Published in final edited form as:
Am J Obstet Gynecol. 2013 November ; 209(5): . doi:10.1016/j.ajog.2013.06.030.

Hypertension in pregnancy is associated with elevated homocysteine levels later in life

Wendy M. White, MD, **Stephen T. Turner, MD**, **Kent R. Bailey, PhD**, **Thomas H. Mosley Jr, PhD**, **Sharon L. R. Kardia, PhD**, **Heather J. Wiste, BA**, **Iftikhar J. Kullo, MD**, and **Vesna D. Garovic, MD**

Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology (Dr White); the Divisions of Nephrology and Hypertension (Drs Turner and Garovic) and Cardiovascular Diseases (Dr Kullo), Department of Internal Medicine; and the Division of Biomedical Statistics and Informatics, Department of Health Sciences Research (Dr Bailey and Ms Wiste), Mayo Clinic, Rochester, MN; the Departments of Geriatrics/Gerontology and Neurology, University of Mississippi Medical Center, Jackson, MS (Dr Mosley); and the Department of Epidemiology, University of Michigan Medical School, Ann Arbor, MI (Dr Kardia).

Abstract

OBJECTIVE—Hyperhomocysteinemia is associated with an elevated cardiovascular disease risk. We examined whether women with a history of hypertension in pregnancy are more likely to have a high level of serum homocysteine decades after pregnancy.

STUDY DESIGN—Serum homocysteine was measured at a mean age of 60 years in nulliparous women (n = 216), and women with a history of normotensive (n = 1825) or hypertensive (n = 401) pregnancies who participated in the Genetic Epidemiology Network of Arteriopathy (GENOA) study. Relationships between homocysteine and pregnancy history were examined by linear and logistic regression, controlling for multiple covariates including personal and family history of hypertension, diabetes, obesity, tobacco use, and demographics.

RESULTS—A history of hypertension in pregnancy, when compared with normotensive pregnancy, was associated with a 4.5% higher serum homocysteine level ($P = .015$) and 1.60-fold increased odds of having an elevated homocysteine (95% confidence interval, 1.15–2.21; $P = .005$) after adjusting for potentially confounding covariates. In contrast, a history of normotensive pregnancy, as compared with nulliparity, was associated with a 6.1% lower serum homocysteine level ($P = .005$) and a 0.49-fold reduced odds of elevated homocysteine levels (95% confidence interval, 0.32–0.74; $P < .001$).

CONCLUSION—Homocysteine levels decades after pregnancy are higher in women with a history of pregnancy hypertension, even after controlling for potential confounders. Thus, pregnancy history may prompt homocysteine assessment and risk modification in an attempt at primary prevention of cardiovascular disease.

Reprints: Vesna D. Garovic, MD, Division of Nephrology and Hypertension, Mayo Clinic, 200 First St. SW, RO-MA-19-E19NEP, Rochester, MN 55905. garovic.vesna@mayo.edu..

The authors report no conflict of interest.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development; the National Heart, Lung, and Blood Institute; or the National Institutes of Health.

Presented as a poster at the 31st annual meeting of the Society for Maternal-Fetal Medicine, San Francisco, CA, Feb. 7-12, 2011.

Keywords

biomarker; cardiovascular disease; homocysteine; hypertension in pregnancy; preeclampsia

Hypertension in pregnancy increasingly is recognized as a predictor of future cardiovascular disease (CVD).[1] A spectrum of hypertensive disorders can be seen in pregnancy including gestational hypertension, preeclampsia, eclampsia, and HELLP syndrome (with clinical features of hemolysis, elevated liver enzymes, and low platelet count), either de novo or superimposed on preexisting chronic hypertension.[2] The signs and symptoms of multisystem organ dysfunction associated with preeclampsia usually abate soon after delivery. However, both retrospective and prospective studies have shown that a history of a hypertensive disorder in pregnancy predicts an increased risk for future cardiovascular morbidity, including hypertension, cardiac disease, and cerebrovascular events.[3] The increased risk for CVD persists even after controlling for traditional risk factors, including hypertension and family history.[4] Clearly, there are mediators that link hypertensive disorders in pregnancy and future CVD risk. Biomarkers, such as homocysteine, may play a role.[5]

