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RESEARCH ARTICLE

The Significance of Serum Levels of Magnesium and Human Chorionic Gonadotropin in Singleton Pregnant Women diagnosed with Pre-eclampsia.

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Abstract

Pre-eclampsia (PE) globally affects many pregnant women causing maternal and perinatal morbidity and mortality. Since there is no treatment for PE, identifying those at risk using markers would be desirable for intensive observation and care. This study therefore aimed at investigating the significance of serum magnesium (Mg^{2+}) and free β-human chorionic gonadotropin (β-hCG) levels as biomarkers for severity of pre-eclampsia.

Methods: This was a case-control study on 150 singleton pregnant women with pre-eclampsia and 150 normotensive matched controls. Data were collected by blood sampling and questionnaire. Serum β-hCG and Mg^{2+} were assayed using quantitative ELISA technique and chemical methods respectively. Outcome parameters were compared using Student's t-test, ANOVA, χ^2 test, and Pearson's correlation coefficient.

Results: Mg^{2+} was significantly lower (0.69 ± 0.11) versus (0.88 ± 0.09) whiles β-hCG was elevated (0.99 ± 0.20) versus (0.85± 0.09) with $p < 0.001$ in the pre-eclampsia group compared with normotensives respectively. Thirteen (8.7%) of the cases which were diagnosed as having severe pre-eclampsia (SPE) had significantly lower Mg^{2+} of (0.48 ± 0.13, $p<0.001$) and significantly elevated β-hCG levels and (1.29 ± 0.33, $p<0.001$) compared with controls respectively. Mg^{2+} levels correlates significantly with both systolic and diastolic blood pressures of the SPEs.

Conclusion: Serum Mg^{2+} and β-hCG levels showed significant reduction and elevation respectively during pre-eclampsia and more so in severe pre-eclampsia. However in the case of SPE only Mg^{2+}showed significant association with systolic and diastolic blood pressures. Serum magnesium seems to be a better marker for SPE and its complications than β-hCG.

Introduction

Pre-eclampsia (PE) is a world-wide pregnancy-specific disorder that affects approximately 3 % of all pregnancies (WHO, 2005). It is a major cause of maternal and perinatal morbidity and mortality (Sibai et al., 2003). In Ghana an incidence of 7% was reported in a teaching hospital (Obed &Aniteye, 2006). The disorder is characterized by the onset of hypertension and proteinuria usually after the twentieth week of gestation in previously normotensive

pregnant women (Wagner, 2004). Additional characteristics of the disorder include maternal endothelial dysfunction, excess inflammatory response and it shares underlying pathophysiological processes with cardiovascular disease and this disorder may be indicative of its later development (Funai et al., 2005). Specific pathogenesis of pre-eclampsia remains unknown, although some contributing factors have been identified including abnormal placentation, oxidative stress, angiogenic factors and inflammation (Roberts & Gammill, 2005). Since there is no cure for PE other than timely delivery, identification of those at risk, those who have subclinical disease or those who have markers of potentially severe disease is desirable for more intensive observation and care.

Several studies have associated high serum levels of β-hCG in the second and third trimesters with adverse pregnancy outcomes including pre-eclampsia (Olsen et al. 2012, Yu et al., 2013). Compared with normal pregnancies, the placentae of patients with unexplained elevated maternal free beta- hCG levels tend to be larger and to have an increase density and intensity of free β-hCG (Al-Sebai et al., 1996). A pregnant woman with elevated β-hCG levels between 16 and 34 weeks of gestation is suggestive of pre-eclampsia (Myatt& Miodovnik, 1999).

Studies have also linked serum magnesium deficiency to pre-eclampsia and magnesium has been used in the management of pre-eclampsia and eclampsia. In a large multicentre trial, it was demonstrated that magnesium sulphate ($MgSO_4$) was superior to other anticonvulsants in the management of both pre-eclampsia and eclampsia (The Eclampsia Trial Collaborative Group, 1995). A placebo-controlled ($MgSO_4$) for Prevention of Eclampsia (MAGPIE) trial clearly demonstrated that $MgSO_4$ reduced the risk of eclampsia in pre-eclamptic women by 50% (Altman et al., 2002). The mechanism of action is unclear, but magnesium may act by improving cerebral vascular perfusion (Greene, 2003).

There are two clinical forms of pre-eclampsia and these are mild pre-eclampsia and severe pre-eclampsia (and its different manifestations such as imminent eclampsia, HELLP syndrome, and eclampsia). Whilst the mild form of the disease can be managed conservatively to await significant fetal maturation, the management of severe pre-eclampsia entails initial stabilization followed by delivery in order to prevent escalation of complications. Currently the classification of mild or severe pre-clampsia is based mainly on blood pressure and urine protein. There are many situations of end organ damage in pre-eclampsia suggestive of severe disease where the blood pressure has not been too high. Urine protein on dipstick varies widely and often unreliable in predicting disease severity. Proteins in a twenty-four hour urine sample though more reliable is cumbersome to carry out in routine clinical practice. A systematic review of 16 studies for estimation of proteinuria as a predictor of complications of pre-eclampsia concluded that measure of proteinuria is a poor predictor of either maternal or fetal complications (Thangaratinam et al., 2009). The search for reliable markers of severe pre-eclampsia therefore continues.

