampsia in Ghanaian women.

Methods

This was a case-control study undertaken at the Korle-Bu Teaching Hospital (KBTH), Ghana between March and June 2016.

The study was conducted at the Korle-Bu Teaching Hospital, the premiere Teaching Hospital and the largest tertiary hospital affiliated with the University of Ghana School of Medicine and Dentistry. The 2000 bed capacity hospital has a 350 bed capacity with 3 operating theatre suites obstetrics and gynaecology department. The department has 65 doctors, 200 nurses and midwives, with a daily antenatal attendance of 100 patients, and a total annual delivery of between 10,000 and 12,000.

Ethical Approval for the study was obtained from the Ethical and Protocol Review Committee of University of Ghana School of Medicine and Dentistry (Protocol Identification Number: CHS-Et/M.4-P4.5/2015-2016). Clearance was also received from the Management of the Korle-Bu Teaching Hospital and Head of Obstetrics and Gynaecology department where the study was conducted.

The study population included third-trimester healthy pregnant women and pre-eclamptics aged 18-35 years attending the obstetrics and gynaecology clinic at the Korle-Bu Teaching Hospital. Patients not eligible for inclusion were:

1. Pregnant and pre-eclamptic on any medical treatment other than iron and folic acid

2. Pregnant and pre-eclamptic with chronic hypertension, history of kidney disease, diabetes mellitus, cardiac diseases and neuromuscular problems.

Pre-eclampsia was diagnosed using the onset of hypertension after 20 weeks of gestation with blood pressure > 140/90 mmHg measured on two separate occasions with the coexistence of proteinuria of at least 2+ on dipstick [13].

The plasma nitric oxide level for healthy pregnant women and pre-eclamptics has been found to be 63.8 and 73.3 µmol/l respectively [14], with a mean difference (d) of 9.5 µmol/l. Using the formula by Charan and Biswas [15], sixty (60) pregnant women in their third trimester (gestation > 30 weeks), consisting of 30 pre-eclamptic as cases and 30 healthy pregnant women as controls were recruited consecutively into the study after obtaining informed consent.

The participants were interviewed using a structured questionnaire to obtain their demographic characteristics after signing an informed consent form. The information collected included their age, parity and gestational age. Participants subsequently had their weight and height measured using mechanical patient weighing scale with height rod (Product: 6003, Italy).

Three ml of blood was drawn from the cubital vein using a sterile 19G hypodermic needle fixed on a 5 ml syringe after cleansing the site to be punctured with methylated spirit. Aseptic conditions were adhered to. The blood sample was transferred into a sodium ethylenediamine tetraacetate (Na EDTA) test tube and prevented from clotting by gently inverting the tube 4 times manually. Nitric oxide levels were assessed in the plasma samples using the Griess Reagent system (Promega, Madison, USA). The

assay relies on a diazotisation reaction that was originally described by Griess in 1879.

Patients' age, weight, height, parity, BMI and plasma nitric oxide levels were entered into Microsoft® Access database 2010 (Microsoft® USA), and analysis was done using statistical package for social science (SPSS®) software version 20.0.

The age, BMI and parity of participants, were presented as means (standard deviations) in a tabular form. The plasma nitric oxide levels between the two groups were presented in a bar chart. Independent t-test was employed to compare the difference between the mean plasma nitric oxide level of pre-eclamptic and healthy pregnant women. A *p*-value ≤ 0.05 was considered statistically significant.

Results

The mean systolic, diastolic and arterial pressures were high in the pre-eclamptic compared to healthy pregnant women (Table 1). Age, parity and BMI were similar among the pre-eclamptic and the healthy pregnant women.

Table 1: Demographic and clinical characteristics of the study sample

Characteristic	Pre-eclamptic Mean(SD)	Healthy pregnant women Mean(SD)	*P*-value
N	30	30	
Age (years)	30.97 (5.51)	29.93 (2.60)	0.358
Parity	1.70 (1.42)	1.13 (1.41)	0.567
BMI	32.03 (7.52)	30.50 (5.50)	0.374
SBP	170.13 (23.69)	116.47 (13.38)	<0.001*
DBP	106.30 (18.79)	67.57 (8.54)	<0.001*
MAP	126.20 (20.86)	83.87 (8.85)	<0.001*

*Significant at *P* < 0.05; n-sample size; SD-standard deviation; BMI-body mass index (kg/m^2); SBP-systolic blood pressure (mmHg); DBP-diastolic blood pressure (mmHg); MAP-mean arterial pressure (mmHg).