Homocysteine is a metabolite of the essential amino acid, methionine. Blood homocysteine levels are influenced by a variety of nutritional factors, including folate and cobalamin intake, and genetic polymorphisms, such as the 677 C → T change in the *MTHFR* gene. Several studies have demonstrated that serum homocysteine levels are elevated in preeclampsia at the time of symptoms compared to gestational age–matched normotensive pregnancies, but these elevations do not appear to predate the disease.[6-15] High levels of serum homocysteine are associated with oxidative stress and endothelial dysfunction, biological processes common to both preeclampsia and CVD.[5,16] Therefore, high homocysteine levels that persist after preeclamptic pregnancies may be mechanistically related to an increased CVD risk in these women.

To date, limited data are available regarding homocysteine level trends after preeclamptic pregnancies. Sep et al[17] showed that 21% of 40 women with preeclampsia had persistent elevations in homocysteine >15 mmol/L at 6-10 months' postpartum. Girouard et al[18] found homocysteine to be increased as much as 8 years' postpartum, although neither of these studies adjusted for known confounders.

The aim of this study was to determine if a history of hypertension in pregnancy is an independent risk factor for hyperhomocysteinemia later in life. We postulate that a high level of plasma homocysteine may serve as a biomarker for an increased risk of CVD and cerebrovascular disease among women with a history of hypertensive pregnancy disorders, years after their affected pregnancies.

Materials And Methods

Participants

The participants were obtained from the Genetic Epidemiology Network of Arteriopathy (GENOA) study, which was part of the Family Blood Pressure Program established in 1995 to investigate the genetics of hypertension across multiple ethnicities. The GENOA study included recruitment sites in Jackson, MI; Starr County, Texas; and Rochester, MN; representing African Americans, Hispanics, and non-Hispanic whites, respectively. The specific recruitment strategy to identify sibships with multiple hypertensive siblings at each site has been described previously.[19] The present study included 2442 women who

participated in the second GENOA study examination (2000 through 2004), which included a questionnaire regarding pregnancy and hypertension in pregnancy (as described below).[4]

All individuals gave informed consent and institutional review board–approved protocols were used. At the second GENOA examination (2000 through 2004), data regarding personal and family medical history, medication use, menopausal status, pregnancy, and hypertension history were gathered using validated questionnaires administered by trained examiners.

Determination of exposure

A validated questionnaire was used to determine history of nulliparity, normotensive pregnancy, or hypertensive pregnancy. Female participants were asked, "Have you had at least 1 pregnancy that lasted >6 months?" Women who answered "yes" were asked to report the number of pregnancies and to answer whether or not they had developed hypertension during any of their pregnancies that had lasted >6 months. Preeclampsia was defined either by self-report or by self-report of proteinuria during the pregnancy with hypertension. This questionnaire was previously shown to be 80% sensitive and 96% specific to assess hypertension in pregnancy.[20]

Determination of confounders

Demographics and covariates were assessed by structured interviews and the information obtained included age at assessment, education level, race, and family history. Standardized physical examinations were performed and the body mass index (BMI) was calculated as weight/height in kg/m^2.

Hypertension was defined in 1 of 2 ways: a history of such and concurrent use of an antihypertensive, or a blood pressure measurement of 140 mm Hg systolic and/or at least 90 mm Hg diastolic. Diabetes mellitus was self-reported.

Smoking was coded as "ever" if >100 cigarettes had been used in the past. The diagnosis of dyslipidemia was confirmed if ≥1 of the following criteria were met: (a) the use of lipid-lowering drugs; or (b) total cholesterol of ≥200 mg/dL, triglycerides of ≥150 mg/dL, or a high-density lipoprotein of ≤40 mg/dL, collected at the time of the phase II examination.

Laboratory methods

Blood work was drawn by venipuncture in the fasting state. Serum homocysteine was measured by the Mayo Clinic high-performance liquid chromatography method and an elevated homocysteine level was defined as >13 μmol/L. Serum total cholesterol, high-density lipoprotein cholesterol, and triglyceride concentrations were measured by standard methods on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN).