Serum biochemical markers have shown some promise. Mumtaz et al., (2008) have demonstrated that serum leptin appears to be a marker of severe pre-eclampsia independently or along with other parameters. Several studies have also reported a positive correlation between elevated maternal serum uric acid levels and adverse maternal and fetal outcomes (Liedholm et al., 1984; Redman et al., 1976; Lancet & Fisher, 1956; McFarlane, 1963). However a meta-analysis of 18 studies concluded that uric acid was a poor predictor of maternal and fetal complications in women with pre-eclampsia (Thangaratinam et al., 2006).

There is the need to investigate additional biomarkers that could predict the severity of pre-eclampsia. The objectives of this study were to: determine the serum values of free β-hCG and Mg^{2+} in second trimester singleton pregnant Ghanaian women diagnosed with pre-eclampsia; evaluate pre-eclampsia severity predictability of these markers and investigate which of these markers better predicts pre-eclampsia severity.

Materials and Methods:

Study Site

A cross-sectional study of 300 Ghanaian singleton pregnant women aged between 16-40 years attending the antenatal clinic at Ridge Regional Hospital, Accra was conducted after recruitments of subjects over a period of 13 months (1st July, 2011 to 31st July, 2012). Ridge Hospital is a tertiary referral center for the Greater Accra Region with a population of over 3 million and about 9000-10000 deliveries annually.

Study Design and Biochemical Assays

Participants were classified as having features of pre-eclampsia (150) or normal controls (150) based on the levels of urine proteins and blood pressure. Pre-eclampsia was presumed when a pregnant woman whose blood pressure was within normal limits during the first half of the pregnancy developed a hypertension of at least 140/90mmhg associated with significant proteinuria during the second half of the pregnancy. Proteinuria was measured using the dipstick. Written informed consent was obtained from all participants. The study was approved by the ethical and protocol review committee of University of Ghana Medical School. The gestational age at recruitment was

estimated from the date of last menstrual period (LMP) and confirmed with ultrasonographic measurements of crown–rump length and/or biparental diameter before 20 weeks of gestation. The abdominal/pelvic ultra sound scan also looked for fetal defects and multiple pregnancies. Questionnaire was completed by all participants to obtain demographic information on maternal age, marital status, educational background, occupation, family history of hypertension. Maternal weight and height were recorded and the body mass index (BMI) (mean ± SD) calculated. Blood pressure (BP) was measured after the subjects had rested for 15 minutes. Pregnant women with gestation < 20 weeks; multifetal gestation; evidence of raised BP before the 20th week of gestation and multigravida/parity were excluded

After recruitment, 5mls of venous blood samples was taken into serum separator vacutainer tubes and stored. Urine protein was determined by the use of dip sticks method (AcuBiotech Co.Ltd, Beijing Airport Industrial Zone Benijing, China).

Serum free β-hCG was assayed using the quantitative sandwich ELISA technique with a test kit obtained from GenWay Biotech, Inc. (San Diego, USA). Absorbance was measured on Multiscan EX Microplate Photometer platform (Thermo Electron Corporation, Shanghai, China) at 450 nm. Similarly, Mg^{2+} was assayed using a test kit obtained from GenWay Biotech, Inc., (San Diego, USA) and measured on Selectra Junior platform (Vital Scientific, Ekkerstijt, Netherlands). Manufacturer's instructions were followed for both analysis.

Hemoglobin (Hb) was immediately determined after blood collection using ABx pentra 60 C+ automated hemocounter (Horiba ABx diagnostics, Ekkerstijt, Netherlands). All tests were performed in duplicates with variation not exceeding 10%.

Serum Mg^{2+} and β-hCG were presented in mmol/ml and MoM respectively as mean with standard deviation (mean ± SD). Gestation-specific multiples of the median (MoM) values were calculated by dividing the β-hCG concentration by the median value for the gestational week at which the sample was taken.

Statistical analysis

Maternal age, BMI, Hb, SBP and DBP were presented as mean ± SD. Socio-demographics data were presented as percentage. Student's t-test was used to test for mean differences and ANOVA for comparison of clinical and biochemical parameters of women with various degree of proteinuria. Test for proportions was performed to compare categorical data. Pearson's correlation coefficient (r) was used to determine the correlation between blood pressures and serum Mg^{2+} and free β-hCG. Statistical significance was determined at $p < 0.05$. Stata software version 10 (Stata Corporation, Texas, USA) was used in all statistical analysis.

