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Evaluation of Zinc and Homocysteine Status in Pregnant Women and Their Association with Pre-eclampsia in Jordan

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ABSTRACT: Pre-eclampsia (PE) is considered a major complication of pregnancy. Hyperhomocyteinemia (H-Hcy) has been proposed to be associated with a number of placenta-mediated diseases, such as PE. Zinc (Zn) is involved in the regulation of total homocysteine (Hcy) levels. A case-control study design was used to examine serum Zn and Hcy statuses, and their association to PE risk. Thirty pregnant women with PE 21～35 years of age, and 30 matched healthy pregnant women were recruited from Amman, Jordan. Plasma Hcy was measured using liquid chromatography-mass spectrometry, and Zn was measured using atomic absorption. Hcy levels were significantly higher among women with PE compared with controls (16.35±0.43 and 7.25±0.21 μmol/L, respectively; $P<0.05$). However, there was no significant difference in Zn levels between women with PE and controls (65.37±1.27 and 63.71±1.24 μg/dL, respectively; $P>0.05$). Blood levels of Hcy (μmol/L) were positively associated with systolic and diastolic blood pressure (β=3.54 and β=1.81, respectively; $P<0.05$), and Zn levels [odds ratios (OR)=0.84; 95% confidence intervals (CI): 0.71～0.98] were significantly associated with PE risk ($P<0.05$). Although women with PE had significantly higher Hcy levels than controls, H-Hcy was not associated with increased PE risk. However, there was a strong association between severity of hypertension and serum Hcy levels, and serum Zn levels were inversely associated with H-Hcy. The likelihood of PE was significantly higher in women who were Zn deficient compared with healthy controls. To conclude, early management of H-Hcy and associated risk factors may be effective in decreasing the incidence of PE.

Keywords: hyperhomocysteinemia, pregnancy and pre-eclampsia, total homocysteine, zinc

INTRODUCTION

Pregnancy and its complications are responsible for about 600,000 deaths worldwide every year, half of which are due to risky pregnancies (Vafaei et al., 2015). Pre-eclampsia (PE) is considered a major cause of maternal morbidity and mortality, and contributes to intrauterine growth restriction, preterm delivery, and prenatal mortality (Harma et al., 2005). Women with pregnancy-induced hypertensive disorders may progress from mild disease to a more serious condition. Types of pregnancy-induced hypertension include hypertension fluid retention mild PE, severe PE, and eclampsia (WHO, 2017). The exact mechanism underlying the etiology of PE remains unclear (Khosrowbeygi and Ahmadvand, 2011), however, the incidence is higher in developing countries than developed countries (Ghulmiyyah and Sibai, 2012).

Although global estimates of some micronutrients deficiencies are not available, population studies have shown that the percentage of pregnant women with zinc (Zn) deficiency ranges from 15～74% in South Asia (Gernand et al., 2016). Micronutrient deficiencies are usually identified in pregnant women, and trace element deficiency may increase susceptibility to developing PE (Akinloye et al., 2010). Some of these trace elements can regulate the balance between free radicals and antioxidants (Roberts et al., 2003). Total homocysteine (Hcy) is a sulfur containing amino acid involved in processes such as lipid peroxidation and oxidative stress. Hcy is metabolized through transsulfuration and remethylation pathways (Khosrowbeygi and Ahmadvand, 2011). Hyperhomocysteinemia (H-Hcy) is a risk factor for cardiovascular diseases and common obstetric problems (Tug et al., 2003). Moreover, some studies have indicated that decreased

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concentrations of Zn is associated with fetal malformation, fetus growth restriction, preterm delivery, PE, and bleeding after delivery (Rafeeinia et al., 2014). H-Hcy is considered a risk factor for endothelial dysfunction and vascular diseases, such as atherosclerosis. It has been hypothesized that maternal H-Hcy is associated with several placenta-mediated diseases such as PE (Khosrowbeygi and Ahmadvand, 2011). Furthermore, Zn is believed to be involved in regulating Hcy levels via mediating methionine synthease and betainehomocysteine methyl transferase, both of which are Zn-dependent metallo-enzymes (Jing et al., 2014). Therefore, it may be hypothesized that Hcy and Zn status are associated with the risk of PE.