Statistical analysis

Women were grouped as nulliparous (no pregnancy lasting >6 months), having a history of a normotensive pregnancy (normotensive in all pregnancies >6 months), and having a history of a hypertensive pregnancy (hypertensive in at least 1 pregnancy >6 months). Means ± SD and counts (percentages) are summarized within the pregnancy group. Pairwise differences in age among the 3 groups were assessed with 2-sample *t* tests and pairwise differences in race and education among the 3 groups were assessed with χ^2 tests. Due to significant differences in age, race, and education among the pregnancy groups, pairwise differences in all other variables among the pregnancy groups were adjusted for age, race, and education using logistic regression for binary variables or linear regression for continuous variables. No adjustment was made for multiple comparisons.

Linear regression was used for predicting continuous homocysteine levels and logistic regression was used for predicting homocysteine levels >13 μmol/L. Associations between the levels of homocysteine and pregnancy groups were assessed using 3 separate models: 1 with no adjustment for covariates; 1 adjusting for age, race, and education; and a full model adjusting for age, race, education, smoking, hypertension, diabetes, BMI, dyslipidemia, statin use, hormone replacement therapy use, family history of hypertension, and family history of coronary heart disease. For the linear regression models, a log transformation was used for homocysteine levels due to a skewed distribution. The adjusted mean ± SD homocysteine and adjusted percentage with homocysteine >13 μmol/L are shown within pregnancy groups for all models. All linear and logistic models were fit with generalized estimating equations to account for relationships among siblings.

Results

There were 2442 women in the GENOA cohort available for analysis. There were a total of 1273 families among the 2442 subjects in the study. The median number of siblings in a family was 2 (minimum 1, maximum 8). The demographic characteristics of the cohort are shown in Table 1. In all, 216 (9%) were nulliparous, 1825 (75%) had histories of normotensive pregnancies, and 401 (16%) had histories of hypertensive pregnancies.

Women were 60 ± 11 (mean ± SD) years of age across the entire cohort, with women with a history of hypertension in pregnancy being younger than those with a normotensive pregnancy. Women with previous hypertensive pregnancies were also more likely to have hypertension and diabetes, less likely to have ever smoked, and had higher BMIs than women in the other 2 groups. Compared to women with previous normotensive pregnancies, women with previous hypertensive pregnancies were more likely to have a family history of hypertension or coronary heart disease. The dyslipidemia rates and the percentage of women taking statins or hormone replacement therapy were not significantly different among the 3 groups. Nulliparous women were significantly more likely to be white and have higher levels of education than women in the other 2 groups, and were significantly younger and were more likely to have hypertension than women with previous normotensive pregnancies (Table 1).

There were no differences in fasting serum homocysteine between women with a history of hypertensive disorders in pregnancy and those who were nulliparous. Women with a history of normotensive pregnancy had the lowest values. This relationship persisted after adjustments for basic demographic factors of age, race, and education, as well as other potential covariates. Results were similar for the categorical variable of elevated homocysteine (Table 2).

The multivariable linear regression model that predicts the impacts of pregnancy history and each of the clinical characteristics on serum homocysteine and the multivariable logistic regression model predicting the odds ratio for having elevated homocysteine are detailed in Tables 3 and 4.

In summary, a history of hypertension in pregnancy, when compared with normotensive pregnancy, was associated with a 4.5% higher serum homocysteine level ($P = .015$) and 1.60-fold increased odds of having elevated homocysteine (95% confidence interval, 1.15–2.21; $P = .005$) levels after adjusting for potentially confounding covariates. In contrast, a history of normotensive pregnancy, as compared with nulliparity, was associated with a 6.1% lower serum homocysteine level ($P = .005$) and a 0.49-fold reduced odds of having elevated homocysteine levels (95% confidence interval, 0.32–0.74; $P < .001$).