Results

The mean age, BMI, systolic BP, and diastolic BP of the study group were significantly higher in the study group compared to the control group [$p<0.001$ (Table 1)]. Illiteracy was higher but not significant in the pre-eclampsia group compared to the controls ($Z = 1.83$, $p < 0.06$) (Table 1). Even though the various levels of educational status did not show statistically significant differences between the two groups there was a tendency towards lower levels of education in the pre-eclampsia group and higher levels of educations in the controls.

Magnesium was significantly lowered (0.69±0.11 versus 0.88±0.09 mmol/ml) whiles free β-hCG was significantly elevated (0.99±0.20 versus 0.85±0.09 MoM) in the pre-eclampsia cases compared with normal controls respectively ($p < 0.001$) (Table2). Moreover, classifying the pre-eclamptic patients based on the level of urine protein indicated that the higher the urine protein the more severe the clinical parameters across board (Table 3). Furthermore severe pre-eclamptic cases had the significantly lowest serum Mg^{2+} and Hb, and the highest BMI, SBP, DBP and β-hCG levels compared with the controls ($p<0.001$ (Table 4). The odds of associating Mg^{2+} and free β-hCG to PE were also very significant at 95%CI of mean difference (Table 4) indicating association of low Mg^{2+} and high free β-hCG to development of SPE. All 13 SPEs have blood pressure greater than 160/110 mmHg, p-value < 0.001 (Tables 3, 4). Interestingly, although there were correlation between Mg^{2+} and free β-hCG with the PE blood pressures, $p<0.001$ (S1,S2, S3,S4), only Mg^{2+} levels correlate significantly with both the systolic and diastolic blood pressures of the SPE women (Figures 1, 2, 3 and 4).

Figure Legends

Figure 1. Relationship between free β-hCG levels and SBP among severe pre-eclamptic women. Linear regression plot of mean β-hCG concentration versus SBP of 13 SPE mothers. There was no significant association between β-hCG and SBP ($R^2 = 0.0935$, $p = 0.306$)

Figure 2. **Association between Mg^{2+} levels and SBP among severe pre-eclamptic women**. Linear regression plot of mean Mg^{2+} concentration versus SBP of 13 SPE mothers. There was significant association between Mg^{2+} levels and SBP ($R^2 = 0.0031$, $p <0.001$).

Figure 3. **Relationship between free β-hCG levels and DBP among severe pre-eclamptic women.** Linear regression plot of mean β-hCG concentration versus DBP of 13 SPE subjects. There was no significant association between β-hCG and DBP ($R^2 = 0.0802$, $p = 0.466$)

Figure 4. Association between Mg^{2+} levels and DBP among severe pre-eclamptic women. Linear regression plot of mean maternal Mg^{2+} concentration versus DBP of SPE. There was positive and significant association between Mg^{2+} levels and DBP ($R^2 = 0.0275$, $p < 0.001$).

Table 1: Clinical and socio-demographic parameters of study population

Parameter	PE (N=150)	Control (N=150)	95% CI of mean difference	p-value
Age (years)	29.65±5.24	26.99±3.03	1.69-3.63	<0.001
BMI (Kg/m^2)	23.37±3.50	21.35±1.58	1.40-2.64	<0.001
SBP (mmHg)	134.78±23.73	119.55±6.31	11.28-19.17	<0.001
DBP (mmHg)	93.78±18.46	84.60±4.94	6.11-12.25	<0.001
Education N(%)			Z-Score	
Illiterates	59(39.3)	30(20.0)	1.83	0.067
Primary	13(8.7)	21(14.0)	-0.46	0.644
Secondary	42(28.0)	54(36.0)	-0.83	0.406
Vocational	34(22.7)	21(14.0)	0.79	0.428
Tertiary	2(1.3)	24(16.0)	-0.56	0.575
Occupation N(%)				
Trader	78(52.0)	99(66.0)	-1.89	0.059
Public Servant	1(0.7)	9(6.0)	-	-
House wife	71(47.3)	36(24.0)	2.33	0.019*
Unemployed	0(0)	6(4.0)	-	-

*p<0.05, PE = pre-eclampsia subjects, BMI = Body Mass Index, SBP = Systolic blood pressure, DBP = Diastolic blood pressure

Table 2: Comparison of serum markers of the study population

Parameter	PE (N=150)	Control (N=150)	95% CI of mean difference	p-value
Mg^{2+} (mmol/ml)	0.69±0.11	0.88±0.09	-0.22-(-0.17)	<0.001
hCG (MoM)	0.99±0.20	0.85±0.09	0.90-0.17	<0.001
Hb (g/dl)	10.82±1.23	13.74±0.78	-3.15-(-2.68)	<0.001

Mg^{2+} = magnesium, hCG = free beta-chorionic gonadotropin, Hb = haemoglobin.

