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TITLE PAGE

Title: A prospective assessment of neurodevelopment in children following a pregnancy complicated by severe preeclampsia

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ABSTRACT

Objective: To prospectively examine whether children of women with a pregnancy affected by severe preeclampsia (PE), compared to children of women without a PE-affected pregnancy, have differences in neurodevelopmental performance up to five years of age.

Design: Prospective cohort study.

Setting: Tertiary care centre.

Participants: Women were recruited into the study following a PE-affected pregnancy. After each PE subject was recruited, the next normotensive woman without prior history of PE and matched by parity, maternal age, and race was invited to participate. Women with a history of chronic hypertension, diabetes, or renal disease were excluded. A total of 129 PE-affected and 140 normotensive mothers were enrolled.

Outcome Measures: The primary outcome measure was failure of the Ages and Stages Questionnaire (ASQ). The ASQ was completed yearly, until age five.

Results: A significant difference was found in the proportion of ASQ categories failed in year 3 ($P<0.05$), and this approached significance in years 1 and 4 ($P<0.10$ and $P<0.15$, respectively). At year 1 the number of ASQ categories failed was significantly greater among children born to PE mothers. A subgroup analysis revealed a significant proportion of PE children born preterm (<37 weeks) failed the ASQ in years 3 and 4 ($P<0.05$), and when failed, those who were preterm failed significantly more categories in years 3 and 4 ($P<0.05$). A trend toward increased failure in the gross motor category was found. There was a significant positive correlation between maternal lifetime CVD risk score and the number of ASQ categories failed at years 1 and 3 ($P<0.05$).

Conclusion: PE was found to be associated with increased child neurodevelopment delays up to

five years of age. Thus PE may be an indicator for early screening and intervention at the neurodevelopmental level, to improve children`s long term health.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Strength: This study follows offspring of pre-eclamptic mothers from birth until age five, filling the knowledge gap regarding the first few years of life.
- Limitation: There was a considerable number of mothers and offspring that were lost to follow-up by three years postpartum, resulting in a sample size too small to provide significant results for certain measures.
- Limitation: The mild PE group was initially limited by sample size, and as a large proportion was lost to follow-up, we were unable to include this group in the analyses.
- Limitation: Some variables were not well collected (child BP), while others were added part way through the study (child waist and hip circumference), resulting in an incomplete set of data for some study participants.
- Strength: Use of the validated Ages and Stages Questionnaire permits early screening and identification of at-risk offspring, to allow for timely intervention and an overall improvement in children's long-term health.

INTRODUCTION

Hypertensive disorders are among the most common complications of pregnancy. Gestational hypertension occurs in 6-17% of pregnancies, and is defined as newly elevated blood pressure (BP) after 20 weeks' gestation in a previously normotensive individual. Preeclampsia (PE) affects 2-7% of otherwise healthy women, and is diagnosed by newly elevated BP after 20 weeks gestation, with associated proteinuria.[1] Presently, the etiology of PE is largely unknown. Many theories have been proposed, including mechanisms involving oxidative stress, angiogenic imbalance, and immunologic intolerance between fetoplacental and maternal tissue.[2] Delivery is the only definitive cure, with other treatments focused on sign and symptom management.[2] It is well known that hypertensive disorders of pregnancy are associated with adverse health outcomes including perinatal deaths, preterm birth, intrauterine growth restriction (IUGR), neonatal morbidity, and infants that are small for gestational age (SGA).[1 3]

Previous studies have demonstrated a positive link between PE and offspring Neurodevelopmental Delay (ND),[4-8] but a number of others have disputed this claim,[6 9 10] thus no conclusions can be made. The inconsistencies between studies investigating the implications of PE pregnancies on offspring indicate a need for further research. Moreover, many studies either examine immediate postnatal or neonatal complications, or follow up with mothers and offspring years down the line, resulting in a knowledge gap regarding the first few years of life. Since earlier detection allows for earlier intervention, there is a need to identify those with increased risk in early childhood.

Using the Preeclampsia New Emerging Team (PE-NET) longitudinal prospective cohort, that has previously been used to investigate maternal outcomes and cardiovascular risk factors [11-13], this study examined whether infants of women with a PE pregnancy, compared to infants of

women without a PE pregnancy, have differences in ND, and whether it changes over time. We hypothesized that infants born to women with PE-affected pregnancies will display markers indicating an increased risk of ND.

METHODS

Study Design

This longitudinal prospective cohort study compared assessments of ND in offspring born to women with/without PE yearly, from ages one to five. Neurodevelopment was assessed via the Ages and Stages Questionnaire (ASQ), the categories of which include gross motor, fine motor, communication, personal social, and problem solving.[14] The ASQ was chosen for its consistency, specificity, cost-effectiveness, and flexibility in administration. Each year parents were mailed the ASQ and asked to complete it through home observation of their child. Additionally, participants were asked to return at one, three, and five years postpartum for a clinical assessment. A study reminder was mailed every six months, and reminders by telephone or email were sent one week prior to a scheduled visit. If subjects missed appointments, they were contacted weekly for the following month before being considered as lost to follow-up.

Participants

The recruitment criteria and process have been previously described.[11] In brief, women were recruited into the Preeclampsia New Emerging Team (PE-NET) longitudinal prospective cohort at the Kingston General Hospital and Ottawa General Hospital between September 2003 and October 2009. All women diagnosed with PE (BP > 140/90 mmHg and proteinuria > 300 mg/24 hours or ≥ 1+ on repeat dipstick) at the time of presentation or admission/transfer were approached to participate. A trained research nurse explained the study and obtained consent.

After each PE subject was recruited, the next normotensive woman without prior history of PE and matched by parity (0 vs. 1 or greater), maternal age (± 5 years), and race was invited to participate. Women with a history of chronic hypertension, diabetes (including development of gestational diabetes), or renal disease were excluded from the study. [15] A total of 129 women with PE and 140 normotensive control women were enrolled in the study. Thirty-four subjects were diagnosed with mild PE, as defined above. Ninety-five subjects met the criteria for severe PE, which included the above definition and one or more of the following: systolic BP ≥ 160 mmHg, diastolic BP ≥ 110 mmHg, proteinuria ≥ 5 g in 24 hours or ≥ 3+ on dipstick, oliguria (≤ 500 ml in 24 hours), cerebral or visual disturbances, epigastric pain, thrombocytopenia (<150,000 10^9/L), increase in AST (>46U/L) and ALT (>40U/L), elevated serum creatinine (>106umol/L), pulmonary edema or cyanosis, IUGR, or eclampsia. All BPs had to be elevated on 2 measurements taken at least 6 hours apart.

The mild PE group (n = 34 at enrollment) is excluded from the current study for a number of reasons: (1) no difference was seen between the mild PE group and the controls in the comparisons done; (2) the number of mild PE patients seen in follow up was small; and (3) we chose to focus on those who experienced more severe disease.

Statistical Methods

Data collected at both time of recruitment and follow-up after one year were used to calculate maternal 30-year[16] and lifetime[17] risk estimates for CVD. For comparisons of categorical variables, the Mantel Haenszel Chi-Square test was used. The Fisher Exact method was used if cell counts were <5. For continuous variables the Mann-Whitney U test was used. A logistic regression analysis was completed for years 1, 2 and 3 of follow up to examine risk factors for ASQ failure. Variables were removed from the model step-wise based on highest p-

value until all those remaining had a p-value less than 0.05. The Pearson correlation coefficient was used to explore the relationships between maternal CVD risk estimates and child neurodevelopment. For all tests a 95% confidence level was used to determine significance. SAS v9.3 and R v2.15.2 were used for all analyses.

This study reviewed data that had been previously collected with approval from the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (OBGY-108-03).

RESULTS

Figure 1 indicates the number of subjects in each group throughout the study.

There were no significant differences in maternal characteristics at baseline, including age, height, weight, race, education level, household income, smoking status, parity, or breastfeeding status. There was a significant difference between groups regarding mode of delivery and having a previous pregnancy with PE, which is expected given the nature of the participants and eligibility criteria for the study (Table 1).

Table 1. Maternal characteristics at baseline visit.

Maternal Characteristics	**Control (n=140)**	**Severe PE (n=95)**	**P-value**
Maternal Age (years), median (IQR)	31.0 (27.75-33.25)	31.0 (28.0-34.0)	0.88
Maternal Height (cm), median (IQR)	165.0 (160.0-170.0)	163.0 (160.0-168.0)	0.13
Maternal Weight (kg), median (IQR)			
Birth	66.5 (58.0-77.0)	68.0 (61.5-82.0)	0.23
Year 1	67.5 (59.4-81.0)	71.95 (63.1-79.8)	0.19
Year 3	72.1 (63.4-90.3)	68.7 (61.9-79.8)	0.33

Maternal BMI (kg/m^2), median (IQR)			
Birth	24.2 (21.7-27.4)	25.3 (21.8-30.5)	0.09
Year 1	25.3 (22.0-29.7)	26.6 (23.0-30.5)	0.08
Year 3	26.0 (22.4-32.3)	25.6 (22.7-30.3)	0.68
Maternal race, n (%)			
White	123 (87.9)	76 (80.0)	0.14
Other	17 (12.1)	19 (20.0)	
Maternal education level, n (%)			
High school or less	9 (6.4)	14 (14.7)	0.11
Post secondary not complete	16 (11.4)	11 (11.6)	
Post secondary complete	115 (82.1)	70 (73.7)	
Household income ($), n (%)			
< 29 999	8 (5.7)	8 (8.4)	0.01
30 000 to 59 999	17 (12.1)	23 (24.2)	
60 000 to 89 999	32 (22.9)	26 (27.4)	
> 90 000	77 (55.0)	33 (34.7)	
Did not respond	6 (4.3)	5 (5.3)	
Maternal smoking, n (%)			
Yes	3 (2.1)	5 (5.3)	0.27
No	137 (97.9)	90 (94.7)	
Parity, n (%)			
Nulliparous	65 (46.4)	44 (46.3)	1.00
Multiparous	75 (53.6)	51 (53.7)	

Previous pregnancy with PE, n (%)			
Yes	0 (0.0)	10 (10.5)	<0.0001
No	140 (100.0)	85 (89.5)	
Mode of delivery, n (%)			
Vaginal	99 (70.7)	30 (31.6)	<0.0001
Caesarian	41 (29.3)	65 (68.4)	
Breastfeeding			
Total, n	98	52	
Yes, n (%)	87 (88.8)	50 (96.2)	0.22
Length of time (weeks), median (IQR)	32.0 (20.0-48.0)	28.0 (8.8-48.0)	0.63

PE - preeclampsia. BMI - Body Mass Index.

