Increased Oxidative Stress in Women With Pregnancy-induced Hypertension

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Objective   To investigate whether pregnancy-induced hypertension (PIH) may increase oxidative stress in women with PIH, and to explore the mechanisms by which PIH may increase oxidative stress and potential free radical damage.   **Methods**   Seventy women with PIH and seventy women with uncomplicated normotensive pregnancy (UNP) whose age, nutritional conditions, levels of hemoglobin and albumin were all matched, were enrolled in a randomized controlled trial. Their plasma concentrations of nitric oxide (NO), vitamin C (VC), vitamin E (VE), and β-carotene (β-CAR) as well as their erythrocyte malondialdehyde (MDA), and activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) were determined by spectrophotometry.   **Results**   Compared with average values of the above experimental parameters in the women with UNP, the average value of erythrocyte MDA in the women with PIH significantly increased ($P<0.0001$), while the average values of plasma NO, VC, VE, and β-CAR as well as those of erythrocyte SOD, CAT, and GPX in the women with PIH significantly decreased ($P<0.0005-0.0001$). The findings from partial correlation analysis (controlling for age) for 70 women with PIH showed that with elevated systolic blood pressure (SBP) and diastolic blood pressure (DBP), MDA value gradually increased ($P<0.001$), and NO, VC, VE, β-CAR, SOD, CAT, and GPX values gradually decreased ($P<0.02-0.001$). The findings from reliability analysis for NO, VC, VE, β-CAR, SOD, CAT, GPX, and MDA values used to reflect increased oxidative stress and potential free radical damage in women with PIH showed that the reliability coefficients (alpha, 8 items) = 0.7062, $P< 0.0001$, and the standardized item alpha = 0.9116, $P< 0.0001$.   **Conclusion**   The findings in the present research suggest that pregnancy-induced hypertension can increase oxidative stress and potential free radical damage in women with pregnancy-induced hypertension.

Key words: Pregnancy-induced hypertension; Oxidative stress; Free radicals; Free radical damage; Oxidation; Lipoperoxidation; Antioxidant; Antioxidase; Malondialdehyde

INTRODUCTION

Hypertension is a common medical complication during pregnancy, and pregnancy-induced hypertension is a common hypertensive disorder during pregnancy[1-8]. Nitric oxide, an important vasodilator and platelet-aggregation-inhibitor, and some antioxidants and antioxidases, and malondialdehyde may be associated with pregnancy-induced hypertension[9-30]. Up to now, there are neither reports on changes of oxidative stress in women with pregnancy-induced hypertension, nor reports about the relationship among SBP, DBP, NO, VC, VE, β-CAR, SOD, CAT, GPX, and MDA in women with pregnancy-induced hypertension. To explore the mechanisms by which pregnancy-induced hypertension would increase oxidative stress and potential free radical damage in women with pregnancy-induced hypertension, 70 women with PIH and 70 women with UNP, whose age, nutritional conditions, levels of hemoglobin and albumin were all matched were enrolled in a randomized controlled trial. Their plasma concentrations of NO, VC, VE, and β-CAR, and erythrocyte MDA, as well as activities of SOD, CAT, and GPX in erythrocytes were determined by spectrophotometry. At the same time, differences in experimental parameters between women with PIH and UNP were detected by independent-samples t test. Relationships between SBP, DBP, and each parameter

for 70 women with PIH were determined by partial correlation analysis (controlling for age), and stepwise regression analysis for SBP, DBP, and parameters, as well as reliability analysis of the experimental parameters were conducted.

MATERIALS AND METHODS

Study Design

A randomized controlled trial was conducted. In order to obtain an objective conclusion, principles of randomized control, replication and equilibrium, and management factor, experimental effect and subjects, and inclusion and exclusion criteria of subjects were taken into consideration, and strictly executed in the research[31-32].