There was no statistically significant difference in plasma nitric oxide levels in pre-eclamptic compared to healthy pregnant women (*P* = 0.160). The plasma nitric oxide levels in pre-eclamptic and healthy pregnant women were 1178.78 (89.70) nM and 1365.43 (95.46) nM respectively (Figure 1).

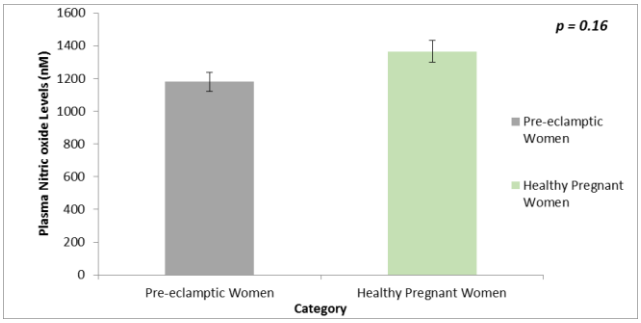

Figure 1: Mean plasma nitric oxide levels for pre-eclamptic and healthy pregnant women

A non-significant negative correlation between mean arterial pressure and plasma nitric oxide levels in pre-eclamptic was noted (Pearson Correlation Coefficient *r* = -0.072; *P* = 0.712) (Figure 2).

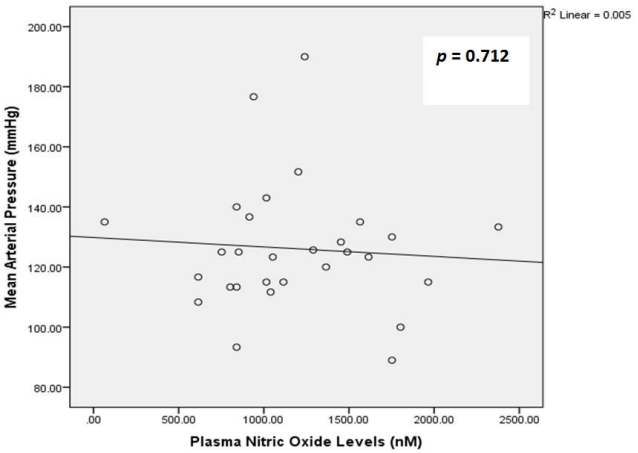

Figure 2: Correlation between mean arterial pressure and plasma nitric oxide levels in pre-eclamptic

Discussion

This study showed a statistically non-significant difference (*P* = 0.358) between the maternal age of pre-eclamptic compared to healthy pregnant women and therefore no association of maternal age with pre-eclampsia. This is similar to the findings of other studies [16] but disagrees with the findings of Macdonald-Wallis and colleagues [17]. The difference in findings may be attributable to sample characteristic differences between the study populations.

This study observed a statistically non-significant difference (*P* = 0.374) in BMI between pre-eclamptic and healthy pregnant women. Therefore BMI may have no association with pre-eclampsia. This is similar to the findings of Onyebule and colleagues [18] but contradicts the observations of other studies which have noted an association of elevated BMI with pre-eclampsia [19].

The systolic, diastolic and mean arterial pressures of the pre-eclamptic were significantly higher compared to that of the healthy pregnant women (*P* < 0.001). This was expected given the criteria used for diagnosis of pre-eclampsia. Mean arterial pressure is said to be predictive of pre-eclampsia even though other studies have noted otherwise [20].

Our study showed a statistically non-significant reduction in plasma nitric oxide levels in pre-eclamptic compared to healthy pregnant women (*P* = 0.160) agreeing with the observations of other studies [8] [10]. Previous studies designed to search for a relationship between nitric oxide production in

pre-eclampsia and healthy pregnancy has shown inconsistent conclusions [9] [11]. The supporters for increased nitric oxide levels in pre-eclamptic compared to healthy pregnant women argue that the increase is as a result of a compensatory mechanism for the occurring endothelial damage in pre-eclampsia hence an attempt to correct the vasospasm effect.