There were significant differences between the majority of infant characteristics at birth, including gestational age (GA), 5 minute Apgar score, admission level and length of stay, and presence of IUGR (Table 2).

Table 2. Infant characteristics at birth.

Infant Characteristics	**Control (n = 140)**	**Severe PE (n = 95)**	P-Value
Sex, n (%)			
Male	75 (53.6)	56 (59.0)	NS
Female	65 (46.4)	39 (41.1)	
Gestational age at birth			
Weeks, median (IQR)	39.5 (38.0-41.0)	36.0 (32.0-38.0)	<0.001
<37 weeks, n (%)	5 (0.04)	59 (0.62)	<0.001

≥37 weeks, n (%)	135 (0.96)	36 (0.38)	
Placental weight			
Grams, median (IQR)	555.0 (480.0-639.0)	413.0 (294.2-596.8)	<0.001
Magnesium Sulfate Usage, n (%)			
Yes	1 (0.7)	48 (50.5)	<0.0001
No	139 (99.3)	47 (49.5)	
If yes, usage <33 weeks gestation, n (%)	0 (0.0)	20 (41.7)	
Apgar score, median (IQR)			
1 min	8.0 (7.0-9.0)	8.0 (6.0-9.0)	<0.01
5 min	9.0 (9.0-9.0)	9.0 (8.0-9.0)	<0.001
Admission info			
Combined Care or Level 1, n (%)	127 (90.7)	36 (37.9)	<0.001
Length of stay (days), median (IQR)	2.0 (2-3)	3.0 (2-4.5)	<0.001
Level 2 or 3, n (%)	13 (9.3)	59 (62.1)	<0.001
Length of stay (days), median (IQR)	4.5 (3-5.5)	15.5 (6.3-32.8)	<0.01
Transferred before discharge, n (%)	0 (0)	9 (9)	
Intrauterine Growth Restriction, n (%)	0 (0.0)	25 (26.3)	<0.001

PE - preeclampsia.

Figure 2 compares both the proportion and number of ASQ categories failed at each year of follow-up between the severe PE and control groups. A significant difference was found in the proportion of categories failed in year 3 ($P<0.05$), and this approached significance in years 1 and 4 ($P<0.10$ and $P<0.15$, respectively). Although a significant difference was not found in year

2, the trend is clearly present. Comparison of the distribution of the number of categories failed, among those who failed at least one category, indicated that severe PE children tended to fail more categories than controls at year 1 ($P<0.10$).

A subgroup analysis was performed looking at only PE patients, categorized by preterm (<37 weeks) and term (≥37 weeks). It was found that GA appears to significantly contribute to the relationship between PE and failure of ASQ categories (Figure 3). A significant proportion of PE children born preterm failed the ASQ in years 3 and 4 ($P<0.05$). Additionally, it was found that when failed, those who were preterm tended to fail more categories. This was significant at years 3 and 4 ($P<0.05$) and approached significance at years 2 and 5 ($P<0.10$).

A logistic regression analysis examining risk factors for ASQ failure was performed for years 1,2 and 3 of follow up, considering the variables severe PE, GA, IUGR, $MgSO_4$ usage, maternal smoking, socioeconomic status (a combination of income, maternal, and paternal education), sex, parity (multiparous or nulliparous) and breastfeeding (did not breastfeed, breastfed <6 months, and breastfed ≥6 months) (Table 3). At year 1, sex, IUGR and $MgSO_4$ usage were retained in the model. Males had a greater risk of ASQ failure than females with an odds ratio of 2.64 (95%CI 1.08, 6.45). The diagnosis of IUGR and $MgSO_4$ usage were also significant risk factors with odds ratios of 3.40 (95%CI 1.00, 11.49) and 3.13 (95%CI 1.26, 7.74), respectively. At year 2, sex and GA were retained in the model. Males had a greater risk of ASQ failure than females with an odds ratio of 3.38 (95%CI 1.40, 8.19), while increasing GA was found to be protective against failure with an odds ratio of 0.87 (95%CI 0.78, 0.97). Finally, at year 3 severe PE and parity (multiparous vs. nulliparous) were retained in the model. Both were found to be risk factors with respective odds ratios of 3.47 (95%CI 1.22, 9.91) and 3.09 (95%CI 1.07, 8.91). However, when controlling for confounding variables including GA,

$MgSO_4$, parity, socioeconomic status, and smoking, the odds ratio estimate for severe PE at year 3 was 2.03 (95% CI 0.48, 8.54), which was not significant.

Table 3. Logistic regression analysis of ASQ failures at year 1, 2, and 3 of follow up.

Variable	OR (95% CI) Year 1 (n=197)	OR (95% CI) Year 2 (n=170)	OR (95% CI) Year 3 (n=99)
Sex (Male vs. Female)	2.64 (1.08, 6.45)	3.38 (1.40, 8.19)	-
IUGR (Yes vs. No)	3.40 (1.00, 11.49)	-	-
$MgSO_4$ (Yes vs. No)	3.13 (1.26, 7.74)	-	-
Gestational Age (Weeks)	-	0.87 (0.78, 0.97)	-
Severe PE vs. Normotensive	-	-	3.47 (1.22, 9.91)
Parity (Multi vs. Nulliparous)	-	-	3.09 (1.07, 8.91)

PE - Preeclampsia; IUGR - Intrauterine Growth Restriction; $MgSO_4$ – magnesium sulphate.

Infants born to PE mothers tended to fail more often in the gross motor ASQ category. This was significant in year 2 (46.15% vs. 5.26%, P = 0.01), and trended toward significance in years 1 (42.86% vs. 23.08%, P = 0.32) and 3 (21.43% vs. 0%, P = 0.24). A larger sample size is needed to confirm this trend.

Significant correlations were noted between maternal lifetime risk score and number of ASQ categories failed at years 1 (r = 0.20, $P = 0.008$) and 3 (r = 0.23, $P = 0.026$). Ordinal regression was explored, but was not possible because the proportional odds assumption did not hold true, and the sample size was not large enough to make proper adjustments.

DISCUSSION

Infants born to mothers with severe PE were more likely to have failed in at least one ASQ category (Figure 2), indicating ND, up to and including 3 years. This is in keeping with Ehrenstein et al.,[18] who found a slightly reduced cognitive performance in adult males exposed to gestational hypertensive disorders. Additionally, Sorensen et al.,[7] revealed that maternal hypertension was an independent risk factor for the development of schizophrenia in offspring later in life, and Tuovinen at al.[19] showed that hypertensive disorders in pregnancy are associated with lower intellectual abilities in twenty-year-old male offspring in a subgroup of the Helsinki Birth Cohort. However, a further investigation of this cohort revealed that it was hypertension without proteinuria that was associated with an increased risk of serious mental disorders requiring hospitalization or contributing to death, while preeclampsia was actually associated with a lower risk.[9] These discrepancies between findings indicate that further studies are needed to better understand this association.

Table 3 suggests that IUGR and MgSO4 are both significant contributors in the first year of life, and more advanced GA is protective in year 2, all of these variables may be considered markers of severe PE. Infants with IUGR are more likely to have significant placental issues often seen with severe PE, MgSO4 is the standard treatment for severe elevations in blood pressure due to PE, and earlier delivery is required when PE is severe and can no longer be medically managed. Severe PE itself was found to be significant in year 3, but when controlling for confounding variables it was no longer significant (odds ratio 2.03; 95% CI 0.48-8.54), likely due to the small sample size (n=99). While previous studies have indicated that GA and IUGR are the primary risk factors in this population,[20] others have shown that among growth-restricted infants, those born to mothers with PE have lower IQ scores than those without PE-complicated

pregnancies, indicating that PE itself is also a contributor.[4] Once again, larger studies are needed to tease out this multifactorial relationship.

Interestingly, our data indicated a slightly decreased gross motor performance compared to the other categories measured. This is in contrast to Whitehouse et al.,[8] who found that gestational hypertension and preeclampsia reduced verbal ability in offspring, but non-verbal performance was unaffected. As with previously discussed findings, further studies are needed to tease out the true nature of developmental deficits experienced in this population

Lastly, there was a significant positive correlation between maternal lifetime CVD risk score and number of ASQ categories failed at years 1 and 3 ($P<0.05$). Likewise, Krakowiak et al.,[21] revealed that children aged 2-5 years exposed to metabolic conditions in pregnancy (diabetes, hypertension, or obesity) scored lower on neurodevelopmental assessments. These persistent neurodevelopmental delays indicate a need for early childhood interventions, to ensure efforts are made to reduce their persistence into school age.

