ORIGINAL ARTICLE

Periodic Assessment of Antenatal and Post Natal Serum Endothelin-1 (ET-1) and Nitric Oxide (NO) Levels in Hypertensive Disorders of Pregnancy (HDP)

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ABSTRACT

Introduction: Hypertensive Disorders of Pregnancy (HDP) is an independent risk factor of cardiovascular (CVS) disease with endothelial dysfunction postulated to be the pathophysiology. Endothelin-1 (ET-1), a potent vasoconstrictor, has been identified as a pivotal mediator in HDP. Disturbances in nitric oxide (NO) bioavailability found in endothelial dysfunction may increase susceptibility to cardiovascular diseases such as hypertension. The study aims to determine serial ET-1 and NO levels in patients with HDP and its role in persistent endothelial dysfunction. **Materials and Methods:** Thirty-six pregnant women from the following categories (i) normal pregnant women (Control) (ii) chronic hypertension during pregnancy (CH) and (iii) pregnancy induced hypertension (PIH) participated in this study. Blood pressure indices measurements and sample collection were done at antepartum (32 weeks) and postpartum (8 weeks and 12 weeks). ET-1 and serum NO were measured using the Human ET-1 (Endothelin-1) ELISA Kit and Nitric Oxide (total) detection kit respectively. **Results:** Serum ET-1 was significantly higher in patients with CH (55.3 pg/ml) and PIH (35.6 pg/ml) compared to Control (11.8 pg/ml) during antenatal until 3 months postpartum (CH 38.3 pg/ml, PIH 29.5 pg/ml, Control 1.9 pg/ml). This was accompanied by significantly lower levels of serum NO in HDP patients. **Conclusion:** Persistently higher than normal levels of ET-1 and lower than normal levels of NO up to 3 months postpartum in patients with history of HDP indicate presence of persistent endothelial dysfunction despite BP normalisation in PIH patients. Long term NO/ET-1 imbalance may account for the increased CVS disease risk.

KEYWORDS: Hypertensive Disorders of Pregnancy (HDP), Endothelin-1 (ET-1), Nitric Oxide (NO), Persistent Endothelial Dysfunction

INTRODUCTION

The prevalence of Hypertensive Disorders of Pregnancy (HDP) in Malaysia is approximately 23.3 per 1000 live births. HDP remains one of the most common disorders in pregnancy, which can lead to both maternal and foetal morbidity and mortality. Additionally, elevated blood pressure (BP) in pregnancy increases the risk of cardiovascular disorders in later life even in the absence of other known risk factors. Earlier studies propose that common antecedent risk factors are the cause however a significant risk is noted even in women with no known risk factors.[1]

HDP is defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg during pregnancy. HDP are generally classified into gestational hypertension (GH), pre-eclampsia (PE), chronic hypertension and chronic hypertension superimposed with pre-eclampsia. GH is diagnosed at ≥ 20 weeks and resolves ≤ 12 weeks postpartum. PE has a proteinuria component and chronic hypertensive includes women already previously diagnosed with essential hypertension prior to the pregnancy.[2] A large population based cohort study in Taiwan determined that women with history of hypertensive disease in pregnancy were at higher risk of developing major adverse cardiovascular events

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(MACEs) up to three years postpartum.[3] Patients with HDP are also at high risk of developing chronic hypertension at an earlier age.[4] The risk of cardiovascular disease is stratified according to the severity of the HDP with women who have history of chronic hypertension with superimposed PE at highest risk.[5] Earlier studies attribute this increased risk to common antecedents or classic cardiovascular (CVS) disease risk factors present even prior to the pregnancy, however more recent studies have found that even women with no known CVS disease risk factors are at higher risk of developing CVS disease later in life.[1,6] Although the reason for this increased risk is as yet, not well understood, the main theory is that it is due to persistent endothelial dysfunction that occurs after the initial insult during pregnancy, which can persist postpartum. Studies using forearm blood flow (FBF) have detected presence of endothelial dysfunction from 6 months to 5 years postpartum.[7]

Endothelin 1 (ET-1) is an endothelial derived peptide with diverse biological action in various systems including renal and cardiovascular. It has a profound vasoconstrictive property and is known as the most potent vasoconstrictor in the human body. Control of ET-1 production and secretion is mainly at gene transcription level. Many CVS diseases have been shown to be associated with elevated ET-1 production, including hypertension, congestive heart failure, and chronic renal failure. It has also been identified as a key mediator in HDP especially in the development of pre-eclampsia. [8-10]

Nitric oxide (NO) is a mediator that induces vasodilatation and inhibits platelet aggregation. It is synthesized from L-arginine through the action of nitric oxide synthase (NOS). NO levels depend on arginine concentrations, NOS activity and superoxide concentration.[11] Disturbances in NO bioavailability found in endothelial dysfunction may increase susceptibility to cardiovascular diseases including hypertension.[12]

The study aims to determine serial ET-1 and NO levels in patients with HDP and its role in persistent endothelial dysfunction.