Subjects

Women with PIH   Seventy women were randomly sampled from 102 women with PIH in the Affiliated Obstetrical and Gynecological Hospital, College of Medicine, Zhejiang University, China, and confirmed by the diagnostic criteria[1-8]. The subjects were enrolled according to the inclusion criteria[1-8], with "Select Cases-Random Sample of Cases" in "SPSS 11.0 for Windows". Their SBP ranged from 141 to 158 mm Hg, and DBP from 91 to 108 mm Hg. Their course of disease was 1 to 5 (2.27 ± 1.23) months, with mild edema and/or proteinuria. Their

age was 20 to 30 years, with their hemoglobin level being 111 to 142 g/L, and serum albumin level being 33.18 to 48.33 g/L. They were all volunteers in this research.

Women with UNP   Seventy women whose age, nutritional condition, hemoglobin, and albumin were matched with the women with PIH were randomly sampled from 107 women with UNP, and confirmed by the diagnostic criteria[1-8]. The subjects were enrolledd according to the inclusion criteria[1-8], with "Select Cases-Random Sample of Cases" in "SPSS 11.0 for Windows". Their SBP ranged from 95 to 138 mm Hg, and DBP from 65 to 89 mm Hg. Their age was 21 to 30 years, with their hemoglobin level being 112 to 141 g/L, and serum albumin level being 35.11 to 48.38 g/L. They were all volunteers in this research.

There was no significant difference between the average values of age, hemoglobin level, and serum albumin level in the women with PIH and UNP by independent-samples t test. There was also no significant difference between annual earning, education level, profession or occupation, residence region, daily diet (food and drink), and dose of sodium[33-34] (sodium chloride and monosodium glutamate) in the daily diet, and mental status during gestation in the two groups by independent-samples t test, or Pearson chi-square test.

The demographic and other data of the 70 women with PIH and the 70 women with UNP are presented in Table 1.

TABLE 1

Demographic and Other Data in Women With PIH and Those With UNP ($\bar{x} \pm s$)

Item	Women With PIH (n=70)	Women With UNP (n=70)	Independent Samples t Test
Age (year)	20～30 (25.45±2.12)	21～30 (25.43±2.13)	t = 0.0548 P = 0.9564
Hemoglobin (g/L)	111～142 (127.2±6.4)	112～141 (126.8±6.0)	t = 0.3525 P = 0.7250
Albumin (g/L)	33.18～48.33 (39.27±2.65)	35.11～48.38 (39.47±2.60)	t = 0.4503 P = 0.6532
Systolic Blood Pressure (mm Hg)	141～158 (149.3±5.1)	95～138 (112.3±9.6)	—
Diastolic Blood Pressure (mm Hg)	91～108 (98.7±4.6)	65～89 (77.6±5.7)	—
Smoking History	No	No	—
Abusing Alcohol History	No	No	—

In medical histories of the above subjects, disorders associated with brain, heart, lungs, liver, and kidneys, and other organs, as well as blood

system, circulatory system, and respiratory system, and other systems, were all excluded by routine blood, urine and stool examinations as well as radiographs,

cardiogram, and other necessary examinations. Medical histories of inflammation, hyperlipidemia, acute or chronic bronchitis, asthma, autoimmune disease, diabetes, atherosclerosis, tumors and other diseases, and subnutrition, malnutrition, and other nutritional diseases were also excluded.

In the previous month, none of the above subjects took any antioxidant supplements, such as vitamin C, vitamin E, β-carotene, ginkgo biloba, and tea polyphenols, and/or other similar substances.

In the above subjects, benign hypertension, essential hypertension, secondary hypertension, adrenal hypertension, portal hypertension, pulmonary hypertension, and renal hypertension, were all excluded[1-8], preeclampsia/eclampsia, chronic hypertension, and chronic hypertension with superimposed pregnancy-induced hypertension or preeclampsia, and HELLP (hemolysis, elevated liver enzymes, low platelets) syndrome, were all excluded[1-8]. In addition, the above subjects all had no smoking history or excessive drinking history.

Methods

Measurement of blood pressure Blood pressure was measured in sitting position at the level of the heart, with an appropriate sphygmomanometer cuff. The fifth Korotkoff sound, phase V (absence of sound) instead of phase IV Korotkoff sound (muffling), was used for the determination of the diastolic value. If the subject was hypertensive, blood pressure was measuredd in both arms at the initial visit. The diagnosis of hypertension was established on the basis of at least two elevated blood pressures on at least two separate occasions[4].