There are a number of limitations to the study that must be addressed. The considerable number of mothers and offspring that were lost to follow-up by three years postpartum (Figure 1) resulted in a sample size too small to provide significant results for certain measures. Based on the failure rates observed at each year, we would need a sample size of 172 severe PE and 172 controls at year 1, 359 severe PE and 359 controls at year 2, and 96 severe PE and 96 controls at year 3, to reach a desired power of 80%.[22] Additionally, the group lost to follow-up by three years postpartum contained a significant number of mild PE subjects, and along with the small amount of subjects in this group to begin with, we were unable to include this group in the analyses. Future studies should include this subgroup, and we would expect the effects found to be lesser than what was observed in the severe PE group. Furthermore, only ~6% of control

mothers were at high risk for cardiometabolic disease,[13] which must be considered as the issue driving the PE. Lastly, some variables were not well collected (child BP), while others were added part way through the study (child waist and hip circumference), resulting in an incomplete set of data for some study participants.

Pregnancy is a useful way to identify women at risk for CVD.[11-13] Our findings indicate that it may also allow us to identify offspring at risk from a neurodevelopmental perspective. This provides a unique opportunity to use maternal health complications to improve whole family outcomes. By identifying these women at time of delivery, early screening and follow-up of offspring can help ensure that those individuals at risk are identified in a timelier manner. This will allow for earlier intervention and an overall improvement in children's long-term health.

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COMPETING INTERESTS

The authors report no conflict of interest.

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DATA SHARING

Extra data is available by emailing Dr. Graeme Smith.

CONTRIBUTORSHIP

MW, GNS and SWW designed the study. GNS, MW, and SWW contributed to acquisition of data. CW and JP completed analysis and interpretation of data. All authors assisted in drafting and/or critically reviewing the manuscript. All authors have approved the final version and agree to be accountable for all aspects of the work.

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LEGENDS

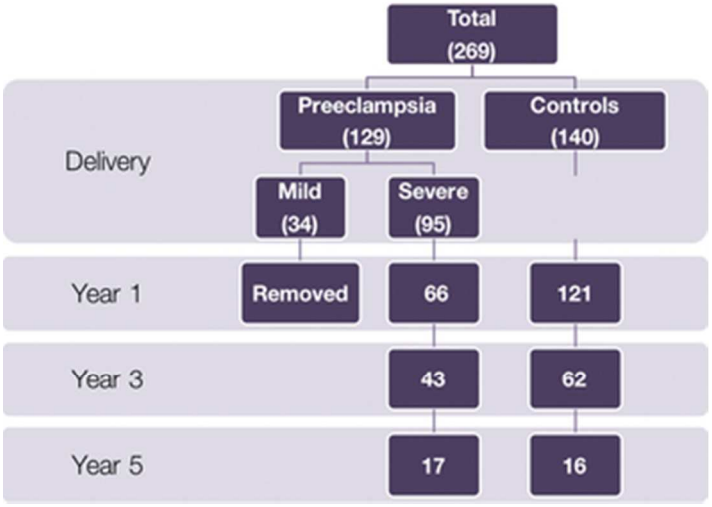
Figure 1. Flow diagram of study subjects at years 1-5.

Figure 2. Comparison of Ages and Stages Questionnaire failures between the severe preeclampsia and control groups at years 1 through 5 of follow up.

★ <0.05, + <0.10, ■ <0.15, p-value based on Mantel-Haenszel Chi Square. Fisher Exact used when cell counts <5. Comparison of the proportion of failures in the severe preeclampsia group to the control group at each year of follow up. ★ <0.05, + <0.10, ■ <0.15, p-value based on Wilcoxon Rank-Sum Test. Comparison of the distribution of the number of categories failed, among those participants who failed at least one category, between the severe preeclampsia group and the control group at each year of follow up. PE- preeclampsia.

Figure 3. Comparison of Ages and Stages Questionnaire failures between the preeclampsia & preterm and preeclampsia & term groups at years 1 through 5 of follow up.

★ <0.05, + <0.10, ■ <0.15, p-value based on Mantel-Haenszel Chi Square. Fisher Exact used when cell counts <5. Comparison of the proportion of failures in the <37 weeks to the ≥37 weeks gestational age group at each year of follow up. ★ <0.05, + <0.10, ■ <0.15, p-value based on Wilcoxon Rank-Sum Test. Comparison of the distribution of the number of categories failed, among those participants who failed at least one category, between the <37 weeks and the ≥37 weeks gestational age group at each year of follow up. PE- preeclampsia; Wks - weeks; GA - gestational age.



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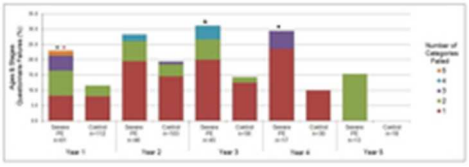

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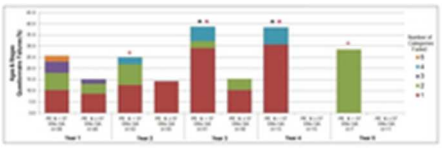

18x6mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Page No	Recommendation
Title and abstract	1	1-3	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	4	Explain the scientific background and rationale for the investigation being reported
Objectives	3	5	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	5	Present key elements of study design early in the paper
Setting	5	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	5-6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	5-6	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*		For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	6	Describe any efforts to address potential sources of bias
Study size	10	6	Explain how the study size was arrived at
Quantitative variables	11	6-7	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	6-7	(*a*) Describe all statistical methods, including those used to control for confounding
			(*b*) Describe any methods used to examine subgroups and interactions
			(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Continued on next page

			Results
Participants	13 *	7	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
			(b) Give reasons for non-participation at each stage
			(c) Consider use of a flow diagram
Descriptive data	14 *	7	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
			(b) Indicate number of participants with missing data for each variable of interest
			(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15 *	9	*Cohort study*—Report numbers of outcome events or summary measures over time
			Case-control study—Report numbers in each exposure category, or summary measures of exposure
			Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	9	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
			(*b*) Report category boundaries when continuous variables were categorized
			(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	10-12	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
			Discussion
Key results	18	13-14	Summarise key results with reference to study objectives
Limitations	19	14-15	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	15	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	15	Discuss the generalisability (external validity) of the study results
			Other information
Funding	22	15	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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A prospective assessment of neurodevelopment in children following a pregnancy complicated by severe preeclampsia

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TITLE PAGE

Title: A prospective assessment of neurodevelopment in children following a pregnancy complicated by severe preeclampsia

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Keywords: preeclampsia, pregnancy, neurodevelopment, neonatology, preventive medicine

Word Count: 3, 094

ABSTRACT

Objective: To prospectively examine whether children of women with a pregnancy affected by severe preeclampsia (PE), compared to children of women without a PE-affected pregnancy, have differences in neurodevelopmental performance up to five years of age.

Design: Prospective cohort study.

Setting: Tertiary care centre.

Participants: Women were recruited into the study following a PE-affected pregnancy. After each PE subject was recruited, the next normotensive woman without prior history of PE and matched by parity, maternal age, and race was invited to participate. Women with a history of chronic hypertension, diabetes, or renal disease were excluded. A total of 129 PE-affected and 140 normotensive mothers were enrolled.

Outcome Measures: The primary outcome measure was failure of the Ages and Stages Questionnaire (ASQ). The ASQ was completed yearly, until age five.

Results: A significant difference was found in the proportion of ASQ categories failed in year 3 ($P<0.05$), and this approached significance in years 1 and 4 ($P<0.10$ and $P<0.15$, respectively). At year 1 the number of ASQ categories failed was significantly greater among children born to PE mothers. A subgroup analysis revealed a significant proportion of PE children born preterm (<37 weeks) failed the ASQ in years 3 and 4 ($P<0.05$), and when failed, those who were preterm failed significantly more categories in years 3 and 4 ($P<0.05$). A trend toward increased failure in the gross motor category was found. There was a significant positive correlation between maternal lifetime CVD risk score and the number of ASQ categories failed at years 1 and 3 ($P<0.05$).

Conclusion: PE was found to be associated with increased child neurodevelopment delays up to

five years of age. Thus PE may be an indicator for early screening and intervention at the neurodevelopmental level, to improve children`s long term health.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Strength: This study follows offspring of pre-eclamptic mothers from birth until age five, filling the knowledge gap regarding the first few years of life.
- Limitation: There was a considerable number of mothers and offspring that were lost to follow-up by three years postpartum, resulting in a sample size too small to provide significant results for certain measures.
- Limitation: Some variables were not well collected (child BP), while others were added part way through the study (child waist and hip circumference), resulting in an incomplete set of data for some study participants.
- Strength: Use of the validated Ages and Stages Questionnaire permits early screening and identification of at-risk offspring, to allow for timely intervention and an overall improvement in children's long-term health.

INTRODUCTION

Hypertensive disorders are among the most common complications of pregnancy. Gestational hypertension occurs in 6-17% of pregnancies, and is defined as newly elevated blood pressure (BP) after 20 weeks' gestation in a previously normotensive individual. Preeclampsia (PE) affects 2-7% of otherwise healthy women, and is diagnosed by newly elevated BP after 20 weeks gestation, with associated proteinuria.[1] Presently, the etiology of PE is largely unknown. Many theories have been proposed, including mechanisms involving oxidative stress, angiogenic imbalance, and immunologic intolerance between fetoplacental and maternal tissue.[2] Delivery is the only definitive cure, with other treatments focused on sign and symptom management.[2] It is well known that hypertensive disorders of pregnancy are associated with adverse health outcomes including perinatal deaths, preterm birth, intrauterine growth restriction (IUGR), neonatal morbidity, and infants that are small for gestational age (SGA).[1 3]

Previous studies have demonstrated a positive link between PE and offspring Neurodevelopmental Delay (ND),[4-8] but a number of others have disputed this claim,[6 9 10] thus no conclusions can be made. The inconsistencies between studies investigating the implications of PE pregnancies on offspring indicate a need for further research. Moreover, many studies either examine immediate postnatal or neonatal complications, or follow up with mothers and offspring years down the line, resulting in a knowledge gap regarding the first few years of life. Since earlier detection allows for earlier intervention, there is a need to identify those with increased risk in early childhood.