PE is considered a major complication of pregnancy, but little is known about its etiology. Many theories about its etiology and pathogenesis have been proposed, including endothelial dysfunction (Rafeeinia et al., 2014). However, limited data are available on plasma Hcy status during pregnancy and on the occurrence of H-Hcy in women with PE in different geographical areas. To date, studies on the impact of Zn status on Hcy levels in women with PE are limited, particularly in Jordan. Therefore, the aim of the present study was to examine the associations between serum levels of Zn and Hcy and risk of PE in a selected sample of healthy, PE Jordanian women.

MATERIALS AND METHODS

Study design and subjects

A case-control study was conducted from December 2015 to April 2016 in Amman, Jordan. Thirty pregnant women with known PE were matched with 30 healthy pregnant women by maternal and gestational age. All women were recruited from the Obstetrics and Gynecology Department, Al-Bashir Hospital and Al-Hussein Medical Center. Eligible participants were pregnant women 21～35 years of age with PE at 20～40 weeks gestational age. Participants were excluded from the study if they met one of the following conditions: smoker, multiple gestation, diabetes mellitus, chronic hypertension, heart failure, renal disease, inflammatory or infective disorders, and infectious disease. A signed consent form was obtained from all subjects who were eligible to participate in this study. The protocol was approved by the Protection of Human Subjects Ethics Committee (No. 1429/3, Amman, Jordan) of the school of graduate studies at the University of Jordan, in accordance with the ethical guidelines described in the Declaration of Helsinki. All data were collected, retrived, and reported in a confidential manner.

Definition of selected variables

Information on selected factors that might influence nutrition during pregnancy, including reproductive history such as age, gestational age, parity, and family history of PE, were obtained. Parity was defined as "yes" if the mother had experienced one or more pregnancies and "no" if she has never previously experienced pregnancy. Additional information on use of supplemental vitamins was obtained for all participants. Supplement use was defined as "yes" if the mother was a supplement user and "no" if the mother was not a supplement user. Anthropometric measurements including weight and height were performed. Body mass index (BMI) before and during pregnancy were calculated: BMI (1) was BMI before pregnancy and BMI (2) was BMI during pregnancy. Systolic and diastolic blood pressure was obtained for all participants from their medical records.

Evaluation of biochemical predictors

Blood samples were obtained from all participants. Samples were treated with ethylene diamine tetra acetic acid and separated on gels to determine serum total Hcy and Zn levels. Total Hcy was defined as the sum of all Hcy species in plasma/serum, including free and protein-bound forms.

Blood samples were transported in an ice box to a private laboratory for analysis. Serum was obtained by centrifuging whole blood samples for 10 min at 4°C within half an hour of collection. Blood samples were stored at −80°C until they were analyzed for serum Hcy and Zn. Serum Hcy was measured using liquid chromatography-mass spectrometer (LCMS-8030, Shimadzu Corporation, Kyoto, Japan), and Zn levels were measured using Atomic Absorption (Shimadzu AA-6300, Shimadzu Corporation). Pregnant women with Zn levels of ≤65 (μg/dL) were considered Zn deficient and those with Zn levels≥ 65 (μg/dL) were considered Zn sufficient (Akhtar, 2013).

Statistical analyses

All statistical analyses were performed using IBM® SPSS® Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY, USA). Means and standard error of the mean (SEM) were calculated for continuous variables, whereas categorical variables were reported as counts and frequency distribution (%). Shapiro Wilk tests were used to test normality for all predictors including serum levels of Zn and Hcy. Therefore, based on normality tests, the group differences in PE risk factors were examined using parametric and non-parametric analyses, as appropriate. Differences between groups were estimated using analysis of variance (ANOVA) or Chi-square (χ^2), as appropriate. To examine the association between serum Hcy levels (dependent) and other indicators (independent), linear regression models were performed. Logistic regression analyses were performed to examine associations between serum Hcy levels, Zn levels, and the risk of PE. The rela-

Table 1. Anthropometric and clinical characteristics of the sample population

Variables	Cases (n=30)	Controls (n=30)	*P*-value
Age (years)	28.3±0.8 (21.0～35.0)	28.0±0.9 (21.0～35.0)	0.75
Gestational age (weeks)	33.1±0.8 (23.0～38.0)	31.7±0.9 (23.0～38.0)	0.23
BMI (1) (kg/m^2)	28.5±1.0 (18.4～41.9)	26.6±1.0 (15.6～37.5)	0.18
BMI (2) (kg/m^2)	34.4±1.1^a (25.0～47.0)	30.8±1.0^b (18.7～45.6)	0.02
Systolic blood pressure (mmHg)	154.2±2.6^a (140.0～180.0)	114.2±1.4^b (100.0～125.0)	0.00
Diastolic blood pressure (mmHg)	95.2±1.1^a (90.0～110.0)	75.3±0.9^b (70.0～80.0)	0.00
Parity[1)]	13 (43.3)^a	6 (20)^b	0.04
Family history[1)]	11 (36.7)^a	0 (0)^b	0.00
Supplement use (yes/no)[1)2)]	15 (50)	22 (73.3)	0.05