MATERIALS AND METHODS

Recruitment

This is a comparative cross-sectional study. A total of thirty-six pregnant women with gestational age between 30 and 36 weeks were recruited to participate in this study from November 2016 to November 2017 from each of the following categories (i) gestational hypertension (GH) (ii) chronic hypertension during pregnancy (CH) and (iii) controls (normal pregnant women). The participants were recruited from the Antenatal Clinic at Hospital Tengku Ampuan Afzan and Klinik Kesihatan Balok, Kuantan, Pahang. For this study, GH was defined as a blood pressure of ≥ 140/90 mmHg on more than two occasions greater than 6 hours apart after 20 weeks of pregnancy and returned to normal blood pressure within 12 weeks postpartum. Patients suffering from any chronic illnesses including diabetes mellitus, renal disease, heart disease, thyroid disorders and systemic lupus erythematosus (SLE) were excluded from the study. Only Malay participants were recruited in this current study.

Prior to recruitment, the prospective follow-up nature of the study and samples required were explained to all subjects and informed consent obtained. Participants were informed that they may choose to voluntarily withdraw from the study at any point. Sample collection was done at antepartum (30-36 weeks) and postpartum (8 weeks and 12 weeks).

This research was approved by the IIUM Research Ethics Committee (IREC 551) and Malaysian Research Ethics Committee and registered under the National Malaysian Medical Research Register (NMRR-16-867-28866).

Measurement of Blood Pressure Indices

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) of all participants were measured with an automatic upper-arm oscillometric device (Dinamap1 Compact T Critikon Vital answers, Johnson & Johnson Medical - CRITIKON, Norderstedt, Germany).

Measurement of Endothelin-1 and Nitric Oxide

Participants were subjected to blood taking to measure serum ET-1 and NO level. ET-1 was measured using the Human ET-1 (Endothelin-1) ELISA Kit (Catalog No: E-EL-H0064) according to the manufacturer's specifications. The kit has a sensitivity of detecting a minimum dose of 0.75 pg/ml. Samples were allowed to clot for 2 hours at room temperature and centrifuged for 15 minutes at

1000 x g. The supernatant was collected and the assay was carried out immediately.

Serum NO was measured using the Nitric Oxide (total) detection kit (Catalog No: ADI-917-020) according to the manufacturer's specifications. The kit has a sensitivity of 0.625 µM/L. Samples were diluted at 1:2 into the reaction buffer then ultrafiltered through a 10 000 MWCO filter and used directly in the assay.

STATISTICAL ANALYSIS

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS), version 21. All continuous data was expressed as mean ± SEM. Multivariate analysis of serial variables using repeated measures ANOVA was performed factoring in the chosen covariates. Tukey's HSD test was used as post hoc for ANCOVA and pairwise comparison using univariate pairwise comparisons, Bonferronni correction and Wilk's Lamda criterion was used as a post hoc tools for repeated measures ANOVA. P value of less than 0.05 ($p < 0.05$) was considered significant.

RESULTS

Table I demonstrates clinical characteristics from control, chronic hypertensive and GH patients. All participants were Malay, since the majority of patients at these clinics were from this ethnicity. There were no significant differences in maternal age or gestational period between the three groups at sampling. Both maternal age and weight at booking were taken as covariates.

Systolic blood pressure, diastolic blood pressure and mean arterial blood pressure was significantly higher in women with HDP compared to Control during all three test intervals. Blood pressure during the three study intervals for the Control group ranged from 106/69 mmHg to 107/70 mmHg, whereas for the CH group was 140/89 mmHg to 143/94 mmHg and for the GH group from 117/71 mmHg to 145/91 mmHg.

Endothelin-1 (ET-1)

As depicted in Figure 1, analysis of ET-1 revealed that the mean serum concentration for the CH at 55.3 pg/ml (n=12) and GH group at 35.6 pg/ml (n=12) was significantly higher ($p<0.05$) compared to the control group at 11.8 pg/ml (n=12) during the antenatal period. The ET-1 levels of the control group were found to be 1.7 pg/ml after 2 months postpartum, significantly lower compared to the chronic hypertensive at 12.7 pg/ml and GH at 8.4 pg/ml. Three months postpartum, ET-1 levels in the control group were 1.9 pg/ml which was statistically significant compared to the chronic hypertensive group at 38.3 pg/ml and GH group at 29.5 pg/ml.

When analysed by period of sampling, ET-1 levels were found to be significantly different between the three groups and at the different time periods of sampling. There is a significant interaction between the group of participants sampled and the period of sampling.