Using the Preeclampsia New Emerging Team (PE-NET) longitudinal prospective cohort, that has previously been used to investigate maternal outcomes and cardiovascular risk factors [11-13], this study examined whether infants of women with a PE pregnancy, compared to infants of

women without a PE pregnancy, have differences in ND, and whether it changes over time. We hypothesized that infants born to women with PE-affected pregnancies will display markers indicating an increased risk of ND.

METHODS

Study Design

This longitudinal prospective cohort study compared assessments of ND in offspring born to women with/without PE yearly, from ages one to five. Neurodevelopment was assessed via the Ages and Stages Questionnaire (ASQ), the categories of which include gross motor, fine motor, communication, personal social, and problem solving.[14] The ASQ was chosen for its consistency, specificity, cost-effectiveness, and flexibility in administration. Each year parents were mailed the ASQ and asked to complete it through home observation of their child. Additionally, participants were asked to return at one, three, and five years postpartum for a clinical assessment. A study reminder was mailed every six months, and reminders by telephone or email were sent one week prior to a scheduled visit. If subjects missed appointments, they were contacted weekly for the following month before being considered as lost to follow-up.

Participants

The recruitment criteria and process have been previously described.[11] In brief, women were recruited into the Preeclampsia New Emerging Team (PE-NET) longitudinal prospective cohort at the Kingston General Hospital and Ottawa General Hospital between September 2003 and October 2009. All women diagnosed with PE (BP > 140/90 mmHg and proteinuria > 300 mg/24 hours or ≥ 1+ on repeat dipstick) at the time of presentation or admission/transfer were approached to participate. A trained research nurse explained the study and obtained consent.

After each PE subject was recruited, the next normotensive woman without prior history of PE and matched by parity (0 vs. 1 or greater), maternal age (± 5 years), and race was invited to participate. Women with a history of chronic hypertension, diabetes (including development of gestational diabetes), or renal disease were excluded from the study. [15] A total of 129 women with PE and 140 normotensive control women were enrolled in the study. Thirty-four subjects were diagnosed with mild PE, as defined above. Ninety-five subjects met the criteria for severe PE, which included the above definition and one or more of the following: systolic BP ≥ 160 mmHg, diastolic BP ≥ 110 mmHg, proteinuria ≥ 5 g in 24 hours or ≥ 3+ on dipstick, oliguria (≤ 500 ml in 24 hours), cerebral or visual disturbances, epigastric pain, thrombocytopenia (<150,000 10^9/L), increase in AST (>46U/L) and ALT (>40U/L), elevated serum creatinine (>106umol/L), pulmonary edema or cyanosis, IUGR, or eclampsia. All BPs had to be elevated on 2 measurements taken at least 6 hours apart.

The mild PE group (n = 34 at enrollment) is excluded from the current study for a number of reasons: (1) no difference was seen between the mild PE group and the controls in the comparisons done; (2) the number of mild PE patients seen in follow up was small; and (3) we chose to focus on those who experienced more severe disease.

Statistical Methods

Data collected at both time of recruitment and follow-up after one year were used to calculate maternal 30-year[16] and lifetime[17] risk estimates for CVD. For comparisons of categorical variables, the Mantel Haenszel Chi-Square test was used. The Fisher Exact method was used if cell counts were <5. For continuous variables the Mann-Whitney U test was used. A logistic regression analysis was completed for years 1, 2 and 3 of follow up to examine risk factors for ASQ failure. Variables were removed from the model step-wise based on highest p-

value until all those remaining had a p-value less than 0.1. PE, IUGR, and GA were forced into the final model, regardless of p-value, to control for their effects. The Pearson correlation coefficient was used to explore the relationships between maternal CVD risk estimates and child neurodevelopment. For all tests a 95% confidence level was used to determine significance. SAS v9.3 and R v2.15.2 were used for all analyses.

This study reviewed data that had been previously collected with approval from the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (OBGY-108-03).

RESULTS

Figure 1 indicates the number of subjects in each group throughout the study.

There were no significant differences in maternal characteristics at baseline, including age, height, weight, race, education level, household income, smoking status, parity, or breastfeeding status. There was a significant difference between groups regarding mode of delivery and having a previous pregnancy with PE, which is expected given the nature of the participants and eligibility criteria for the study (Table 1).

Table 1. Maternal characteristics at baseline visit.

Maternal Characteristics	**Control (n=140)**	**Severe PE (n=95)**	**P-value**
Maternal Age (years), median (IQR)	31.0 (27.75-33.25)	31.0 (28.0-34.0)	0.88
Maternal Height (cm), median (IQR)	165.0 (160.0-170.0)	163.0 (160.0-168.0)	0.13
Maternal Weight (kg), median (IQR)			
Birth	66.5 (58.0-77.0)	68.0 (61.5-82.0)	0.23
Year 1	67.5 (59.4-81.0)	71.95 (63.1-79.8)	0.19

Year 3	72.1 (63.4-90.3)	68.7 (61.9-79.8)	0.33
Maternal BMI (kg/m^2), median (IQR)			
Birth	24.2 (21.7-27.4)	25.3 (21.8-30.5)	0.09
Year 1	25.3 (22.0-29.7)	26.6 (23.0-30.5)	0.08
Year 3	26.0 (22.4-32.3)	25.6 (22.7-30.3)	0.68
Maternal race, n (%)			
White	123 (87.9)	76 (80.0)	0.14
Other	17 (12.1)	19 (20.0)	
Maternal education level, n (%)			
High school or less	9 (6.4)	14 (14.7)	0.11
Post secondary not complete	16 (11.4)	11 (11.6)	
Post secondary complete	115 (82.1)	70 (73.7)	
Household income ($), n (%)			
< 29 999	8 (5.7)	8 (8.4)	0.01
30 000 to 59 999	17 (12.1)	23 (24.2)	
60 000 to 89 999	32 (22.9)	26 (27.4)	
> 90 000	77 (55.0)	33 (34.7)	
Did not respond	6 (4.3)	5 (5.3)	
Maternal smoking, n (%)			
Yes	3 (2.1)	5 (5.3)	0.27
No	137 (97.9)	90 (94.7)	
Parity, n (%)			
Nulliparous	65 (46.4)	44 (46.3)	1.00

Multiparous	75 (53.6)	51 (53.7)	
Previous pregnancy with PE, n (%)			
Yes	0 (0.0)	10 (10.5)	<0.0001
No	140 (100.0)	85 (89.5)	
Mode of delivery, n (%)			
Vaginal	99 (70.7)	30 (31.6)	<0.0001
Caesarian	41 (29.3)	65 (68.4)	
Breastfeeding			
Total, n	98	52	
Yes, n (%)	87 (88.8)	50 (96.2)	0.22
Length of time (weeks), median (IQR)	32.0 (20.0-48.0)	28.0 (8.8-48.0)	0.63

PE - preeclampsia. BMI - Body Mass Index.

There were significant differences between the majority of infant characteristics at birth, including gestational age (GA), 5 minute Apgar score, admission level and length of stay, and presence of IUGR (Table 2).

Table 2. Infant characteristics at birth.

Infant Characteristics	**Control (n = 140)**	**Severe PE (n = 95)**	P-Value
Sex, n (%)			
Male	75 (53.6)	56 (59.0)	NS
Female	65 (46.4)	39 (41.1)	
Gestational age at birth			
Weeks, median (IQR)	39.5 (38.0-41.0)	36.0 (32.0-38.0)	<0.001

<37 weeks, n (%)	5 (0.04)	59 (0.62)	<0.001
≥37 weeks, n (%)	135 (0.96)	36 (0.38)	
Placental weight			
Grams, median (IQR)	555.0 (480.0-639.0)	413.0 (294.2-596.8)	<0.001
Magnesium Sulfate Usage, n (%)			
Yes	1 (0.7)	48 (50.5)	<0.0001
No	139 (99.3)	47 (49.5)	
If yes, usage <33 weeks gestation, n (%)	0 (0.0)	20 (41.7)	
Apgar score, median (IQR)			
1 min	8.0 (7.0-9.0)	8.0 (6.0-9.0)	<0.01
5 min	9.0 (9.0-9.0)	9.0 (8.0-9.0)	<0.001
Admission info			
Combined Care or Level 1, n (%)	127 (90.7)	36 (37.9)	<0.001
Length of stay (days), median (IQR)	2.0 (2-3)	3.0 (2-4.5)	<0.001
Level 2 or 3, n (%)	13 (9.3)	59 (62.1)	<0.001
Length of stay (days), median (IQR)	4.5 (3-5.5)	15.5 (6.3-32.8)	<0.01
Transferred before discharge, n (%)	0 (0)	9 (9)	
Intrauterine Growth Restriction, n (%)	0 (0.0)	25 (26.3)	<0.001

PE - preeclampsia.

Figure 2 compares both the proportion and number of ASQ categories failed at each year of follow-up between the severe PE and control groups. A significant difference was found in the proportion of categories failed in year 3 (P<0.05), and this approached significance in years 1

and 4 (P<0.10 and P<0.15, respectively). Although a significant difference was not found in year 2, the trend is clearly present. Comparison of the distribution of the number of categories failed, among those who failed at least one category, indicated that severe PE children tended to fail more categories than controls at year 1 (P<0.10).