Continuous variable are presented as mean±SEM with the range in parentheses.
Different letters (a,b) indicated statistically significant difference at $P<0.05$.
BMI (1), body mass index before pregnancy; BMI (2), body mass index during pregnancy.
[1)]Categorical variables are presented as the number and percentage (%) in each group.
[2)]Supplement use was coded as "yes" if the mother was a supplement user and "no" if the mother was not a supplement user.

tive risks were reported as odd ratios (OR) with 95% confidence intervals (CI). *P*-values of <0.05 were considered significant.

RESULTS

The general anthropometric and selected clinical characteristics of the study subjects are shown in Table 1. There were no significant differences ($P>0.05$) between cases and controls in terms of age (years), gestational age (weeks), BMI before pregnancy (kg/m^2), and supplement use during pregnancy. However, BMI during pregnancy was significantly higher in women with PE compared with controls (34.35±1.09 vs 30.81±1.04, respectively, $P<0.05$). Approximately 43.3% of women with PE and 20% of controls had carried pregnancies to a viable gestational age (Parity) ($P>0.05$). The number of women with a family history of PE was significantly higher in those with PE compared with controls. Indeed, none of the healthy pregnant women had documented a family history of PE ($P<0.05$). Furthermore, systolic and diastolic blood pressure was significantly higher in women with PE than in controls (154.16±2.57 and 114.16±1.44 mmHg vs 95.16±1.05 and 75.33±0.89 mmHg, respectively, $P<0.001$).

The *P*-value for normality testing using Shapiro-Wilk tests for plasma Hcy and Zn levels was $P<0.05$ for both groups. We concluded that Zn and Hcy levels for this subset of subjects are not normally distributed. Mann-mean differences in Zn and Hcy levels among groups are presented in Fig. 1. Serum levels of Hcy (μmol/L) was significantly higher in women with PE compared with controls (16.35±0.43 vs 7.25±0.21, respectively; $P<0.001$). Although the mean concentration of Zn (μg/dL) in controls was higher than in women with PE, the difference was not statistically significant (65.37±1.27 vs 63.71±1.24, respectively; $P<0.05$).

Association between serum Hcy levels and selected predictors

Linear regression analyses were performed to assess the association between serum Hcy levels (μmol/L) and selected predicators in women with PE, controls, and the full cohort. In these analyses, the selected predicators were introduced as independent variables and serum Hcy as the dependent variable. The changes in Hcy levels (μmol/L) per unit change of the selected predicators are presented as β-coefficient with corresponding *P*-values (Table 2). Hcy levels were significantly associated with plasma Zn levels in pregnant women with PE but not in controls or in the full cohort ($\beta=0.18$, $P>0.05$). None of the predictors were associated with serum Hcy levels (μmol/L) within the control group.

BMI before and during pregnancy was significantly associated with Hcy levels ($P<0.05$). BMI of all women before pregnancy was associated with 0.25 μmol/L increases in Hcy levels ($P<0.05$) in the full cohort. After adjusting for BMI before pregnancy and family history of PE, BMI during pregnancy was positively and significantly associated with 0.22 μmol/L increases in Hcy levels ($P<0.05$) in the full cohort. Both parity and family history of PE were strongly associated with Hcy levels in the full cohort ($\beta=3.29$ and $\beta=4.61$, respectively, $P<0.05$). To assess the association between serum Zn status and selected predictors, linear regression analyses were per-