Table 3: Comparison of mean clinical and serum markers of pre-eclamptic women with different degrees of proteinuria

Parameter	Pregnant women with different degrees of proteinuria (N=150)		Severe preeclamptic patients	p-value
	(+1) (N=99)	(+2) (N=38)	(+3) (N=13)	
Age (years)	29.8 ± 5.41	28.4 ±4.80	31.77 ± 4.53	0.212
BMI (Kg/m^2)	22.68 ± 2.98	23.40 ± 3.35	28.58 ± 3.21	<0.001
SBP (mmHg)	128.2 ±18.92	137.90 ± 22.20	175.92 ± 18.01	<0.001
DBP(mmHg)	88.9 ±15.30	96.60 ±17.47	122.69 ±15.89	<0.001
Mg^{2+}(mmol/ml)	0.72 ±0.07	0.68±0.09	0.48±0.13	<0.001
hCG (MoM)	0.95 ±0.15	0.98 ±0.20	1.29 ±0.33	<0.001

Hb (g/dl)	11.07 ±1.17	10.63 ±1.05	9.45 ±1.19	<0.001

Table 4: Clinical and serum markers of women diagnosed with severe pre-eclampsisa compared with controls

Parameter	Severe preeclamptic patients (N=13)	Control (N=150)	95% CI of mean difference	p-value
Age (years)	31.77±4.53	26.99±3.03	2.97-6.59	<0.001
BMI (Kg/m^2)	29.53±1.64	21.35±1.58	7.28-9.08	<0.001
SBP (mmHg)	175.92±18.01	119.55±6.31	51.91-60.83	<0.001
DBP (mmHg)	122.69±15.89	84.60±4.94	34.42-41.77	<0.001
Mg^{2+} (mmol/ml)	0.48±0.13	0.88±0.09	-0.46-(-0.35)	<0.001
hCG (MoM)	1.29±0.33	0.85±0.09	0.37-0.51	<0.001
Hb (g/dl)	9.45±1.19	13.74±0.78	-4.76-(-3.82)	<0.001
Urine protein	3.15±0.38	0	3.10-3.21	<0.001

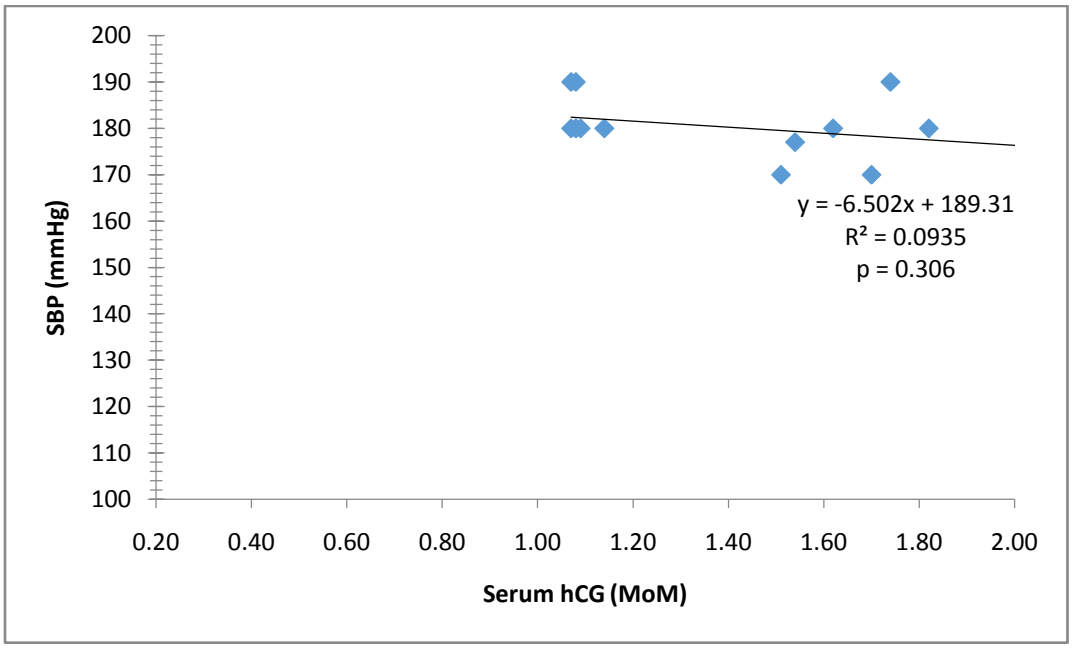


Figure 1: Relationship between free β-hCG levels and SBP among women with severe pre-eclampsia