A subgroup analysis was performed looking at only PE patients, categorized by preterm (<37 weeks) and term (≥37 weeks). It was found that GA appears to significantly contribute to the relationship between PE and failure of ASQ categories (Figure 3). A significant proportion of PE children born preterm failed the ASQ in years 3 and 4 (P<0.05). Additionally, it was found that when failed, those who were preterm tended to fail more categories. This was significant at years 3 and 4 (P<0.05) and approached significance at years 2 and 5 (P<0.10).

A logistic regression analysis examining risk factors for ASQ failure was performed for years 1,2 and 3 of follow up, considering the variables $MgSO_4$ usage, maternal smoking, socioeconomic status (a combination of income, maternal, and paternal education), sex, parity (multiparous or nulliparous) and breastfeeding (did not breastfeed, breastfed <6 months, and breastfed ≥6 months) (Table 3). As well, severe PE, GA, and IUGR were forced into the model regardless of p-value, due to their well-known known effects. Male sex had a greater risk of ASQ failure than females with in odds ratio of 2.31 (95%CI 0.88, 6.05) at year 1 and 2.72 (95%CI 1.11, 6.70) at year 2. This relationship was not significant by year 3. $MgSO_4$ usage was retained in the model at year 1 only, with an odds ratio of 2.69 (95%CI 0.73, 9.99). The diagnosis of IUGR increased the risk of ASQ failures in year 1, 2, and 3, with odds ratios of 2.22 (95%CI 0.53, 9.22), 1.63 (95%CI 0.30, 8.85), and 3.96 (0.71, 21.93), respectively. Increasing gestational age was protective against ASQ failure with odds ratios of 0.96 (95%CI 0.83, 1.10), 0.84 (95%CI 0.73, 0.98), and 0.94 (95%CI 0.79, 1.11) at years 1, 2, and 3, respectively. Interestingly,

severe PE appeared to be protective in the first two years, while it increased the risk of ASQ failures in year 3 with an odds ratio of 2.31 (95%CI 0.63, 8.53). As well, multiparity was a risk factor at year 3 with odds ratio 2.74 (95%CI 0.92, 8.17).

Table 3. Logistic regression analysis of ASQ failures at year 1, 2, and 3 of follow up

Variable	**Year 1 (n=197)**	**Year 2 (n=170)**	**Year 3 (n=99)**
	OR (95% CI)	*OR (95% CI)*	*OR (95% CI)*
Sex (Male vs. Female)	2.31 (0.88, 6.05)	2.72 (1.11, 6.70)	-
IUGR (Yes vs. No)	2.22 (0.53, 9.22)	1.63 (0.30, 8.85)	3.96 (0.71, 21.93)
$MgSO_4$ (Yes vs. No)	2.69 (0.73, 9.99)	-	-
Gestational Age (Weeks)	0.96 (0.83, 1.10)	0.84 (0.73, 0.98)	0.94 (0.79, 1.11)
Severe PE vs. Normotensive	0.90 (0.24, 3.34)	0.63 (0.19, 2.09)	2.31 (0.63, 8.53)
Parity (Multi vs. Nulliparous)	-	-	2.74 (0.92, 8.17)

IUGR – Intrauterine Growth Restriction; $MgSO_4$ – magnesium sulphate; PE – Preeclampsia.

Infants born to PE mothers tended to fail more often in the gross motor ASQ category. This was significant in year 2 (46.15% vs. 5.26%, P = 0.01), and trended toward significance in years 1 (42.86% vs. 23.08%, P = 0.32) and 3 (21.43% vs. 0%, P = 0.24). A larger sample size is needed to confirm this trend.

Significant correlations were noted between maternal lifetime risk score and number of ASQ categories failed at years 1 (r = 0.20, *P* = 0.008) and 3 (r = 0.23, *P* = 0.026). Ordinal regression was explored, but was not possible because the proportional odds assumption did not hold true, and the sample size was not large enough to make proper adjustments.

DISCUSSION

Infants born to mothers with severe PE were more likely to have failed in at least one ASQ category (Figure 2), indicating ND, up to and including 3 years. This is in keeping with Ehrenstein et al.,[18] who found a slightly reduced cognitive performance in adult males exposed to gestational hypertensive disorders. Additionally, Sorensen et al.,[7] revealed that maternal hypertension was an independent risk factor for the development of schizophrenia in offspring later in life, and Tuovinen at al.[19] showed that hypertensive disorders in pregnancy are associated with lower intellectual abilities in twenty-year-old male offspring in a subgroup of the Helsinki Birth Cohort. However, a further investigation of this cohort revealed that it was hypertension without proteinuria that was associated with an increased risk of serious mental disorders requiring hospitalization or contributing to death, while preeclampsia was actually associated with a lower risk.[9] These discrepancies between findings indicate that further studies are needed to better understand this association.

Table 3 suggests that IUGR and earlier GA are contributors to ASQ failure in years 1-3, with $MgSO_4$ usage also impacting this finding in year 1, but all of these variables may be considered markers of severe PE. Infants with IUGR are more likely to have significant placental issues often seen with severe PE, $MgSO_4$ is the standard treatment for severe elevations in blood pressure due to PE, and earlier delivery is required when PE is severe and can no longer be medically managed. Severe PE itself was trending toward significant in year 3 (odds ratio 2.31; 95%CI 0.63-8.53) but did not reach it, likely due to the small sample size (n=99). While previous studies have indicated that earlier GA and IUGR are the primary risk factors in this population,[20] others have shown that among growth-restricted infants, those born to mothers with PE have lower IQ scores than those without PE-complicated pregnancies, indicating that PE itself is also a is a major contributor.[4] Follow up studies conducted on the PE-NET cohort also support the

effects of severe PE on cognitive ability. Ratsep et al.[21], found impairment in both working memory in the offspring of PE mothers, based on psychometric testing, as well as visuospatial processing. A smaller cohort of subjects was followed up with brain magnetic resonance imaging at a mean age of 9.66 years for PE offspring and 9.79 years for controls. This study revealed a number of structural and vascular anatomic changes in the brains of PE offspring that shared similarities with alterations found in autism.[22] The deficits in higher level cognitive functioning reveal that the increased risk seen with severe PE in year 3 is likely the beginning of a trend, but larger studies with longer follow-up are needed to further define this relationship.

Interestingly, our data indicated a slightly decreased gross motor performance compared to the other categories measured. This is in contrast to Whitehouse et al.,[8] who found that gestational hypertension and preeclampsia reduced verbal ability in offspring, but non-verbal performance was unaffected. As with previously discussed findings, further studies are needed to tease out the true nature of developmental deficits experienced in this population

Lastly, there was a significant positive correlation between maternal lifetime CVD risk score and number of ASQ categories failed at years 1 and 3 ($P<0.05$). Likewise, Krakowiak et al.,[23] revealed that children aged 2-5 years exposed to metabolic conditions in pregnancy (diabetes, hypertension, or obesity) scored lower on neurodevelopmental assessments. These persistent neurodevelopmental delays indicate a need for early childhood interventions, to ensure efforts are made to reduce their persistence into school age.

There are a number of limitations to the study that must be addressed. The considerable number of mothers and offspring that were lost to follow-up by three years postpartum (Figure 1) resulted in a sample size too small to provide significant results for certain measures. Based on the failure rates observed at each year, we would need a sample size of 172 severe PE and 172

controls at year 1, 359 severe PE and 359 controls at year 2, and 96 severe PE and 96 controls at year 3, to reach a desired power of 80%.[24] Additionally, the group lost to follow-up by three years postpartum contained a significant number of mild PE subjects, and along with the small amount of subjects in this group to begin with, we were unable to include this group in the analyses. Future studies should include this subgroup, and we would expect the effects found to be lesser than what was observed in the severe PE group. Furthermore, only ~6% of control mothers were at high risk for cardiometabolic disease,[13] which must be considered as the issue driving the PE. Lastly, some variables were not well collected (child BP), while others were added part way through the study (child waist and hip circumference), resulting in an incomplete set of data for some study participants.

Pregnancy is a useful way to identify women at risk for CVD.[11-13] Our findings indicate that it may also allow us to identify offspring at risk from a neurodevelopmental perspective. This provides a unique opportunity to use maternal health complications to improve whole family outcomes. By identifying these women at time of delivery, early screening and follow-up of offspring can help ensure that those individuals at risk are identified in a timelier manner. This will allow for earlier intervention and an overall improvement in children's long-term health.

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COMPETING INTERESTS

The authors report no conflict of interest.

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DATA SHARING

Extra data is available by emailing Dr. Graeme Smith.

CONTRIBUTORSHIP

MW, GNS and SWW designed the study. GNS, MW, and SWW contributed to acquisition of data. CW and JP completed analysis and interpretation of data. All authors assisted in drafting and/or critically reviewing the manuscript. All authors have approved the final version and agree to be accountable for all aspects of the work.

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LEGENDS

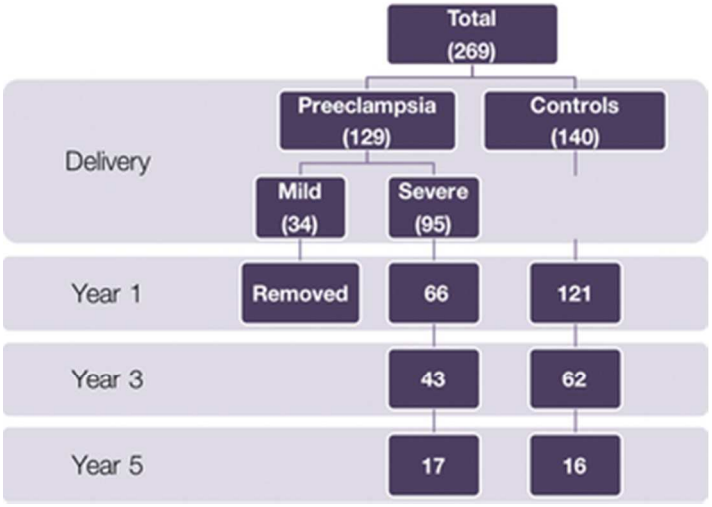
Figure 1. Flow diagram of study subjects at years 1-5.