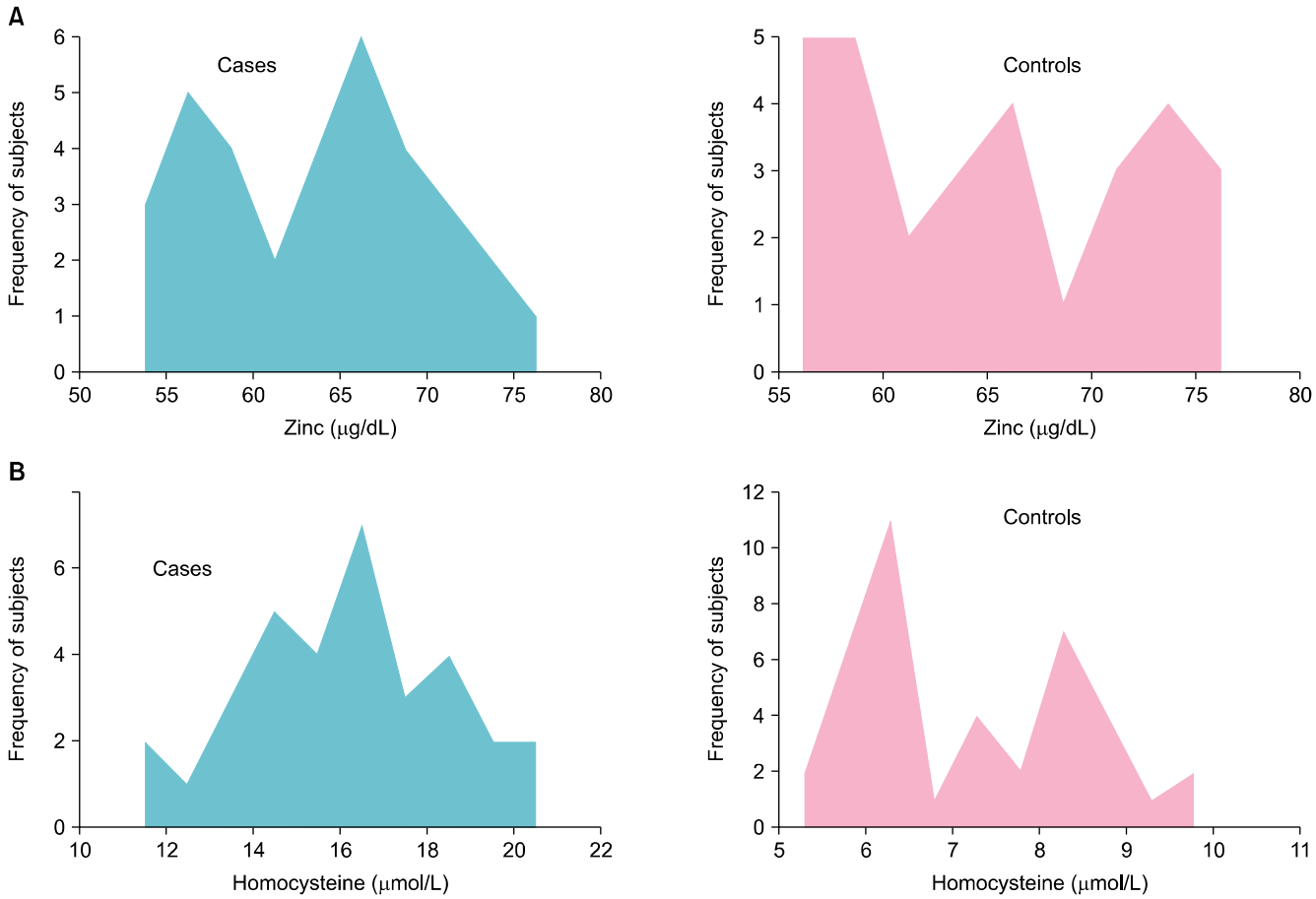

Fig. 1. Mean differences in serum zinc and homocysteine levels in cases and controls. (A) Mean concentration of zinc (μg/dL) was not significantly different in cases as compared to controls (P>0.05). (B) Homocysteine (μmol/L) was significantly higher in cases as compared to controls (P<0.001).

Table 2. Association between serum homocysteine levels and selected predictors

Indicator	Cases (n=30)		Controls (n=30)		Full cohort (n=60)	
	β-Coefficient[1)]	P	β-Coefficient	P	β-Coefficient	P
Age (years)	0.21	0.09	0.18	0.40	0.04	0.78
BMI (1) (kg/m^2)	0.03	0.72	0.07	0.07	0.25	0.01
BMI (2) (kg/m^2)	0.00	0.97	0.07	0.05	0.22[2)]	0.03
Parity	0.11	0.90	−0.40	0.47	3.29	0.01
Family history of PE	−0.33	0.73	−[3)]	−	4.61	0.00
Supplement use	0.26	0.61	−0.15	0.87	−2.21	0.09
Zinc levels (μg/dL)	0.18	0.02	0.03	0.31	−0.07	0.92

P-values <0.05 were considered statistically significant.
BMI (1), body mass index before pregnancy; BMI (2), body mass index during pregnancy; PE, pre-eclampsia.
[1)]Indicates the change in Hcy serum levels per unit change in the predicators.
[2)]Controlled for family history and BMI before pregnancy.
[3)]Family history was not introduced to the model as no subject had a family history.

formed. In these models, only age was significantly associated with Zn levels in the full cohort (β=−0.49, P<0.05) (Table 3).