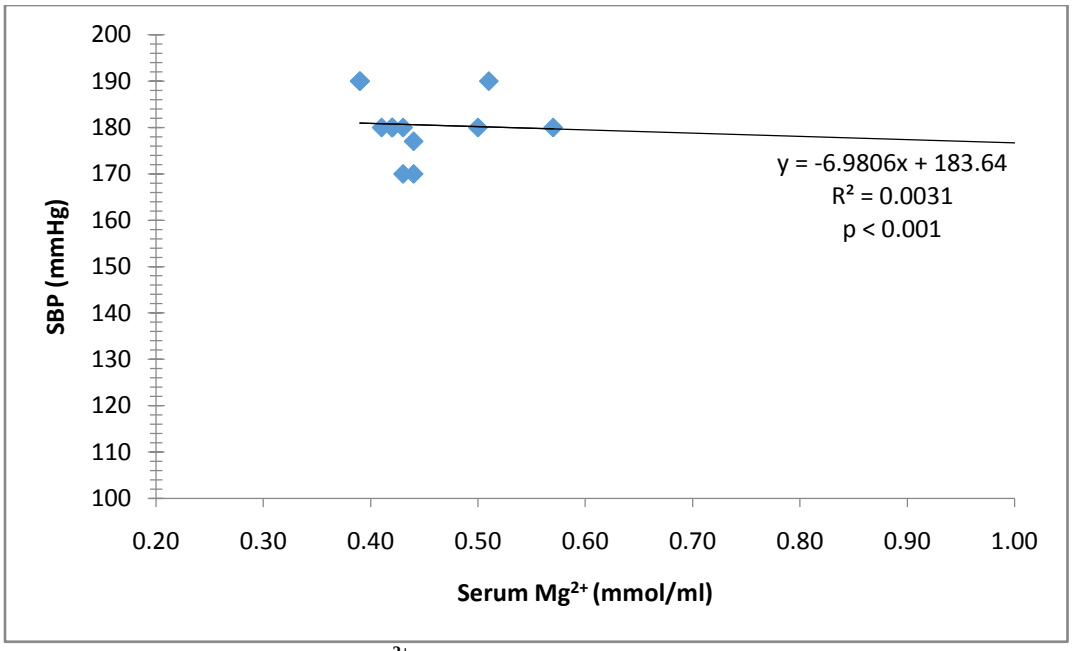


Figure 2: Association between Mg^{2+} levels and SBP among women with severe pre-eclampsia

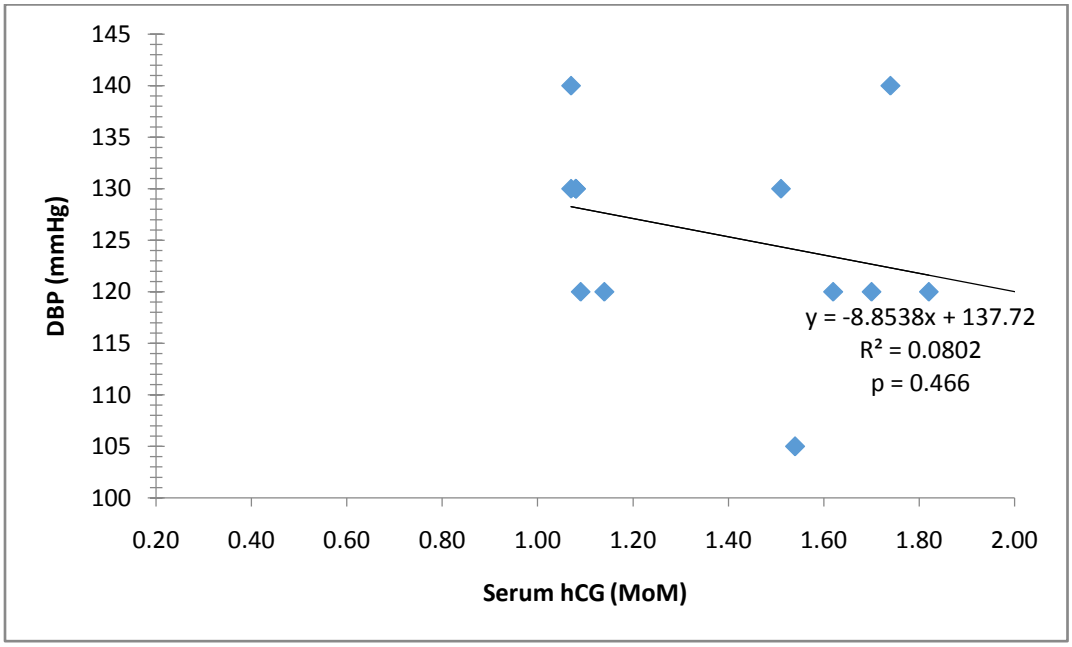


Figure 3: Relationship between free β-hCG levels and DBP among women with severe pre-eclampsia