Collection and pretreatment of blood samples Fasting venous blood samples were collected from all the subjects in the morning. Heparin sodium was added as an anticoagulant, plasma and promptly-separated erythrocytes were stored at −50 ℃ immediately. The blood samples did not undergo any hemolysis[31-32].

Determination of biochemical substances and enzymes Spectrophotometry for α-naphthylamine coloration was used to determine plasma NO level expressed as nmol/L[35-36]. Spectrophotometry for ferrozine coloration was used to determine plasma VC and VE levels expressed as μmol/L[31-32]. Plasma β-CAR level was measured by spectrophotometry and expressed as μmol/L[31-32]. Spectrophotometry for pyrogallol auto-oxidation inhibition was used to determine erythrocyte SOD activity expressed as U/g·Hb[31-32]. Spectrophotometry for coloration of hydrogen peroxide and acetic acid-potassium dichromate was used to determine erythrocyte CAT activity expressed as K/g·Hb[31-32]. The modified

Hafeman's spectrophotometry for determination of glutathione peroxidase was used to determine erythrocyte GPX activity expressed as U/mg·Hb[35-36]. Spectrophotometry for thiobarbituric acid reactive substances (TBARS) was used to determine erythrocyte MDA level expressed as nmol/g·Hb[31-32].

Analytical reagents used to determine the above biochemical substances and enzymes, such as vitamin C, vitamin E, β-carotene, 5,6-diphenyl-3-(2-pyridyl)-1,2,4-triazinedisulfonic acid disodium salt (ferrozine), Cu·Zn-superoxide dismutase, catalase, α-naphthylamine, 1,2,3-trihydroxybenzene (pyrogallol), and 1,1,3,3-tetraethoxypropane, 2-thiobarbituric acid (TBA), were purchased from SIGMA® Chemical Company, USA. Other analytical reagents were produced in China. Fresh quadruply distilled water was prepared with a quartz glass distilling apparatus. In determing the above biochemical substances and enzymes, main analytical instruments included Hewlett Packard 8453-spectrophotometer (USA), and others[31-32].

In determining the above biochemical substances and enzymes, the standardization during experiment, such as the same batch number of each reagent, same quality control, same laboratory assistant, and same analytical apparatus, was strictly used for each experiment in order to decrease errors and ensure the analytical quality[31-32].

Medical Statistic Analysis

All experimental data were analyzed with SPSS 11.0 for Windows statistic software using a Compaq Pentium Ⅳ/2.4 GHz computer. The experimental parameters presented normal distributions by Kolmogorov-Smirnov Z test, and were expressed as $\bar{x} \pm s$ and 95% confidence interval (95% CI). Hypothesis testing methods included independent-samples t test, Pearson chi-square test (χ^2 test), partial correlation analysis (controlling for age), stepwise regression, and reliability analysis. In the statistical analysis, the level of hypothesis testing (α) was ≤0.05 in order to avoid false positives (type Ⅰ error), and the power of hypothesis testing (power) was ≥ 0.85 to avoid false negatives (type Ⅱ error)[31-32].

RESULTS

Comparison Between the Average Values of Biochemical Parameters ($\bar{x} \pm s$) in Women With PIH and Those With UNP

Compared with the average values of biochemical parameters in women with UNP, the average values

of plasma NO, VC, VE, and β-CAR as well as those of erythrocyte SOD, CAT, and GPX in women with PIH significantly decreased (*P*<0.0005-0.0001), while the average value of erythrocyte MDA in women with PIH significantly increased (*P*<0.0001) (Table 2).