To examine the association between Hcy levels and Zn status in women with PE and controls, each group, were stratified into two according to indicative cutoffs of serum Zn (μg/dL). Fig. 2 illustrates mean Hcy levels (μmol /L) of Zn deficient and sufficient pregnant women with PE and controls. No significant difference were found within the control group (P>0.05), whereas there was a trend toward significance among women with PE (P= 0.05).

Bivariate linear regression analysis was performed to assess the association between blood pressure (mmHg) and serum Hcy levels (μmol/L) in the full cohort. Serum Hcy levels (μmol/L) were entered as independent variables

Table 3. Association between zinc status and selected predictors

Indicator	Cases (n=30)		Controls (n=30)		Full cohort (n=60)	
	β-Coefficient[1)]	*P*	β-Coefficient	*P*	β-Coefficient	*P*
Age (years)	−0.49	0.08	−0.34	0.32	−0.49	0.03
BMI (1) (kg/m^2)	0.45	0.15	0.09	0.87	0.36	0.18
BMI (2) (kg/m^2)	−0.02	0.92	−0.14	0.78	−0.21	0.40
Parity	2.04	0.48	1.33	0.76	0.62	0.77
Family history of PE	5.23[2)]	0.05	−[3)]	−	2.44	0.32
Supplement use	−2.73	0.35	1.82	0.58	−0.09	0.95

P-values <0.05 were considered statistically significant.
BMI (1), body mass index before pregnancy; BMI (2), body mass index during pregnancy; PE, pre-eclampsia.
[1)]Indicates the change in serum zinc levels per unit change in the predicators.
[2)]Controlled for supplement use.
[3)]Family history was not introduced to the model as no subject had a family history.

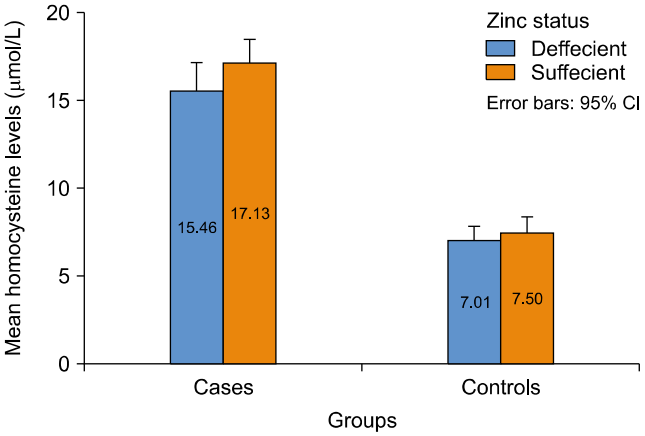

Fig. 2. Homocysteine levels (μmol/L) in zinc deficient pregnant women and zinc sufficient pregnant women in both cases and controls. Zinc deficient defined as ≤65 (μg/dL); zinc sufficient defined as ≥65 (μg/dL). No significant difference was found within cases and controls (*P*>0.05). Mean±95% confidence intervals (CI).

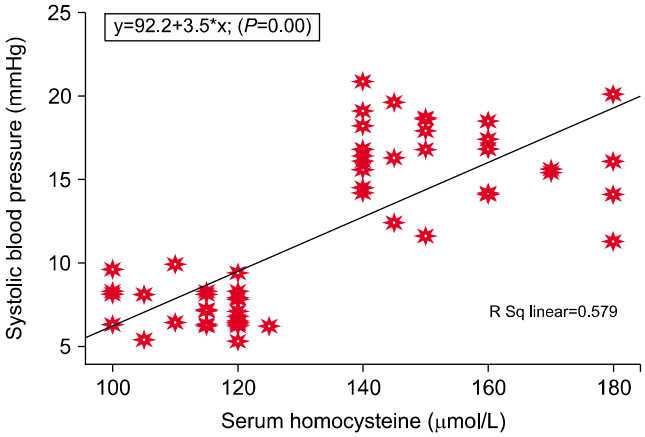

Fig. 3. Predicted systolic blood pressure per unit change of serum homocysteine levels. The straight line represents the linear regression calculations with the corresponding regression equation of the model. Systolic blood pressure (mmHg) was entered as the dependent variables.