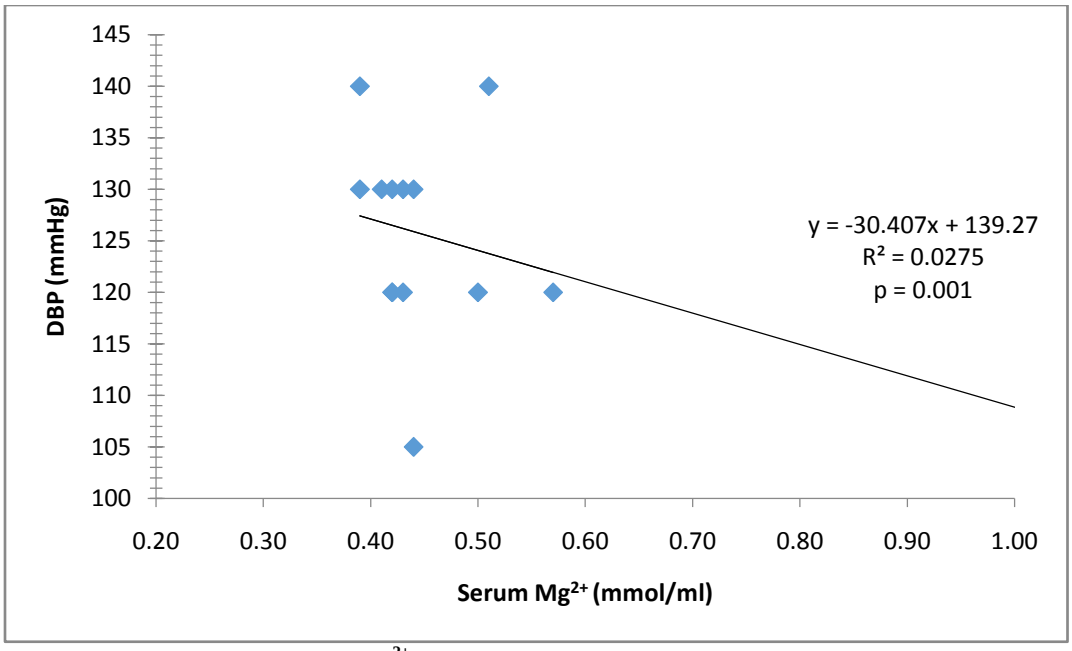
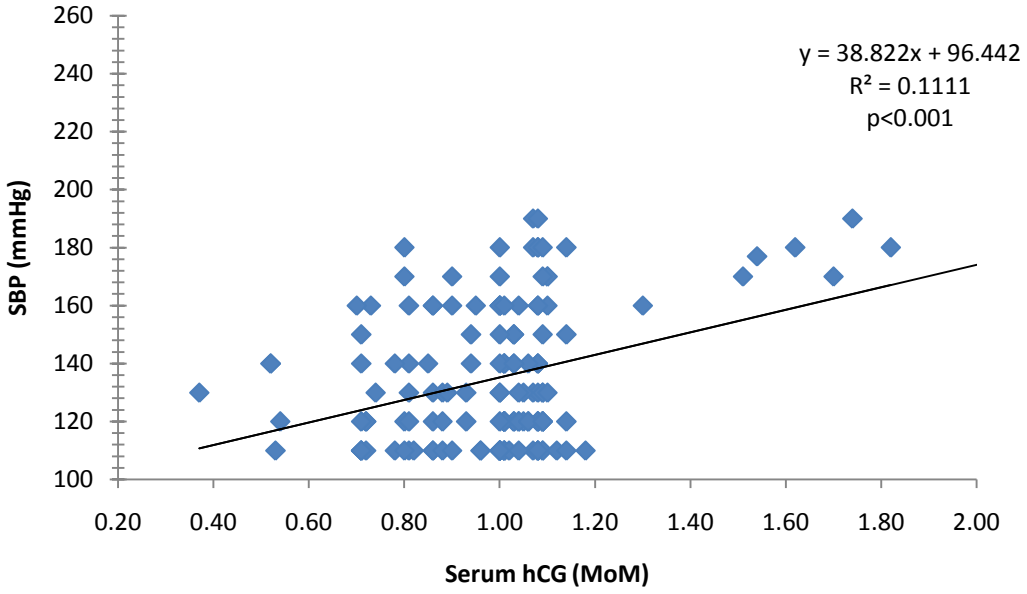
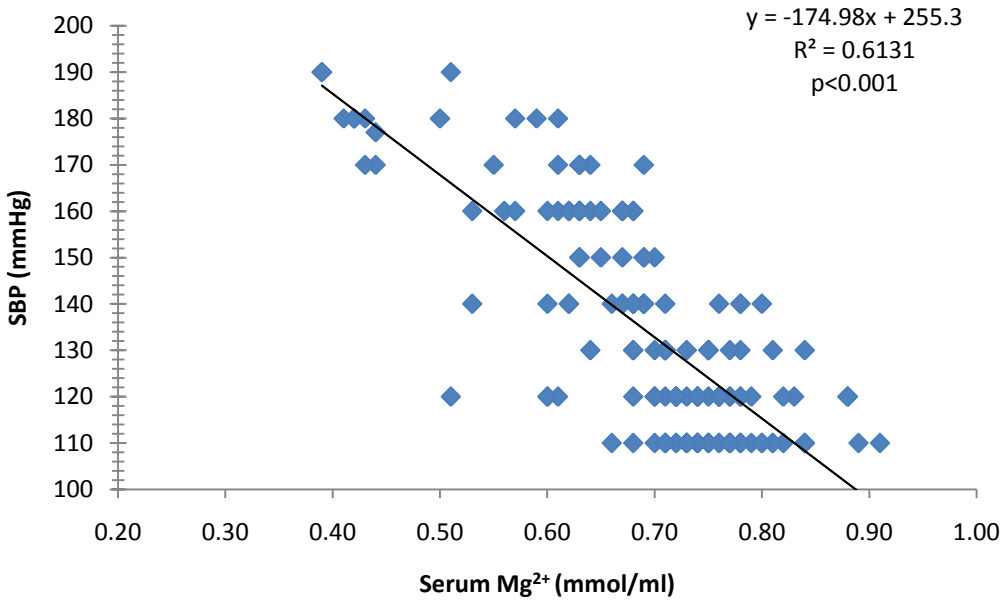