TABLE 2

Comparison of Average Values of Biochemical Parameters Between Women With PIH and Those With UNP ($\bar{x} \pm s$), and Their 95% CI

Group	*n*	Plasma				Erythrocytes			
		NO	VC	VE	β-CAR	SOD	CAT	GPX	MDA
		nmol/L	μmol/L	μmol/L	μmol/L	U/g·Hb	K/g·Hb	U/mg·Hb	nmol/g·Hb
Women with PIH	70	292.9± 31.0 (285.6-300.3)	46.05± 11.52 (43.30-48.80)	18.42± 4.61 (17.32-19.52)	1.39±0.44 (1.29-1.50)	1866±214 (1815-1917)	246.1± 65.8 (230.4-261.8)	25.38± 4.85 (24.22-26.53)	33.42± 4.50 (32.35-34.50)
Women with UNP	70	384.3± 36.5 (375.6-393.0)	54.29± 13.25 (51.13-57.45)	24.81± 4.47 (23.74-25.88)	1.66±0.50 (1.54-1.78)	2055±201 (2007-2103)	294.4± 75.8 (276.3-312.5)	28.39± 4.94 (27.22-29.57)	28.27± 4.44 (27.21-29.33)
F[*] *P*		2.0724 0.1522	0.6162 0.4338	0.0032 0.9547	0.1968 0.8886	2.1102 0.1486	1.3277 0.2512	0.0158 0.9003	0.6971 0.4052
t[**] *P*		15.9770 < 0.0001	3.9262 0.0001	8.3305 < 0.0001	3.3978 0.0009	5.3800 < 0.0001	4.0210 < 0.0001	3.6460 0.0004	6.8237 < 0.0001

Note. [*]Levene's test for equality of variances. [**] Independent-samples *t* test. Figures in parentheses are 95% CI.

95% CI of the Average Values of Biochemical Parameters ($\bar{x} \pm s$) in Women With PIH and Those With UNP

The upper limits of 95% CI of the average values of NO, VC, VE, β-CAR, SOD, CAT, and GPX in women with PIH were tess than the lower limits of 95% CI of those in women with UNP. The lower limit of 95% CI of the average value of MDA in women with PIH was greater than the upper limit of 95% CI of that in women with UNP (Table 2).

Partial Correlation Analysis of SBP and Each Experimental Parameter in 70 Women With PIH

The findings in partial correlation analysis (controlling for age) of SBP and each experimental parameter in women with PIH suggested that, MDA value gradually increased (*P*<0.001), and NO, VC, VE, β-CAR, SOD, CAT, and GPX values gradually decreased when SBP increased (*P*<0.005-0.001) (Table 3).

Partial Correlation Analysis of DBP and Each Experimental Parameter in 70 Women With PIH

The findings in the partial correlation analysis (controlling for age) of DBP and each experimental parameter in 70 women with PIH suggested that,

MDA value gradually increased (*P*<0.001), and NO, VC, VE, β-CAR, SOD, CAT, and GPX values gradually decreased when DBP increased (*P*<0.02-0.001) (Table 3).

Stepwise Regression Analysis of SBP and Experimental Parameters in 70 Women With PIH

Supposing SBP value in each woman with PIH was the dependent variable Y, and NO, VC, VE, β-CAR, SOD, CAT, GPX, and MDA values were the different independent variables, respectively, the findings in the stepwise regression analysis of SBP and experimental parameters in 70 women with PIH suggested that the model of stepwise regression was $Y = 233.4478 - 0.4268_{NO} + 0.8867_{VC}$, $F = 27.2921$, *P*<0.0001 (Table 4).

Stepwise Regression Analysis of DBP and Experimental Parameters in 70 Women With PIH

Supposing DBP value in each woman with PIH was the dependent variable Y, and NO, VC, VE, β-CAR, SOD, CAT, GPX, and MDA values were the different independent variables, respectively, the findings in the stepwise regression analysis of DBP and experimental parameters in 70 women with PIH suggested that the model of stepwise regression was $Y = 178.5721 - 0.4127_{NO} + 0.9031_{VC}$, $F = 15.5900$, *P*<0.0001 (Table 4).