whereas systolic and diastolic blood pressure (mmHg) were entered as dependent variables. An increase of serum Hcy levels of 1 μmol/L was associated with an 3.5 mmHg increase in systolic blood pressure and a 1.8 mmHg increase in diastolic blood pressure (*P*<0.05). These associations between Hcy (μmol/L) and systolic and diastolic blood pressure (mmHg) are shown in Fig. 3 and 4, respectively. Logistic regression analyses were performed to examine the associations between H-Hcy and selected predictors. In this model, H-Hcy was entered as the dependent variable and the model was adjusted for blood pressure. When all predictors were entered, only BMI before and during pregnancy was significantly associated with H-Hcy risk (BMI (1): OR=1.27, 95% CI: 1.12∼1.51; BMI (2): OR=1.28, 95% CI: 1.13∼1.54; *P*≤0.05). Zn levels were inversely associated with increased H-Hcy risk after further adjustment for supplement use. Both systolic and diastolic blood pressure were positively associated with H-Hcy risk (Table 4).

Table 5 shows the risk of selected risk factors on PE in multiple models. Data are presented as OR with 95% CI

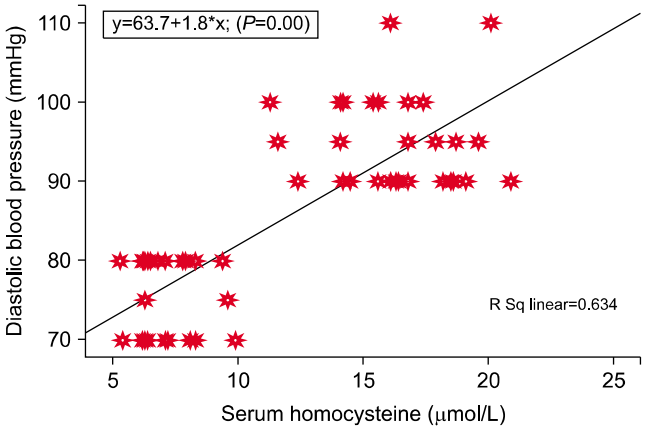

Fig. 4. Predicted diastolic blood pressure per unit change of serum homocysteine levels. The straight line represents the linear regression calculations with the corresponding regression equation of the model. Diastolic blood pressure (mmHg) was entered as the dependent variables.

and *P*-values. All values were adjusted for serum Hcy levels and blood pressure. Zn (μg/dL) levels were inversely associated with risk of PE (OR=0.84, 95% CI: 0.71∼

Table 4. Association between hyperhomocysteinemia risk and selected predictors[1)]

Variable	OR (95% CI)	*P*-value
Age (years)	0.93 (0.77～1.14)	NS
BMI (1) (kg/m^2)[2)]	1.27 (1.12～1.51)	0.006
BMI (2) (kg/m^2)[2)]	1.28 (1.13～1.54)	0.005
Gestational age (weeks)	0.96 (0.83～1.27)	NS
Zinc (μg/dL)[3)]	0.87 (0.77～0.99)	0.044
Parity (yes/no)[4)]	0.21 (0.05～0.88)	0.03
Supplement use (yes/no)[4)]	0.26 (0.06～0.99)	0.04
Family history (yes/no)	22.47 (0.01～1.44)	NS
Systolic blood pressure (mmHg)	1.14 (1.12～1.18)	0.003
Diastolic blood pressure (mmHg)	1.21 (1.07～1.34)	0.002

[1)]Model adjusted for blood pressure.
[2)]Controlling for BMI (1) or BMI (2).
[3)]Controlled for supplement use.
[4)]Controlled for age.
BMI (1), body mass index before pregnancy; BMI (2), body mass index during pregnancy; OR, odd ratios; CI, confidence intervals; NS, not significant.

Table 5. Risk of pre-eclampsia and selected predictors of pre-eclampsia[1)]

Variable	OR (95% CI)	*P*-value
Age (years)	0.99 (0.87～1.12)	NS
BMI (1) (kg/m^2)	1.20 (1.15～1.44)	0.02
BMI (2) (kg/m^2)	1.14 (1.12～1.33)	0.02
Gestational age (weeks)	1.04 (0.96～1.25)	NS
Zinc (μg/dL)	0.84 (0.71～0.98)	0.03
Homocysteine (μmol/L)	13.64 (0.90～23.03)	NS
Parity (yes/no)	7.42 (1.34～41.06)	0.02
Supplement use (yes/no)	0.21 (0.05～0.81)	0.02
Family history (yes/no)[2)]	22.42 (0.08～1.46)	NS
Systolic blood pressure (mmHg)	1.57 (0.03～4.47)	NS
Diastolic blood pressure (mmHg)	0.18 (0.06～0.99)	NS

[1)]Model adjusted for homocysteines and blood pressure.
[2)]Controlled for family history.
BMI (1), body mass index before pregnancy; BMI (2), body mass index during pregnancy; NS, not significant.