Nitric Oxide (NO)

The mean serum concentration of NO as shown in Figure 2 shows that during the antenatal period those in the control group had the highest levels at 214.9 µM/L compared to the CH group at 150.6 µM/L and the GH group at 167.2 µM/L. During 2 months postpartum levels of serum NO in both chronic hypertensive group at 73.3 µM/L and GH group at 78.1 µM/L was significantly lower than the control group at 107.8 µM/L. After 3 months postpartum serum NO levels in the control group was significantly higher at 55.5 µM/L compared to the chronic hypertensive group at 24.8 µM/L and GH group at 32.8 µM/L.

When analysed by period of sampling, NO levels were found to be significantly different between the three groups and at the different time periods of sampling. However, there was no significant interaction between the group of participants sampled and the period of sampling.

DISCUSSION

ET-1 has been associated with numerous cardiovascular diseases including hypertension, congestive heart failure and chronic renal failure. This is mainly due to its extreme vasoconstrictive potency.[12-14]

Table 1: Clinical characteristics of patients in each group during antenatal and postnatal period. CH: chronic hypertension during pregnancy, PIH: pregnancy induced hypertension, MAP: mean arterial pressure. 'a' significant compared to control ($p<0.05$), b significant compared to CH ($p<0.05$) (Adjusted for covariates age and weight at booking. Covariates evaluated at the following values Age = 34.64, Weight = 59.53)

Groups	Mean Blood Pressure ± SEM (mmHg)									Maternal Age ± SEM (years)
	Antenatal			2 months post partum			3 months post partum			
	Systolic	Diastolic	MAP	Systolic	Diastolic	MAP	Systolic	Diastolic	MAP	
Control	107 ± 2.8	70 ± 2.5	82 ± 2.5	108 ± 2.8	75 ± 2.3	86 ± 2.4	106 ± 2.2	69 ± 2.1	81 ± 2.0	34.6 ± 0.9
CH	143 ± 3.5[a]	94 ± 1.9[a]	110 ± 2.3[a]	143 ± 3.4[a]	92 ± 1.7[a]	109 ± 2.1[a]	140 ± 2.8[a]	89 ± 1.2[a]	106 ± 1.5[a]	35.2 ± 1.4
GH	145 ± 5.7[a]	91 ± 3.9[a]	109 ± 4.3[a]	123 ± 4.2[a,b]	75 ± 2.2[b]	91 ± 2.7[b]	117 ± 2.4[b]	71 ± 1.4[b]	88 ± 3.1[b]	34.3 ± 0.9

Previous studies have mostly focused on ET-1 levels in patients with PE as researchers try to evaluate its value as a biomarker for predicting PE and severity of disease, and found that circulating ET-1 is indeed raised in these patients however correlation with severity was not a universal finding.[11,15,16] This study found that ET-1 levels in patients with HDP who did not develop PE were also significantly higher during antenatal period compared to healthy controls. There was however, no difference between the pregnant chronic hypertensive patients and those with GH, which may indicate that elevation of ET-1 levels serve as a common end pathway for HDP.

GH group. Despite the numerous studies on ET-1 in PE women, most studies on levels postpartum are on anti-angiogenic factors and markers of oxidative stress. However, due to the overwhelming evidence of ET-1 involvement in pathophysiology of hypertension, these increased levels despite normal BP is important to note.[9,13,17]

Normal pregnancy is associated with reduced vascular resistance and increased NO levels which serves to alleviate the stress on the cardiovascular system due to physiological increase in maternal blood volume.[18]

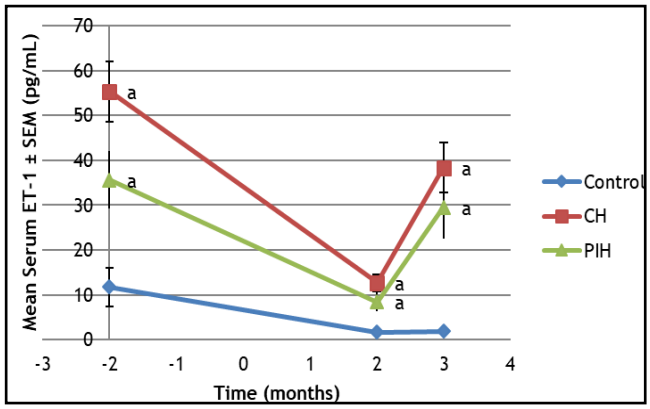

Figure 1: Mean serum Endothelial-1 ± SEM (pg/mL) in each group during antenatal and postnatal period. '0 on x axis represents parturition. CH: chronic hypertension during pregnancy, GH: gestational hypertension. 'a' significant compared to control ($p<0.05$). (Adjusted for covariates age and weight at booking. Covariates evaluated at the following values Age = 34.64, Weight = 59.53)