However, the supporters for decreased nitric oxide levels in pre-eclamptic as compared to healthy pregnant women suggest that the reduction is as a result of down-regulation of the nitric oxide synthase enzyme and/or occurrence of endothelial damage in the development of the disorder. The results of a statistically non-significant difference in plasma nitric oxide levels between pre-eclamptic and healthy pregnant women in this study is supported by other studies [8] [10]. This finding, however, may not mean that there is no association between plasma nitric oxide levels and pre-eclampsia but then as per this study, the change in nitric oxide level may not significantly impact the pathophysiology of pre-eclampsia. This may be because the determination of nitric oxide levels is confounded by several factors including a source of sample (plasma, serum, urine), a method of assaying, diet, alcohol consumption, atmospheric pollution, exercise and cigarette smoking [21].

In disorders where there may be a very small difference in nitric oxide production, it may be impossible to find a significant change over the uncontrolled external factors stated above. Considering the study site, it may be very difficult eliminating the above inter-subject variations, and therefore a longitudinal study on selected subjects where inter/intra personal variations can be seen as well as any differences in nitric oxide levels throughout pregnancy between pre-eclamptic and healthy pregnant women is advised. Other studies have also admitted the contradictory reports regarding the involvement of nitric oxide in maternal adaptation to pregnancy and suggested possible multi-mechanism physiology acting in concert to maintain the pregnant mother and the foetus with the input from each mechanism being genetically determined [22].

A non-significant (P = 0.712) negative correlation (Pearson correlation coefficient r = -0.072) was found between mean arterial pressure and plasma nitric oxide levels of pre-eclamptic with an R^2 value of 0.5% implying that changes in plasma nitric oxide levels are a poor predictor of mean arterial pressures in pre-eclamptic patients.

In conclusion, this study failed to demonstrate any significant difference in plasma nitric oxide levels in pre-eclamptic compared to healthy pregnant women. Therefore, plasma nitric oxide levels may not play a significant role in the pathophysiology of pre-eclampsia.

Acknowledgements

The authors express their sincere gratitude to Prof. S. A. Obed (Head of Department of Obstetrics and Gynaecology-Korle-Bu Teaching Hospital) for his guidance and advice during the development of this manuscript. Also, we are grateful to patients who participated in the study.

References

1. Duley L. The Global Impact of Pre-eclampsia and Eclampsia. Semin Perinatol. 2009; 3(33):130–7. https://doi.org/10.1053/j.semperi.2009.02.010 PMid:19464502

2. Khan KS, Wojdyla D, Say L, Gülmezoglu AM, Van Look PF. WHO analysis of causes of maternal death: a systematic review. Lancet. 2006; 367(9516):1066–74. https://doi.org/10.1016/S0140-6736(06)68397-9

3. Lain KY, Roberts JM. Contemporary concepts of the pathogenesis and management of preeclampsia. J Am Med Assoc. 2002; 287(24):3183–6. https://doi.org/10.1001/jama.287.24.3183

4. Goldenberg RL, Rouse DJ. Prevention of premature birth. N Engl J Med. 1998; 339(5):313–20. https://doi.org/10.1056/NEJM199807303390506 PMid:9682045

5. Smith RA, Kenny LC. Current thoughts on the pathogenesis of pre-eclampsia. Obstet Gynaecol. 2006; 8(1):7–13. https://doi.org/10.1576/toag.8.1.007.27202

6. George EM, Granger JP. Recent insights into the pathophysiology of preeclampsia. Expert Rev Obstet Gynecol. 2010; 5(5):557–66. https://doi.org/10.1586/eog.10.45 PMid:21170149 PMCid:PMC3001629

7. Krause B, Hanson MA, Casanello P. Role of nitric oxide in placental vascular development and function. Placenta. 2011; 32(11):797–805. https://doi.org/10.1016/j.placenta.2011.06.025 PMid:21798594 PMCid:PMC3218217

8. Diejomaoh F, Omu A, Al-Busiri N, Taher S, Al-Othman S, Fatinikun T, et al. Nitric oxide production is not altered in preeclampsia. Arch Gynecol Obstet. 2004; 269(4):237–43. https://doi.org/10.1007/s00404-002-0465-6 PMid:15221318