0.98; P=0.03). Supplement use during pregnancy was negatively associated with the risk of PE (OR=0.21, 95% CI: 0.05～0.81; P=0.02) after adjusting for family history. Parity was associated with an increased odds of PE (OR =7.42, 95% CI: 1.34～41.06; P=0.02). Furthermore, increased BMI during pregnancy was positively associated with risk of PE (OR=1.14, 95% CI: 1.12～1.33; P=0.02). Although serum Hcy levels (μmol/L) were significantly higher among women with PE than controls, these differences did not affect the odds of PE reaching the desired significance level (P>0.05) in the risk analysis.

DISCUSSION

PE is a public health risk in both developed and developing countries, and accounts for most maternal and perinatal morbidity and mortality globally (Yelikar et al., 2016). In the present study, there were no significant differences between PE pregnant women and healthy pregnant women in terms of age, gestational age, and BMI before pregnancy. However, BMI during pregnancy was significantly higher in pregnant women with PE than that in healthy pregnant women. This difference could be related to increased water retention (edema) and protein in urine (proteinuria) in PE pregnant women, which is associated with swelling and an abnormal increment in weight (Kanagal et al., 2014).

In our study, serum Hcy was higher in women with PE than in controls (16.35 μmol/L vs 7.25 μmol/L, respectively; P<0.001). A similar trend was found by Makedos et al. (2007) in which mean Hcy levels were 11.11 μmol/L in pregnant women with PE compared with 6.4 μmol/L in healthy pregnant women. Furthermore, Harma et al. (2005) investigated the association between levels of Hcy, Zn, copper, and PE using a case control design. The study included 24 pregnant women with PE and 44 normotensive pregnant controls. The authors found that Hcy levels were higher in pregnant women with PE than in normotensive controls (16.39±9.32 nmol/mL vs 9.45±3.64 nmol/mL, respectively). Another study conducted in Iran by Hasanzadeh et al. (2008) showed that women with PE have higher Hcy levels than normal pregnant women.

However, results from studies investigating the association between serum Hcy and PE are contradictory. For example, a case control study conducted by Shilpa et al. (2017) demonstrated significantly lower serum Hcy level in PE patients (8.90±4.33 μmol/L) compared with normal non-pregnant women (13.31±5.81 μmol/L). However, mean serum Hcy levels of the PE patients (8.90±4.33 μmol/L) were not significantly higher than those of normal pregnant women (7.52±2.25 μmol/L). The authors indicated that this insignificant minor difference in serum Hcy levels may be due to PE patients receiving folic acid supplementation during their first trimester of gestation. In our study, serum Hcy levels did not significantly differ between supplement users and non-supplement users in women with PE and controls; however, the likelihood of H-Hcy was significantly lower in supplement users vs non-users (OR=0.26,95% CI: 0.06～0.99; P=0.04).

Our study demonstrated the effect of serum Hcy levels on the severity of the disease. There was a strong association between the severity of hypertension and levels of serum Hcy. An increase in serum Hcy of 1 μmol/L was associated with a 3.5 mmHg increase in systolic blood pressure and 1.8 mmHg increase in diastolic blood pressure (P<0.05). Our results are consistent with a previous study that showed higher maternal serum Hcy levels in women with severe PE compared with women with mild PE (6.38±0.3 vs. 14.05±1.43, respectively) (Khosrowbeygi and Ahmadvand, 2011). A recent case control

study involving 51 PE pregnant women examined the association between serum Hcy levels and the severity of PE. The authors reported significantly higher serum Hcy levels in pregnant women with severe PE compared with women with non-severe disease (10.67±1.49 vs 7.76±1.88, respectively; $P<0.05$) (Shahbazian et al., 2016). Similarly, in a 2-year prospective cohort study, the association between Hcy levels and severity of hypertension and complications of PE and eclampsia was confirmed (Maru et al., 2016).