Figure 4: Association between Mg^{2+} levels and DBP among women with severe pre-eclampsia

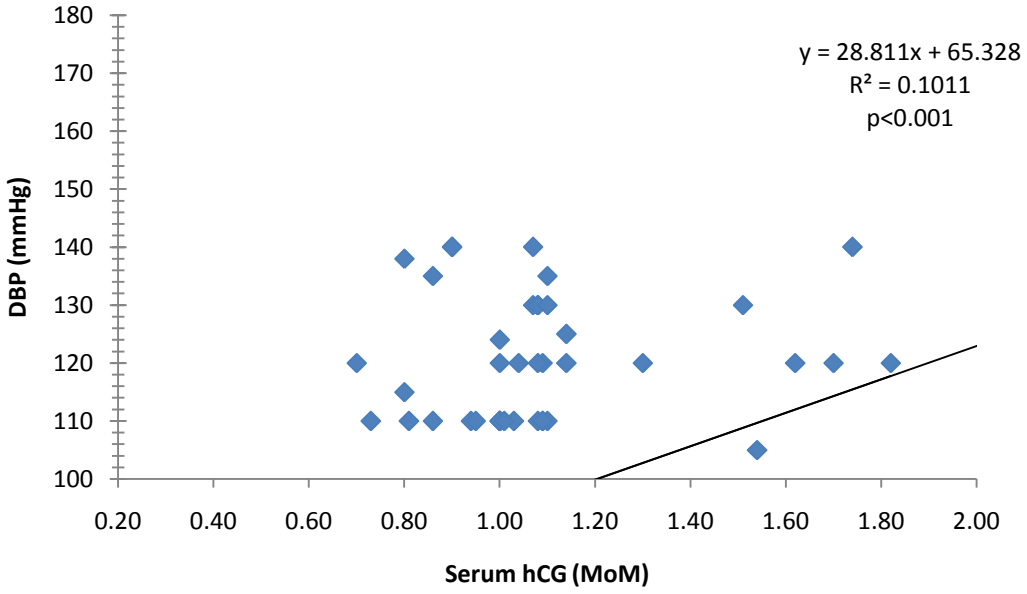
Supplementary Materials



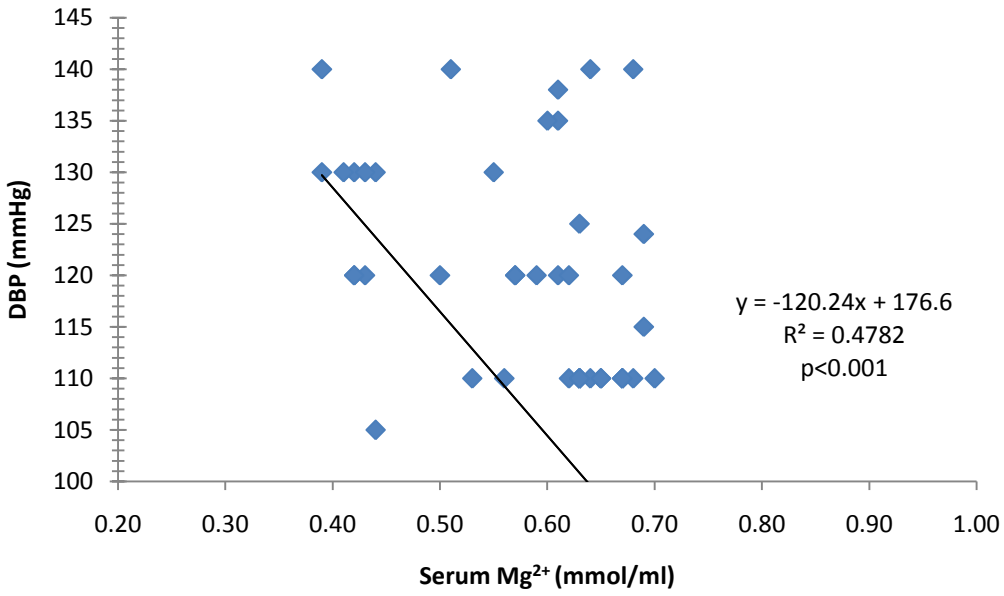
S1: Relationship between free β-hCG levels and SBP among pre-eclamptic subjects