Figure 2. Comparison of Ages and Stages Questionnaire failures between the severe preeclampsia and control groups at years 1 through 5 of follow up.

★ <0.05, + <0.10, ■ <0.15, p-value based on Mantel-Haenszel Chi Square. Fisher Exact used when cell counts <5. Comparison of the proportion of failures in the severe preeclampsia group to the control group at each year of follow up. ★ <0.05, + <0.10, ■ <0.15, p-value based on Wilcoxon Rank-Sum Test. Comparison of the distribution of the number of categories failed, among those participants who failed at least one category, between the severe preeclampsia group and the control group at each year of follow up. PE- preeclampsia.

Figure 3. Comparison of Ages and Stages Questionnaire failures between the preeclampsia & preterm and preeclampsia & term groups at years 1 through 5 of follow up.

★ <0.05, + <0.10, ■ <0.15, p-value based on Mantel-Haenszel Chi Square. Fisher Exact used when cell counts <5. Comparison of the proportion of failures in the <37 weeks to the ≥37 weeks gestational age group at each year of follow up. ★ <0.05, + <0.10, ■ <0.15, p-value based on Wilcoxon Rank-Sum Test. Comparison of the distribution of the number of categories failed, among those participants who failed at least one category, between the <37 weeks and the ≥37 weeks gestational age group at each year of follow up. PE- preeclampsia; Wks - weeks; GA - gestational age.



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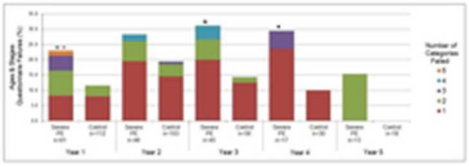

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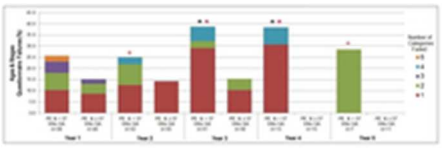

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Page No	Recommendation
Title and abstract	1	1-3	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	4	Explain the scientific background and rationale for the investigation being reported
Objectives	3	5	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	5	Present key elements of study design early in the paper
Setting	5	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	5-6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	5-6	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*		For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	6	Describe any efforts to address potential sources of bias
Study size	10	6	Explain how the study size was arrived at
Quantitative variables	11	6-7	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	6-7	(*a*) Describe all statistical methods, including those used to control for confounding
			(*b*) Describe any methods used to examine subgroups and interactions
			(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Continued on next page

Results			
Participants	13 *	7	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
			(b) Give reasons for non-participation at each stage
			(c) Consider use of a flow diagram
Descriptive data	14 *	7	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
			(b) Indicate number of participants with missing data for each variable of interest
			(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15 *	9	*Cohort study*—Report numbers of outcome events or summary measures over time
			Case-control study—Report numbers in each exposure category, or summary measures of exposure
			Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	9	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
			(*b*) Report category boundaries when continuous variables were categorized
			(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	10-12	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	13-14	Summarise key results with reference to study objectives
Limitations	19	14-15	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	15	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	15	Discuss the generalisability (external validity) of the study results
Other information			
Funding	22	15	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

A prospective assessment of neurodevelopment in children following a pregnancy complicated by severe preeclampsia

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TITLE PAGE

Title: A prospective assessment of neurodevelopment in children following a pregnancy complicated by severe preeclampsia

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Keywords: preeclampsia, pregnancy, neurodevelopment, neonatology, preventive medicine

Word Count: 3, 094

ABSTRACT

Objective: To prospectively examine whether children of women with a pregnancy affected by severe preeclampsia (PE), compared to children of women without a PE-affected pregnancy, have differences in neurodevelopmental performance up to five years of age.

Design: Prospective cohort study.

Setting: Tertiary care centre.

Participants: Women were recruited following a PE-affected pregnancy. After each PE subject was recruited, the next normotensive woman without prior history of PE and matched by parity, maternal age, and race was invited to participate. Women with a history of chronic hypertension, diabetes, or renal disease were excluded. Total enrollment included 129 PE-affected and 140 normotensive mothers.

Outcome Measures: The primary outcome measure was failure of the Ages and Stages Questionnaire (ASQ). The ASQ was completed yearly, until age five.

Results: A significant difference was found in the proportion of ASQ categories failed in year 3 ($P<0.05$), and this approached significance in years 1 and 4 ($P<0.10$ and $P<0.15$, respectively). At year 1 the number of ASQ categories failed was significantly greater among children born to PE mothers. A subgroup analysis revealed a significant proportion of PE children born preterm (<37 weeks) failed the ASQ in years 3 and 4 ($P<0.05$), and when failed, those who were preterm failed significantly more categories ($P<0.05$). A trend toward increased failure in the gross motor category was found. There was a significant positive correlation between maternal lifetime CVD risk score and number of ASQ categories failed at years 1 and 3 ($P<0.05$).

Conclusion: Severe PE is associated with other adverse pregnancy outcomes, including IUGR and preterm birth, all of which are associated with increased neurodevelopment delays. Thus PE

indicates a need for early screening and intervention at the neurodevelopmental level to improve children`s long term health, with larger studies required to tease out contributing factors.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- Strength: This study follows offspring of pre-eclamptic mothers from birth until age five, filling the knowledge gap regarding the first few years of life.
- Limitation: There was a considerable number of mothers and offspring that were lost to follow-up by three years postpartum, resulting in a sample size too small to provide significant results for certain measures.
- Limitation: Some variables were not well collected (child BP), while others were added part way through the study (child waist and hip circumference), resulting in an incomplete set of data for some study participants.
- Strength: Use of the validated Ages and Stages Questionnaire permits early screening and identification of at-risk offspring, to allow for timely intervention and an overall improvement in children's long-term health.

INTRODUCTION

Hypertensive disorders are among the most common complications of pregnancy. Gestational hypertension occurs in 6-17% of pregnancies, and is defined as newly elevated blood

pressure (BP) after 20 weeks' gestation in a previously normotensive individual. Preeclampsia (PE) affects 2-7% of otherwise healthy women, and is diagnosed by newly elevated BP after 20 weeks gestation, with associated proteinuria.[1] Presently, the etiology of PE is largely unknown. Many theories have been proposed, including mechanisms involving oxidative stress, angiogenic imbalance, and immunologic intolerance between fetoplacental and maternal tissue.[2] Delivery is the only definitive cure, with other treatments focused on sign and symptom management.[2] It is well known that hypertensive disorders of pregnancy are associated with adverse health outcomes including perinatal deaths, preterm birth, intrauterine growth restriction (IUGR), neonatal morbidity, and infants that are small for gestational age (SGA).[1 3]

Previous studies have demonstrated a positive link between PE and offspring Neurodevelopmental Delay (ND),[4-8] but a number of others have disputed this claim,[6 9 10] thus no conclusions can be made. The inconsistencies between studies investigating the implications of PE pregnancies on offspring indicate a need for further research. Moreover, many studies either examine immediate postnatal or neonatal complications, or follow up with mothers and offspring years down the line, resulting in a knowledge gap regarding the first few years of life. Since earlier detection allows for earlier intervention, there is a need to identify those with increased risk in early childhood.

Using the Preeclampsia New Emerging Team (PE-NET) longitudinal prospective cohort, that has previously been used to investigate maternal outcomes and cardiovascular risk factors [11-13], this study examined whether infants of women with a PE pregnancy, compared to infants of women without a PE pregnancy, have differences in ND, and whether it changes over time. We hypothesized that infants born to women with PE-affected pregnancies will display markers indicating an increased risk of ND.

METHODS

Study Design

This longitudinal prospective cohort study compared assessments of ND in offspring born to women with/without PE yearly, from ages one to five. Neurodevelopment was assessed via the Ages and Stages Questionnaire (ASQ), the categories of which include gross motor, fine motor, communication, personal social, and problem solving.[14] The ASQ was chosen for its consistency, specificity, cost-effectiveness, and flexibility in administration. Each year parents were mailed the ASQ and asked to complete it through home observation of their child. Additionally, participants were asked to return at one, three, and five years postpartum for a clinical assessment. A study reminder was mailed every six months, and reminders by telephone or email were sent one week prior to a scheduled visit. If subjects missed appointments, they were contacted weekly for the following month before being considered as lost to follow-up.

Participants

The recruitment criteria and process have been previously described.[11] In brief, women were recruited into the Preeclampsia New Emerging Team (PE-NET) longitudinal prospective cohort at the Kingston General Hospital and Ottawa General Hospital between September 2003 and October 2009. All women diagnosed with PE (BP > 140/90 mmHg and proteinuria > 300 mg/24 hours or ≥ 1+ on repeat dipstick) at the time of presentation or admission/transfer were approached to participate. A trained research nurse explained the study and obtained consent. After each PE subject was recruited, the next normotensive woman without prior history of PE and matched by parity (0 vs. 1 or greater), maternal age (± 5 years), and race was invited to participate. Women with a history of chronic hypertension, diabetes (including development of

gestational diabetes), or renal disease were excluded from the study. [15] A total of 129 women with PE and 140 normotensive control women were enrolled in the study. Thirty-four subjects were diagnosed with mild PE, as defined above. Ninety-five subjects met the criteria for severe PE, which included the above definition and one or more of the following: systolic BP ≥ 160 mmHg, diastolic BP ≥ 110 mmHg, proteinuria ≥ 5 g in 24 hours or ≥ 3+ on dipstick, oliguria (≤ 500 ml in 24 hours), cerebral or visual disturbances, epigastric pain, thrombocytopenia (<150,000 10^9/L), increase in AST (>46U/L) and ALT (>40U/L), elevated serum creatinine (>106umol/L), pulmonary edema or cyanosis, IUGR, or eclampsia. All BPs had to be elevated on 2 measurements taken at least 6 hours apart.