Comment

In our study, a history of hypertension in pregnancy compared to normotensive pregnancy was associated with higher homocysteine levels decades later in life, even after adjusting for confounders, such as a personal or family history of hypertension, BMI, and diabetes. Normotensive pregnancy, in contrast, functioned as a "negative stress test" and was associated with lower homocysteine levels compared to nulliparous women. Elevated serum homocysteine levels previously have been associated with the acute preeclampsia state, and these elevations have been shown to persist in some women up to 1 year postpartum,[17,18] but this is the first report demonstrating persistent elevations decades after pregnancy. Thus, hyperhomocysteinemia is yet another independent biomarker in the pathway that links a history of hypertension in pregnancy with future cardiovascular risk.

The main strengths of our study are the large number of participants with accurate outcomes measured decades from pregnancy exposure, the ethnic diversity of the population, and the appropriate measurement of and correction for known confounders such as hypertension–both personal and family history, BMI, age, and race.[5]

Other factors that may affect homocysteine levels include use of hormone replacement and statins. The literature is conflicted as to whether the use of hormone replacement (estrogen alone, estrogen + progestin, oral or non-oral formulations all being pertinent variables) results in lower homocysteine levels or has no effect.[21-24] Whether statins and other cholesterol-lowering agents affect homocysteine levels is also controversial.[25] As their covariate effects are not known definitively, we adjusted for these potential confounders. In our multivariate models, statin use was not independently associated with log(homocysteine) or elevated homocysteine. However, women with hormone replacement therapy had lower log(homocysteine) after adjusting for other variables in the model.

Our study has several limitations. The prevalence of hypertensive disorders in pregnancy was 16% in this cohort, higher than the commonly reported 8% in the general population.[19] This was likely a result of our use of a cohort with a personal or family history of hypertension. Although the presence of hypertension and other known confounders were adjusted for in the analysis, this cohort may not be generalizable to other populations in terms of their homocysteine metabolism.

Also, the exposure of hypertension in pregnancy was self-reported and not confirmed by chart review. This would not have been possible in this study given the significant amount of time that had elapsed since these pregnancy events, the large number of delivery hospitals involved, and the large size of the cohort. As in all case-control or cross-sectional studies with retrospective components, differential recall between those affected by hypertensive disorders of pregnancy and normotensive controls may lead to inaccurate, usually inflated, risk estimates.[26] The use of standardized protocols to obtain this information using a validated questionnaire was done to reduce error. Others have shown that recall bias associated with pregnancy-related complications is low.[27] The tool we used to report a history of preeclampsia had particularly high sensitivity and even higher specificity.[20] Given this, preeclamptic women would be more likely to be misclassified as normotensive, actually leading to an underestimation of the association between future hyperhomocysteinemia and history of hypertensive disorders of pregnancy. The tool is also limited in that it is not able to distinguish between gestational hypertension and preeclampsia, or to stratify based on preeclampsia severity.

There is also no information about other characteristics of this cohort at the time of exposure, such as BMI, age at pregnancy, or presence of other cardiovascular risk factors, such as diabetes. Baseline homocysteine levels before the index pregnancy were also not

known though previous work has failed to show hyperhomocysteinemia as a risk factor for future preeclampsia. For example, median homocysteine levels measured at 22-24 weeks in 683 women, 80 of whom went on to develop preeclampsia, showed no difference between hypertensive and normotensive pregnancies.[28] A large cohort study in The Netherlands did not show a relationship between baseline homocysteine levels and adjusted risk for subsequent preeclampsia, although low folic acid levels were associated with an increased risk.[29]

One unmeasured confounder is the potential for different genetic variants in the *MTHFR* gene, which can alter homocysteine levels, albeit to a lesser degree than folate deficiency. The metabolism of folic acid is controlled, in part, by the functionality of the MTHFR enzyme, and the functionality is influenced by the type of genetic variant. Plasma levels of homocysteine are affected by both diet and genetics: the homozygous TT genotype for the c667T allele of *MTHFR* occurs in 5-14% of the population and results in higher plasma levels of homocysteine. This TT variant has been shown in a few studies to have a higher incidence in women with preeclampsia; however, the majority of studies have not demonstrated this association.[30-32] Other genetic variants that alter enzyme function or factors that affect the availability of cofactors may explain the relationships among hyperhomocysteinemia, hypertensive disorders of pregnancy, and CVD risk.