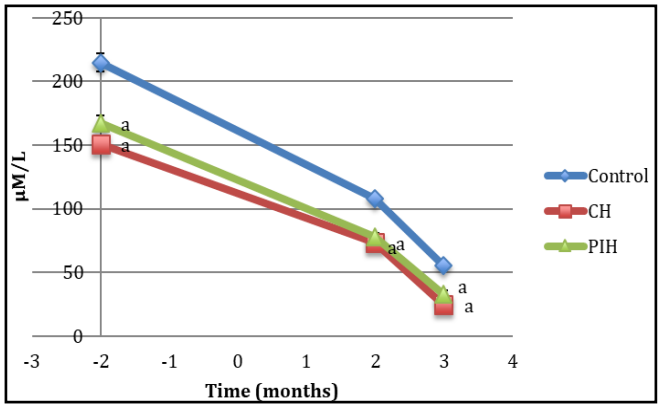

Figure 2: Mean serum Serum NO ± SEM (µM/L) in each group during antenatal and postnatal period. '0 on x axis represents parturition. CH: chronic hypertension during pregnancy, GH: pregnancy induced hypertension. 'a' significant compared to control ($p<0.05$). (Adjusted for covariates age and weight at booking. Covariates evaluated at the following values Age = 34.64, Weight = 59.53)

Our results also indicated that levels of ET-1 remained significantly high in women with HDP up to 3 months postpartum with no difference between GH group and CH group despite BP normalisation in the

However, in HDP and specifically PE circulating levels of NO have been found to be reduced.[11,19] NO is synthesized via the action of NOS from L-arginine and causes vascular smooth muscle relaxation and

other beneficial effects on the maternal vascular system. Its levels are incumbent upon several factors including L-arginine levels, NOS substrate concentrations and oxidative stress. Oxidative stress found in HDP is attributed to placental ischaemia causing an increase in antiangiogenic factors, reactive oxygen species (ROS) and endothelium derived contractile factors (EDCF). There is also concurrent reduction in pro-aniogenic factors and endothelium derived relaxing factors (EDRF) such as NO.[11,12] This study also found a similar reduction in NO levels in HDP patients as compared to control, which persisted up to 3 months postpartum.

One of the main functions of NO is to limit the potent constrictor capacity of ET-1. In conditions such as HDP, where bioavailability of NO are low and circulating ET-1 is high, ET-1 dependent increased vascular tone occurs. Studies have shown that both increased ET-1 mediated arterial pressure elevation after acute NOS inhibition and diminished vasoconstrictor response to NOS inhibition during non-selective blockade of ET-1 receptors.[11,18-20]

Addressing the normalization of BP in GH groups despite significantly higher levels of ET-1 and lower levels of NO compared to control during postpartum, may suggest that even low levels of NO act to antagonize ET-1 activity. However, exposure to long term unmitigated actions of ET-1 may cause adaptive changes which are likely persistent or irreversible.[21] As this study shows, there is evidence to suggest that although the initial insult occurs during pregnancy leading to GH, the endothelial dysfunction persists even during postpartum (Figure 3).

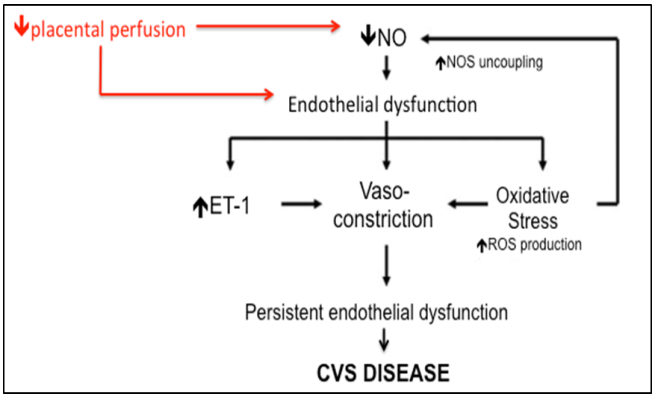

Figure 3: Possible mechanism of persistent endothelial dysfunction in patients with previous history of hypertensive disease in pregnancy. NO: nitric oxide, NOS: nitric oxide synthase, ET-1: endothelin-1, ROS: reactive oxygen species, CVS: cardiovascular

CONCLUSION

Persistently higher than normal levels of ET-1 and lower than normal levels of NO up to 3 months postpartum in patients with history of HDP indicate presence of persistent endothelial dysfunction despite BP normalization in GH patients. Long term NO/ET-1 imbalance may account for the increased CVS disease risk. A larger study more representative of this country's multi-ethnic composition as well as more prospective in nature may shed more light as to the clinical significance of these findings and possible therapeutic, prognostic and preventative implications.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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