TABLE 3

Partial Correlation Analysis of SBP, DBS, and Each Experimental Parameter in Women With PIH

Correlative Item	Partial Correlation Coefficients (Controlling for Age)				
	r	P	Correlative Item	r	P
SBP and NO	− 0.4415	<0.001	DBP and NO	− 0.4391	<0.001
SBP and VC	− 0.4872	<0.001	DBP and VC	− 0.3877	0.001
SBP and VE	− 0.4712	<0.001	DBP and VE	− 0.4050	0.001
SBP and β-CAR	− 0.3547	0.003	DBP and β-CAR	− 0.2828	0.019
SBP and SOD	− 0.3653	0.002	DBP and SOD	− 0.2902	0.016
SBP and CAT	− 0.4157	<0.001	DBP and CAT	− 0.3572	0.003
SBP and GPX	− 0.4235	<0.001	DBP and GPX	− 0.3440	0.004
SBP and MDA	0.5085	<0.001	DBP and MDA	0.4130	<0.001

TABLE 4

Stepwise Regression Analysis of SBP, DBP, and Experimental Parameters in 70 Women With PIH

	Model	Unstandardized Coefficients (B)	Standardized Coefficients (Beta)	t	P
SBP[*]	Constant	233.4478		12.6417	<0.0001
	NO	-0.4268	-2.5965	3.9563	0.0002
	VC	0.8867	2.0058	3.0561	0.0032
DBP[**]	Constant	178.5721		8.6896	<0.0001
	NO	-0.4127	-2.5109	3.4380	0.0010
	VC	0.9031	2.0428	2.7970	0.0067

Note. Dependent variable: [*]Systolic blood pressure, [**]Diastolic blood pressure.

Reliability Analysis of Biochemical Parameters Reflecting Oxidative Stress and Potential Free Radical Damage in 70 Women With PIH

The findings in reliability analysis suggested that the reliability coefficient (8 items) alpha = 0.7062, P<0.0001, and the standardized item alpha = 0.9116, P<0.0001.

DISCUSSION

VC, VE, and β-CAR are important antioxidants, and SOD, CAT, and GPX are important antioxidases in humans, and MDA is an important metabolic product of lipoperoxidative reactions of lipids exposed to oxygen[7,31-32,35-51]. VC, VE, β-CAR, SOD, CAT, GPX, and MDA play important roles in the normal metabolism and health states in humans[7,31-32,35-51]. NO is one of the very important neurotransmitter molecules, and can directly modify enzymes that produce second messengers[35-36,38,44-45,50-51] and also a major paracrine mediator and important regulatory agent in various female reproductive processes, such as ovulation, implantation, pregnancy maintenance, labor and delivery[9-23]. Therefore, NO plays an important role in human metabolism[9-23,30,35-36,38,44-45,50-51]. Markedly abnormal metabolism of NO and MDA as well as significantly decreased antioxidants and antioxidases may induce metabolic disorders and pathological aggravation of a series of free radical chain reactions, thus resulting in increased oxidative stress and potential free radical damage, and a variety of diseases associated with abnormal reactions of free radicals (FRs) in humans[7,9-32,35-51].

The findings of this research show that there exist abnormal metabolism of NO, imbalance between oxidation and antioxidation, and increased oxidative stress and potential free radical damage in women with pregnancy-induced hypertension. There might be several interpretations.

The elementary pathologic change in pregnancy-induced hypertension is the spasm of systemic small arteries, which leads to circulatory disturbance and blood supply insufficiency, thereby resulting in ischemia and hypoxia, necrosis and dysfunction in

organs and tissues[1-8]. Vasospasm not only results in widespread endothelium dysfunction and damage, and elevation of blood pressure, but also leads to decreased production of vasodilator factors, such as NO, endothelium-derived relaxing factors (EDRF), prostaglandin E_2 (PGE$_2$), prostaglandin I_2 (PGI$_2$), and prostacyclin, as well as increased production of vasoconstrictive products, such as endothelin (ET), thromboxane A_2 (TXA$_2$), and platelet-derived growth factor (PDGF)[1-23]. As a result, there is increased vasoconstriction of small blood vessels, with hypoperfusion and ischemia of downstream tissues and systemic hypertension[1-8].