In summary, higher homocysteine levels decades later in life may be one of the pathways that link a history of hypertension in pregnancy with future CVD risk. Normotensive pregnancy, in contrast, serves as a “negative stress test” and is associated with lower homocysteine levels, a biomarker of cardiovascular risk, later in life. Thus, pregnancy history may prompt homocysteine assessment and risk modification in an attempt at primary prevention of CVD.

Future work will focus on further refining of the associations between specific hypertensive disorders in pregnancy (gestational hypertension, mild preeclampsia, severe preeclampsia, HELLP syndrome, and superimposed preeclampsia) and their individual associations with blood homocysteine levels throughout the lifespan.

Acknowledgments

This research was supported by grant number K08HD051714 from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (V.D.G.) and grants from the National Heart, Lung, and Blood Institute, National Institutes of Health: U01HL054481, U01HL054471, U01HL054512, and U01HL054498 (S.T.T., T.H.M., S.L.R.K.).

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TABLE 1

Subject characteristics

Variable	Nulliparous (n = 216)	Normotensive pregnancy (n = 1825)	Hypertensive pregnancy (n = 401)
Race, n (%)			
Hispanic	69 (32)	543 (30)	112 (28)
Non-Hispanic white	83 (38)	508 (28)	112 (28)
Non-Hispanic black	64 (30)	774 (42)	177(44)
Significance		*a*	*a*
Age, y	57 ± 13	61 ± 10[a]	57 ± 11[b]
BMI, kg/m^2	33 ± 8	32 ± 7	34 ± 7[a,b]
Education, n (%)			
<High school (≤8 y)	39 (18)	456 (25)	85 (21)
Some high school (9-11 y)	14(6)	208 (11)	61 (15)
High school graduate or GED (12 y)	51 (24)	543 (30)	116(29)
>High school (>12 y)	112 (52)	618(34)	139 (35)
Significance		*a*	*a*
Ever smoked, n (%)	69 (32)	574 (31)	106 (26)[a,b]
Diabetes, n (%)	63 (29)	592 (32)	152 (38)[a,b]
Hypertension, n (%)	152 (70)	1245 (68)[a]	329 (82)[a,b]
Dyslipidemia, n (%)	176(81)	1414(78)	297 (74)
Statins, n (%)	40 (19)	402 (22)	92 (23)
Family history of hypertension, n (%)	162 (75)	1291 (71)	331 (83)[b]
Family history of coronary heart disease, n (%)	108(50)	786 (43)	210 (52)[b]
Hormone replacement therapy, n (%)	56 (26)	452 (25)	91 (23)

P values are adjusted for age, race, and education for all variables except age, race, and education. Values are mean ± SD or n (%).

BMI, body mass index; *GED*, general educational development.

[a]Significant difference from nulliparous, $P < .05$

[b]Significant difference from normotensive pregnancy, $P < .05$.

TABLE 2

Relationship between pregnancy history and serum homocysteine levels

Parameter	Model	Nulliparous	Normotensive pregnancy	HTN pregnancy	*P* value overall	*P* value normal vs null	*P* value HTN vs null	*P* value HTN vs normal
Log (homocysteine)	Unadjusted	2.24 ± 0.37	2.21 ± 0.32	2.24 ± 0.36	.11	.15	.88	.09
Log (homocysteine)	Age, race, education adjusted	2.26 ± 0.34	2.19 ± 0.30	2.25 ± 0.33	< .001	.002	.69	< .001
Log (homocysteine)	Full	2.26 ± 0.33	2.19 ± 0.29	2.24 ± 0.33	.002	.005	.47	.015
Homocysteine >13 μmol/L	Unadjusted	16%	11%	15%	.012	.018	.74	.011
Homocysteine >13 μmol/L	Age, race, education adjusted	19%	11%	17%	< .001	< .001	.64	< .001
Homocysteine >13 μmol/L	Full	18%	11%	15%	< .001	< .001	.34	.005

HTN, hypertensive.