9. Smárason A, Allman KG, Young D, Redman CWG. Elevated levels of serum nitrate, a stable end product of nitric oxide, in women with pre-eclampsia. An Int J Obstet Gynaecol. 1997; 104(5):538–43. https://doi.org/10.1111/j.1471-0528.1997.tb11528.x

10. Di Iorio R, Marinoni E, Emiliani S, Villaccio B, Cosmi E V. Nitric oxide in preeclampsia: lack of evidence for decreased production. Eur J Obstet Gynecol Reprod Biol. 1998; 76(1):65–70. https://doi.org/10.1016/S0301-2115(97)00159-0

11. Seligman SP, Buyon JP, Clancy RM, Young BK, Abramson SB. The role of nitric oxide in the pathogenesis of preeclampsia. Am J Obstet Gynecol. 1994; 171(4):944–8. https://doi.org/10.1016/S0002-9378(94)70064-8

12. Bellamy L, Casas J-P, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. Br Med J. 2007; 335(7627):974. https://doi.org/10.1136/bmj.39335.385301.BE PMid:17975258 PMCid:PMC2072042

13. Tranquilli AL, Dekker G, Magee L, Roberts J, Sibai BM, Steyn W, et al. The classification, diagnosis and management of the hypertensive disorders of pregnancy: a revised statement from the ISSHP. Pregnancy Hypertens Int J Women's Cardiovasc Heal. 2014; 4(2):97–104. https://doi.org/10.1016/j.preghy.2014.02.001

14. Adekanle DA, Adeyemo OT, Adeniyi AA, Okere RA, Jimoh AK, Adebara IO, et al. Serum Magnesium Levels in Healthy Pregnant and Pre-Eclamptic Patients—A Cross-Section Study. Open J Obstet Gynecol. 2104; 4(9):56–568.

15. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian J Psychol Med. 2013; 35(2):121. https://doi.org/10.4103/0253-7176.116232 PMid:24049221 PMCid:PMC3775042

16. Shamsi U, Hatcher J, Shamsi A, Zuberi N, Qadri Z, Saleem S. A multicentre matched case control study of risk factors for preeclampsia in healthy women in Pakistan. BioMed Cent Womens Heal. 2010; 10:14(1).

17. Macdonald-Wallis C, Lawlor DA, Heron J, Fraser A, Nelson SM, Tilling K. Relationships of risk factors for pre-eclampsia with patterns of occurrence of isolated gestational proteinuria during normal term pregnancy. PLoS One. 2011; 6(7):e22115. https://doi.org/10.1371/journal.pone.0022115 PMid:21789220 PMCid:PMC3138774

18. Onyegbule OA, Meludu SC, Dioka CE, Udigwe GO, Udo JN, Ezidigboh AN, et al. Comparison of serum levels of calcium and magnesium among preeclamptic and normotensive pregnant women at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria. Int J Res Med Sci. 2014; 2(2):404–8. https://doi.org/10.5455/2320-6012.ijrms20140506

19. Poorolajal J, Jenabi E. The association between body mass index and preeclampsia: a meta-analysis. J Matern Neonatal Med. 2016; 29(22):3670–6. https://doi.org/10.3109/14767058.2016.1140738 PMid:26762770

20. Redman C, Beilin L, Bonnar J, Wilkinson R. Plasma-urate measurements in predicting fetal death in hypertensive pregnancy. Lancet. 1976; 307(7974):1370–4. https://doi.org/10.1016/S0140-6736(76)93024-5

21. Baylis C, Suto T, Conrad K. Importance of nitric oxide in control of systemic and renal hemodynamics during normal pregnancy: studies in the rat and implications for preeclampsia. Hypertens Pregnancy. 1996; 15(2):147–69. https://doi.org/10.3109/10641959609015699

22. Buhimschi IA, Saade GR, Chwalisz K, Garfield RE. The nitric oxide pathway in pre-eclampsia: pathophysiological implications. Hum Reprod Update. 1998; 4(1):25–42. https://doi.org/10.1093/humupd/4.1.25 PMid:9622411