The mild PE group (n = 34 at enrollment) is excluded from the current study for a number of reasons: (1) no difference was seen between the mild PE group and the controls in the comparisons done; (2) the number of mild PE patients seen in follow up was small; and (3) we chose to focus on those who experienced more severe disease.

Statistical Methods

Data collected at both time of recruitment and follow-up after one year were used to calculate maternal 30-year[16] and lifetime[17] risk estimates for CVD. For comparisons of categorical variables, the Mantel Haenszel Chi-Square test was used. The Fisher Exact method was used if cell counts were <5. For continuous variables the Mann-Whitney U test was used. A logistic regression analysis was completed for years 1, 2 and 3 of follow up to examine risk factors for ASQ failure. Variables were removed from the model step-wise based on highest p-value until all those remaining had a p-value less than 0.1. PE, IUGR, and GA were forced into the final model, regardless of p-value, to control for their effects. The Pearson correlation coefficient was used to explore the relationships between maternal CVD risk estimates and child

neurodevelopment. For all tests a 95% confidence level was used to determine significance. SAS v9.3 and R v2.15.2 were used for all analyses.

This study reviewed data that had been previously collected with approval from the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (OBGY-108-03).

RESULTS

Figure 1 indicates the number of subjects in each group throughout the study.

There were no significant differences in maternal characteristics at baseline, including age, height, weight, race, education level, household income, smoking status, parity, or breastfeeding status. There was a significant difference between groups regarding mode of delivery and having a previous pregnancy with PE, which is expected given the nature of the participants and eligibility criteria for the study (Table 1).

Table 1. Maternal characteristics at baseline visit.

Maternal Characteristics	**Control (n=140)**	**Severe PE (n=95)**	**P-value**
Maternal Age (years), median (IQR)	31.0 (27.75-33.25)	31.0 (28.0-34.0)	0.88
Maternal Height (cm), median (IQR)	165.0 (160.0-170.0)	163.0 (160.0-168.0)	0.13
Maternal Weight (kg), median (IQR)			
Birth	66.5 (58.0-77.0)	68.0 (61.5-82.0)	0.23
Year 1	67.5 (59.4-81.0)	71.95 (63.1-79.8)	0.19
Year 3	72.1 (63.4-90.3)	68.7 (61.9-79.8)	0.33
Maternal BMI (kg/m^2), median (IQR)			
Birth	24.2 (21.7-27.4)	25.3 (21.8-30.5)	0.09

Year 1	25.3 (22.0-29.7)	26.6 (23.0-30.5)	0.08
Year 3	26.0 (22.4-32.3)	25.6 (22.7-30.3)	0.68
Maternal race, n (%)			
White	123 (87.9)	76 (80.0)	0.14
Other	17 (12.1)	19 (20.0)	
Maternal education level, n (%)			
High school or less	9 (6.4)	14 (14.7)	0.11
Post secondary not complete	16 (11.4)	11 (11.6)	
Post secondary complete	115 (82.1)	70 (73.7)	
Household income ($), n (%)			
< 29 999	8 (5.7)	8 (8.4)	0.01
30 000 to 59 999	17 (12.1)	23 (24.2)	
60 000 to 89 999	32 (22.9)	26 (27.4)	
> 90 000	77 (55.0)	33 (34.7)	
Did not respond	6 (4.3)	5 (5.3)	
Maternal smoking, n (%)			
Yes	3 (2.1)	5 (5.3)	0.27
No	137 (97.9)	90 (94.7)	
Parity, n (%)			
Nulliparous	65 (46.4)	44 (46.3)	1.00
Multiparous	75 (53.6)	51 (53.7)	
Previous pregnancy with PE, n (%)			
Yes	0 (0.0)	10 (10.5)	<0.0001

No	140 (100.0)	85 (89.5)	
Mode of delivery, n (%)			
Vaginal	99 (70.7)	30 (31.6)	<0.0001
Caesarian	41 (29.3)	65 (68.4)	
Breastfeeding			
Total, n	98	52	
Yes, n (%)	87 (88.8)	50 (96.2)	0.22
Length of time (weeks), median (IQR)	32.0 (20.0-48.0)	28.0 (8.8-48.0)	0.63

PE - preeclampsia. BMI - Body Mass Index.

There were significant differences between the majority of infant characteristics at birth, including gestational age (GA), 5 minute Apgar score, admission level and length of stay, and presence of IUGR (Table 2).

Table 2. Infant characteristics at birth.

Infant Characteristics	**Control (n = 140)**	**Severe PE (n = 95)**	P-Value
Sex, n (%)			
Male	75 (53.6)	56 (59.0)	NS
Female	65 (46.4)	39 (41.1)	
Gestational age at birth			
Weeks, median (IQR)	39.5 (38.0-41.0)	36.0 (32.0-38.0)	<0.001
<37 weeks, n (%)	5 (0.04)	59 (0.62)	<0.001
≥37 weeks, n (%)	135 (0.96)	36 (0.38)	
Placental weight			

Grams, median (IQR)	555.0 (480.0-639.0)	413.0 (294.2-596.8)	<0.001
Magnesium Sulfate Usage, n (%)			
Yes	1 (0.7)	48 (50.5)	<0.0001
No	139 (99.3)	47 (49.5)	
If yes, usage <33 weeks gestation, n (%)	0 (0.0)	20 (41.7)	
Apgar score, median (IQR)			
1 min	8.0 (7.0-9.0)	8.0 (6.0-9.0)	<0.01
5 min	9.0 (9.0-9.0)	9.0 (8.0-9.0)	<0.001
Admission info			
Combined Care or Level 1, n (%)	127 (90.7)	36 (37.9)	<0.001
Length of stay (days), median (IQR)	2.0 (2-3)	3.0 (2-4.5)	<0.001
Level 2 or 3, n (%)	13 (9.3)	59 (62.1)	<0.001
Length of stay (days), median (IQR)	4.5 (3-5.5)	15.5 (6.3-32.8)	<0.01
Transferred before discharge, n (%)	0 (0)	9 (9)	
Intrauterine Growth Restriction, n (%)	0 (0.0)	25 (26.3)	<0.001

PE - preeclampsia.

Figure 2 compares both the proportion and number of ASQ categories failed at each year of follow-up between the severe PE and control groups. A significant difference was found in the proportion of categories failed in year 3 ($P<0.05$), and this approached significance in years 1 and 4 ($P<0.10$ and $P<0.15$, respectively). Although a significant difference was not found in year 2, the trend is clearly present. Comparison of the distribution of the number of categories failed,

among those who failed at least one category, indicated that severe PE children tended to fail more categories than controls at year 1 ($P<0.10$).

A subgroup analysis was performed looking at only PE patients, categorized by preterm (<37 weeks) and term (≥37 weeks). It was found that GA appears to significantly contribute to the relationship between PE and failure of ASQ categories (Figure 3). A significant proportion of PE children born preterm failed the ASQ in years 3 and 4 ($P<0.05$). Additionally, it was found that when failed, those who were preterm tended to fail more categories. This was significant at years 3 and 4 ($P<0.05$) and approached significance at years 2 and 5 ($P<0.10$).

A logistic regression analysis examining risk factors for ASQ failure was performed for years 1, 2 and 3 of follow up, considering the variables $MgSO_4$ usage, maternal smoking, socioeconomic status (a combination of income, maternal, and paternal education), sex, parity (multiparous or nulliparous) and breastfeeding (did not breastfeed, breastfed <6 months, and breastfed ≥6 months) (Table 3). As well, severe PE, GA, and IUGR were forced into the model regardless of p-value, due to their well-known known effects. Male sex had a greater risk of ASQ failure than females with in odds ratio of 2.31 (95%CI 0.88, 6.05) at year 1 and 2.72 (95%CI 1.11, 6.70) at year 2. This relationship was not significant by year 3. $MgSO_4$ usage was retained in the model at year 1 only, with an odds ratio of 2.69 (95%CI 0.73, 9.99). The diagnosis of IUGR increased the risk of ASQ failures in year 1, 2, and 3, with odds ratios of 2.22 (95%CI 0.53, 9.22), 1.63 (95%CI 0.30, 8.85), and 3.96 (0.71, 21.93), respectively. Increasing gestational age was protective against ASQ failure with odds ratios of 0.96 (95%CI 0.83, 1.10), 0.84 (95%CI 0.73, 0.98), and 0.94 (95%CI 0.79, 1.11) at years 1, 2, and 3, respectively. Interestingly, severe PE appeared to be protective in the first two years, while it increased the risk of ASQ

failures in year 3 with an odds ratio of 2.31 (95%CI 0.63, 8.53). As well, multiparity was a risk factor at year 3 with odds ratio 2.74 (95%CI 0.92, 8.17).