In blood vessels, NO is a potent vasodilator and platelet-aggregation-inhibitor, and the deficiency of NO is closely related to the development of pregnancy-induced hypertension in gestation[1-23]. During pregnancy, activity of nitric oxide synthase (NOS), especially activity of inducible nitric oxide synthase (iNOS), could be severely inhibited by a large amount of free radicals (FRs) such as superoxide anion radical (O_2^-) and hydroxyl radical ($\cdot$OH), as well as reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) generated and induced by hypoperfusion, ischemia in organs and tissues, placental ischemia, and inflammatory process, thereby leading to markedly decreased NO formation[7,9,12,16-17,35-36,38,44-45,50-51]. At the same time, during pregnancy, NOS and iNOS could be inactivated by a large amount of FRs and ROS generated and induced by decreased EDRF, PGE$_2$, PGI$_2$, prostacyclin, and platelet function, and by increased ET, TXA$_2$, and PDGF, and by release of cytokines, such as tumor necrosis factor-alpha during pregnancy, thereby leading to significantly decreased NO synthesis[7,9,12,16-17,35-36,38,44-45,50-51]. In addition, NOS and iNOS could be deactivated by metabolic products in lipoperoxidative reactions, such as MDA and conjugated diene (CD), resulting in decreased NO synthesis[7,25,35-36,38,44-45,50-51].

During gestation, a large amount of FRs, ROS, MDA, and CD could be generated and released by a series of biochemical reactions, such as increased activation of the xanthine oxidase system, aggravated reactions of the xanthine/xanthine oxidase system, and enhanced oxidative decomposition, peroxidative modification, as well as increased lipoperoxidative reactions of polyunsaturated fatty acids, unsaturated phospholipids, glycolipids, and cholesterol, and other lipids in blood, tissues, and cellular membranes in women with PIH, which could inactivate NOS[7,25-28,35-36,38,44-45,50-51], attack and destroy the active sites and groups in the molecular structures of antioxidants and antioxidases, deactivate or inactivate

them[7,25-28,31-32,35-51], thereby resulting in significant overproduction of MDA and CD, markedly impaired VC, VE, and β-CAR levels as well as SOD, CAT, and GPX activities, and decreased defense against excessive FRs and ROS[31-32,35-51].

The above abnormal biochemical and biophysical changes during gestation not only induce increased oxidative stress and aggravate a series of FRs chain reactions, but also cause injury and contraction of arterial smooth muscle cells, damage and dysfunction of endothelial cells, and increased vascular resistance[10-11,13]. At the same time, these changes could reduce blood flow, increase turbulent flow in hemodynamics[21,40,52], decrease the fluidity, viscoelasticity, and deformability of cellular membranes, such as erythrocytes, vessel endothelial cells, and smooth muscle cells in hemorheology[21,40,52]. As a result SBP and DBP in women with PIH are further elevated.

The relationship among SBP, DBP, and each of the above biochemical parameters can not be really compared by a bivariate correlation analysis, because there exists a close correlation between age and each parameter in humans[31-32,35-36,38-42,44-45,48-51]. In this research, therefore, partial correlation analysis was used to compute the correlation between SBP, DBP, and each parameter in 70 women with PIH in order to eliminate the effect of age on the correlation analysis[51-53]. The findings indicate that the above parameters are closely related to SBP and DBP. When SBP and/or DBP were elevated, MDA gradually increased, and NO, VC, VE, β-CAR, SOD, CAT, and GPX gradually decreased. In other words, the higher the SBP and/or DBP, the severer the oxidative stress and potential free radical damage in their bodies.

The findings in stepwise regression analysis indicate that in the above biochemical substances and enzymes, NO and VC are most closely related to SBP and DBP in women with PIH, suggesting that significantly decreased NO is an important factor for elevated SBP and/or DBP in women with PIH, and significantly decreased VC is an elementary factor for increased oxidative stress and potential free radical damage in women with PIH.

In addition, the findings in both partial correlation analysis and stepwise regression analysis suggest that pregnancy-induced hypertension would be a risk factor for pregnant women and their fetuses.

In conclusion, the findings in this research suggest that pregnancy-induced hypertension increases oxidative stress and potential free radical damage in women with pregnancy-induced hypertension.

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