First 3 rows show mean ± SD of log(homocysteine) in each pregnancy group estimated from linear regression models where serum homocysteine was analyzed as continuous variable with log transformation due to nonnormality. Three models are: unadjusted; adjusted for demographics of age, race, and education; and adjusted for demographics + smoking, HTN, log body mass index, diabetes, dyslipidemia, statins, hormone replacement therapy, family history of HTN, and coronary heart disease. Last 3 rows show proportion of women in each pregnancy group with elevated homocysteine (>13 μmol/L) estimated from logistic regression models with same 3 levels of adjustment. Accounting for sibling relationships is done using generalized estimating equations.

TABLE 3

Pregnancy history, clinical characteristics, and serum homocysteine levels

Parameter	Beta coefficient	% difference	*P* value
Normotensive pregnancy (vs nulliparous)	−0.0633	−6.1	.005
Hypertensive pregnancy (vs normotensive)	0.0435	4.5	.015
Race			.001
Non-Hispanic, white	ref		
Hispanic	−0.0876	−8.4	< .001
Non-Hispanic, black	−0.0293	−2.9	.07
Age, per 10 y	0.1022	10.8	< .001
Education level			.032
High school graduate or GED (12 y)	ref		
<High school education (≤8 y)	0.0515	5.3	.017
Partial high school education (9-11 y)	0.0651	6.7	.011
>High school education (>12 y)	0.0164	1.7	.22
Ever smoked	0.0454	4.6	< .001
Current hypertension	0.0727	7.5	< .001
Diabetes	0.0352	3.6	.028
Log (BMI)	0.0520	5.3	.12
Dyslipidemia	0.0048	0.5	.74
Statins	−0.0116	−1.2	.49
Hormone replacement therapy	−0.0704	−6.8	< .001
Family history of hypertension	−0.0175	−1.7	.25
Family history of coronary heart disease	0.0153	1.5	.29
Intercept	1.4255		< .001

Data show linear regression results for serum homocysteine analyzed as continuous variable and adjusted for several covariates. Models were fit with generalized estimating equations to account for sibling relationships. For categorical variables, negative estimate indicates that homocysteine is lower in comparison group than in reference group. For continuous variables, positive estimate indicates that homocysteine is higher for each increase in specified units (ie, homocysteine is higher with each 10-y age increase). As homocysteine was modeled with log transformation, percent difference in homocysteine values is also shown.

BMI, body mass index; *GED*, general educational development; *ref*, reference.

TABLE 4

Multivariable logistic regression model predicting elevated homocysteine (>13 μmol/L)

Parameter	OR (95% CI)	*P* value
Normotensive pregnancy (vs nulliparous)	0.49 (0.32–0.74)	< .001
Hypertensive pregnancy (vs normotensive)	1.60 (1.15–2.21)	.005
Race		.78
Non-Hispanic, white	ref	
Hispanic	0.88 (0.55–1.42)	.61
Non-Hispanic, black	1.01 (0.70–1.47)	.95
Age, per 10 y	1.76 (1.48–2.08)	< .001
Education level		.009
High school graduate or GED (12 y)	ref	
<High school education (≤8 y)	1.60 (1.08–2.37)	.019
Partial high school education (9-11 y)	1.52 (0.99–2.32)	.05
>High school education (>12 y)	0.86 (0.60–1.22)	.40
Ever smoked	1.33 (1.01–1.74)	.043
Current hypertension	2.02 (1.32–3.10)	.001
Diabetes	1.50 (1.13–2.00)	.005
Log (BMI)	1.19 (0.59–2.38)	.62
Dyslipidemia	1.08(0.75–1.53)	.69
Statins	1.04 (0.76–1.43)	.80
Hormone replacement therapy	0.75 (0.53–1.04)	.09
Family history of hypertension	0.95 (0.71–1.28)	.75
Family history of coronary heart disease	1.24 (0.92–1.67)	.15

Data show logistic regression results for OR (95% CI) for having elevated serum homocysteine (>13 μmol/L) accounting for sibling relationships using generalized estimating equations.

BMI, body mass index; *CI*, confidence interval; *GED*, general educational development; *OR*, odds ratio; *ref*, reference.