S2: Relationship between Mg^{2+} levels and SBP among pre-eclamptic subjects



S3: Relationship between free β-hCG levels and DBP among pre-eclamptic subjects



S4: Relationship between Mg^{2+} levels and DBP among pre-eclamptic subjects

Discussions

This study has shown that elevated free β-hCG and lowered Mg^{2+} serum levels in the second trimester were associated with development of pre-eclampsia in the Ghanaian singleton pregnant women. Additionally, low levels of Mg^{2+} was consistently associated with the degree of proteinuria and strongly associated with the level of SBPs and DBPs of the PE and SPE. It supports several others which also found that serum Mg^{2+} levels were significantly lower in pre-eclamptic women compared with non-pre-eclamptics (Escenet al., 2003; Dahleet al., 1995; Mageeet al., 2005). This study further established that the more severe the pre-eclamptic disease is the lower the level of serum Mg^{2+}.

Deficiency in magnesium is believed to significantly facilitate contractile responses to bradykinins, angiotensin II, serotonin and Prostanglandin F2 alpha and could possibly lead to spasms in the placental and the umbilical cord vasculature (Altura, 1983). These effects could explain some of the manifestations of PE such as *intrauterine growth restriction (*IUGR), premature labor, and systemic hypertension. Magnesium repletion helps to relax smooth muscles which may be primary to the various therapeutic effects of the element.

Serum free β-hCG levels were also reported to be significantly elevated in pre-eclamptic women (Myatt& Miodovnik., 1999; Mizejewski, 2007). An elevation in free β-hCG levels in the second and third trimesters has been linked to the development of pre-eclampsia and other adverse pregnancy outcomes such as increased risk of perinatal death, low birth weight, small-for-gestational-age infants, preterm premature rupture of membranes, and preterm birth (Myatt& Miodovnik., 1999). This is thought to be due to disorder in the formation of uteroplacental vasculature resulting in inadequate blood supply to the fetoplacental unit (Roberts & Gammill, 2005). There is then, fetoplacental hypoxia, leading to imbalances in the release and metabolism of prostaglandins, endothelin, and nitric oxide by placental and extraplacental tissues (Roberts & Gammill, 2005). These as well as enhanced lipid peroxidation and other undefined factors contribute to the hypertension, platelet activation and systemic endothelial dysfunction characteristics of pre-eclampsia (Obed &Aniteye, 2006). It is believed that increased free β-hCG production is in response to placental hypoxia since β-hCG production has been shown to increase when normal placental villi in organ cultures were maintained under hypoxic conditions (Thompson et al., 2004).

The significantly higher age bracket of the PE and SPE groups compared to the control agrees with studies elsewhere. Maternal age between 30-40 years is among the clinical risk factors for the development of PE, believed to probably reflect undiagnosed chronic hypertension with superimposed PE (Escen et al., 2003; O'Brien, 1992). The BMI association with high free β-hCG and low Mg^{2+} levels together with development of pre-eclampsia in this study is consistent with observations by Thompson and associates in 2004 that pre-eclampsia risk is doubled from a

BMI of 26 and nearly tripled at a BMI of 30. High BMI and pre-eclampsia share certain pathophysiological features, including endothelial dysfunction, oxidative stress, and an increased state of inflammation (Poon et al., 2010).

Moreover, there was a significant association between both free β-hCG and Mg^{2+} levels on one hand and both systolic and diastolic blood pressures in PE subjects. However, using blood pressure as an adverse pregnancy outcome, only Mg^{2+} level correlated consistently and significantly with both SBP and DBP. Even though serum free β-hCG was significantly elevated in SPE the serum values failed to correlate significantly with the severity of diastolic and systolic blood pressures in this study.

Furthermore, elevated free β-hCG and lowered Mg^{2+} corresponds significantly with low hemoglobin levels in the PE as well as the SPE when compared with the controls. Severe anemia (Hb < 10g/dl) has been associated with a higher risk for pre-eclampsia and poor perinatal outcomes (Ali & Adam, 2011).

In conclusion this study showed a significant reduction and elevation of serum Mg^{2+} and β-hCG levels respectively during pre-eclampsia and more so in cases of severe pre-eclampsia. However, in the case of severe pre-eclampsia and using BP as an index of severity, only serum magnesium showed significant association with systolic and diastolic blood pressures. Serum magnesium may be a better biomarker for the severity of pre-eclampsia and its complications as compared to free β-hCG since there was consistent significant increase in proteinuria together with higher systolic and diastolic blood pressures as the Mg^{2+} level decreases in the SPE. We recommend large cohort research to confirm the present study so that serum magnesium levels can be included as a biomarker to routinely determine the severity of the disease. Confirmation will also implies the need for magnesium supplementation in those areas where pre-eclampsia take its greatest toll.

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Disclosure

The authors have no conflict of interest.

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