Many studies have indicated that H-Hcy is a well-recognized risk factor for cardiovascular diseases. Accordingly, many studies have addressed the possible causal relationship between H-Hcy and adverse pregnancy outcomes (Dodds et al., 2008). Plasma Hcy concentrations normally decrease during pregnancy via an unknown mechanism, which may involve normal increases in glomerular filtration rates that accompany pregnancy, increases in plasma volume and associated hemodilution, and increased uptake of Hcy by the fetus (López-Quesada et al., 2003; Harma et al., 2005). Elevated levels of Hcy have been postulated to produce oxidative stress and endothelial dysfunction, which are associated with PE (Mignini et al., 2005). However, there are contradictory data on the potential of Hcy as a marker for subsequent PE. Some studies suggest that H-Hcy in early pregnancy can increase the risk of developing PE (Cotter et al., 2003; López-Quesada et al., 2003; Dodds et al., 2008). However, Zeeman et al. (2003) showed that Hcy concentrations do not significantly differ between women with PE and normal pregnant women, and that maternal Hcy concentrations cannot be used as a predicator for PE (Zeeman et al., 2003). In this study, serum Hcy levels (μmol/L) were significantly higher among pregnant women with PE, but their differences did not affect the odds of PE reaching the desired significance level (OR=13.64, 95% CI: 0.90～23.03; $P>0.05$) in the risk analysis. In the current study, the small sample size or other unconsidered confounders may have masked the association. In addition, pregnancy is associated with a decrease in serum Zn concentration (Kumru et al., 2003); this alteration may include physiological adjustment to pregnancy, a response to hormone changes, hemodilution, or combination of these factors (Tamura et al., 2000).

The demands for micronutrients during pregnancy often cannot be met in low-income countries due to poor diets (Gernand et al., 2016). For example in South Asia, the prevalence of Zn deficiencies ranges from 15～74% (Gernand et al., 2016). In our study, approximately 50% of the participants were Zn deficient. Age, parity, socioeconomic status and other factors might influence nutrition during gestation (Gernand et al., 2016). We showed that Zn was associated with age in the full sample but not in women with PE or controls. Indeed, a one-year increase in age was significantly associated with a 0.49 μg/dL decrease in serum Zn levels ($P=0.03$); however, there was no association between serum Zn levels and other predictors in women with PE and controls ($P>0.05$). Unfortunately, no information was available about dietary intake of Zn in our sample population, which is the main factor that determines Zn status. In this context, many previous studies have explored the relationship between changes in serum Zn levels in pregnant women and PE. However, results from these studies are conflicting. Some studies found lower serum Zn is significantly higher in pregnant women with PE compared with healthy pregnant women (Ilhan et al., 2002; Farzin and Sajadi, 2012; Sarwar et al., 2013). However, other studies showed that serum Zn levels are higher in pregnant women with PE than in healthy pregnant women (Mahomed et al., 2000; Harma et al., 2005; Katz et al., 2012). Our data did not show any significant differences in Zn levels between pregnant women with PE and controls (63.71±1.24 μg/dL and 65.37±1.27 μg/dL, respectively; $P>0.05$).

Our findings are in agreement with Golmohammadlou et al. (2008) and Vigeh et al. (2006), who showed that the serum Zn concentrations do not significantly differ between pregnant women with PE and healthy pregnant women. These results may be explained by the negative impact of iron on Zn absorption and status that could further exacerbate Zn status (Lönnerdal, 2000). In our study, approximately 63% of full cohort used supplements (calcium, iron, or multivitamins), which could explain why both pregnant women with PE and controls were at borderline of the normal range of the Zn cutoff level (i.e., 65.00 μg/dL) (Álvarez et al., 2007). However, elevated Zn in blood was significantly associated with H-Hcy in PE, and Hcy concentrations were positively correlated with plasma Zn concentrations in women with PE ($r=0.588$; $P=0.003$) but not healthy controls (Harma et al., 2005). In pregnant women with PE, a one unit increase in serum Zn was significantly associated with 0.18 μmol/L decrease in serum Hcy levels ($P=0.02$); however, there was no association between serum Zn and serum Hcy in controls or the full cohort ($P>0.05$).

Limitations of the present study include the small sample and a lack of information about dietary Zn intake, both of which may mask the influence of Zn status on Hcy levels and PE risk in pregnant women. A greater prospective and more population based studies are needed to further explore the association between elevated Hcy, low vitamins and Zn status, and the risk of PE. Overall, early management of H-Hcy and the associated risk factors may be effective in decreasing the incidence of PE in our population.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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