Table 3. Logistic regression analysis of ASQ failures at year 1, 2, and 3 of follow up

Variable	**Year 1 (n=197)**	**Year 2 (n=170)**	**Year 3 (n=99)**
	OR (95% CI)	*OR (95% CI)*	*OR (95% CI)*
Sex (Male vs. Female)	2.31 (0.88, 6.05)	2.72 (1.11, 6.70)	-
IUGR (Yes vs. No)	2.22 (0.53, 9.22)	1.63 (0.30, 8.85)	3.96 (0.71, 21.93)
$MgSO_4$ (Yes vs. No)	2.69 (0.73, 9.99)	-	-
Gestational Age (Weeks)	0.96 (0.83, 1.10)	0.84 (0.73, 0.98)	0.94 (0.79, 1.11)
Severe PE vs. Normotensive	0.90 (0.24, 3.34)	0.63 (0.19, 2.09)	2.31 (0.63, 8.53)
Parity (Multi vs. Nulliparous)	-	-	2.74 (0.92, 8.17)

IUGR – Intrauterine Growth Restriction; $MgSO_4$ – magnesium sulphate; PE – Preeclampsia.

Infants born to PE mothers tended to fail more often in the gross motor ASQ category. This was significant in year 2 (46.15% vs. 5.26%, P = 0.01), and trended toward significance in years 1 (42.86% vs. 23.08%, P = 0.32) and 3 (21.43% vs. 0%, P = 0.24). A larger sample size is needed to confirm this trend.

Significant correlations were noted between maternal lifetime risk score and number of ASQ categories failed at years 1 (r = 0.20, *P* = 0.008) and 3 (r = 0.23, *P* = 0.026). Ordinal regression was explored, but was not possible because the proportional odds assumption did not hold true, and the sample size was not large enough to make proper adjustments.

DISCUSSION

Mild PE is not associated with adverse ND outcomes in offspring. Infants born to mothers with severe PE were more likely to have failed in at least one ASQ category (Figure 2),

indicating ND, up to and including 3 years. This is in keeping with Ehrenstein et al.,[18] who found a slightly reduced cognitive performance in adult males exposed to gestational hypertensive disorders. Additionally, Sorensen et al.,[7] revealed that maternal hypertension was an independent risk factor for the development of schizophrenia in offspring later in life, and Tuovinen at al.[19] showed that hypertensive disorders in pregnancy are associated with lower intellectual abilities in twenty-year-old male offspring in a subgroup of the Helsinki Birth Cohort. However, a further investigation of this cohort revealed that it was hypertension without proteinuria that was associated with an increased risk of serious mental disorders requiring hospitalization or contributing to death, while preeclampsia was actually associated with a lower risk.[9] These discrepancies between findings indicate that further studies are needed to better understand this association.

Severe PE-affected offspring can be viewed as having a 'severe PE syndrome', which includes other adverse pregnancy outcomes, including IUGR and earlier GA. For instance, Table 3 suggests that these outcomes are contributors to ASQ failure in years 1-3, with $MgSO_4$ usage also impacting this finding in year 1, but all of these variables may simply be considered markers of severe PE. Infants with IUGR are more likely to have significant placental issues often seen with severe PE, $MgSO_4$ is the standard treatment for severe elevations in blood pressure due to PE, and earlier delivery is required when PE is severe and can no longer be medically managed. Severe PE itself was trending toward significant in year 3 (odds ratio 2.31; 95%CI 0.63-8.53) but did not reach it, potentially due to the small sample size (n=99). While previous studies have indicated that earlier GA and IUGR are the primary risk factors in this population,[20] others have shown that among growth-restricted infants, those born to mothers with PE have lower IQ scores than those without PE-complicated pregnancies, indicating that PE itself is also a is a major

contributor.[4] Follow up studies conducted on the PE-NET cohort also support the effects of severe PE on cognitive ability. Ratsep et al.[21], found impairment in both working memory in the offspring of PE mothers, based on psychometric testing, as well as visuospatial processing. A smaller cohort of subjects was followed up with brain magnetic resonance imaging at a mean age of 9.66 years for PE offspring and 9.79 years for controls. This study revealed a number of structural and vascular anatomic changes in the brains of PE offspring that shared similarities with alterations found in autism.[22] The deficits in higher level cognitive functioning reveal that the increased risk seen with severe PE in year 3 is likely the beginning of a trend, but larger studies with longer follow-up are needed to further define this relationship.

Interestingly, our data indicated a slightly decreased gross motor performance compared to the other categories measured. This is in contrast to Whitehouse et al.,[8] who found that gestational hypertension and preeclampsia reduced verbal ability in offspring, but non-verbal performance was unaffected. As with previously discussed findings, further studies are needed to tease out the true nature of developmental deficits experienced in this population

Lastly, there was a significant positive correlation between maternal lifetime CVD risk score and number of ASQ categories failed at years 1 and 3 ($P<0.05$). Likewise, Krakowiak et al.,[23] revealed that children aged 2-5 years exposed to metabolic conditions in pregnancy (diabetes, hypertension, or obesity) scored lower on neurodevelopmental assessments. These persistent neurodevelopmental delays indicate a need for early childhood interventions, to ensure efforts are made to reduce their persistence into school age.

There are a number of limitations to the study that must be addressed. The considerable number of mothers and offspring that were lost to follow-up by three years postpartum (Figure 1) resulted in a sample size too small to provide significant results for certain measures. Based on

the failure rates observed at each year, we would need a sample size of 172 severe PE and 172 controls at year 1, 359 severe PE and 359 controls at year 2, and 96 severe PE and 96 controls at year 3, to reach a desired power of 80%.[24] Additionally, the group lost to follow-up by three years postpartum contained a significant number of mild PE subjects, and along with the small amount of subjects in this group to begin with, we were unable to include this group in the analyses. Future studies should include this subgroup, and we would expect the effects found to be lesser than what was observed in the severe PE group. Furthermore, only ~6% of control mothers were at high risk for cardiometabolic disease,[13] which must be considered as the issue driving the PE. Lastly, some variables were not well collected (child BP), while others were added part way through the study (child waist and hip circumference), resulting in an incomplete set of data for some study participants.

Pregnancy is a useful way to identify women at risk for CVD.[11-13] Our findings indicate that it may also allow us to identify offspring at risk from a neurodevelopmental perspective. This provides a unique opportunity to use maternal health complications to improve whole family outcomes. By identifying these women at time of delivery, early screening and follow-up of offspring can help ensure that those individuals at risk are identified in a timelier manner. This will allow for earlier intervention and an overall improvement in children's long-term health.

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COMPETING INTERESTS

The authors report no conflict of interest.

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DATA SHARING

Extra data is available by emailing Dr. Graeme Smith.

CONTRIBUTORSHIP

MW, GNS and SWW designed the study. GNS, MW, and SWW contributed to acquisition of data. CW and JP completed analysis and interpretation of data. All authors assisted in drafting and/or critically reviewing the manuscript. All authors have approved the final version and agree to be accountable for all aspects of the work.

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LEGENDS

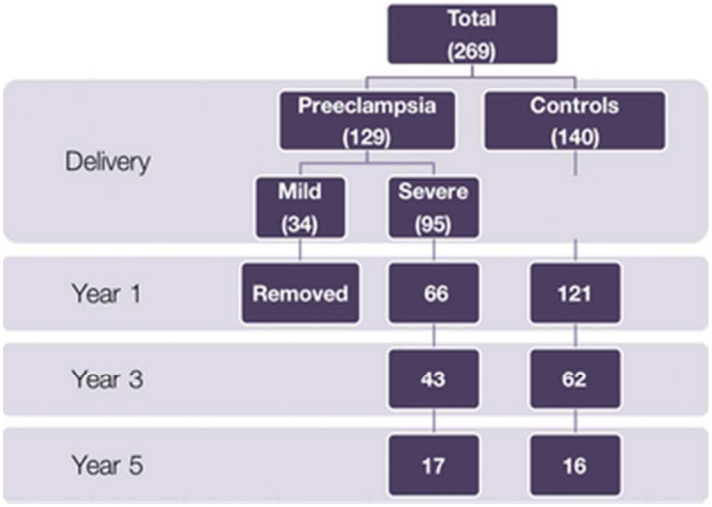
Figure 1. Flow diagram of study subjects at years 1-5.

Figure 2. Comparison of Ages and Stages Questionnaire failures between the severe preeclampsia and control groups at years 1 through 5 of follow up.

★ <0.05, + <0.10, ■ <0.15, p-value based on Mantel-Haenszel Chi Square. Fisher Exact used when cell counts <5. Comparison of the proportion of failures in the severe preeclampsia group to the control group at each year of follow up. ★ <0.05, + <0.10, ■ <0.15, p-value based on Wilcoxon Rank-Sum Test. Comparison of the distribution of the number of categories failed, among those participants who failed at least one category, between the severe preeclampsia group and the control group at each year of follow up. PE- preeclampsia.

Figure 3. Comparison of Ages and Stages Questionnaire failures between the preeclampsia & preterm and preeclampsia & term groups at years 1 through 5 of follow up.

★ <0.05, + <0.10, ■ <0.15, p-value based on Mantel-Haenszel Chi Square. Fisher Exact used when cell counts <5. Comparison of the proportion of failures in the <37 weeks to the ≥37 weeks gestational age group at each year of follow up. ★ <0.05, + <0.10, ■ <0.15, p-value based on Wilcoxon Rank-Sum Test. Comparison of the distribution of the number of categories failed, among those participants who failed at least one category, between the <37 weeks and the ≥37 weeks gestational age group at each year of follow up. PE- preeclampsia; Wks - weeks; GA - gestational age.



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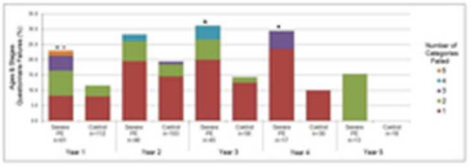

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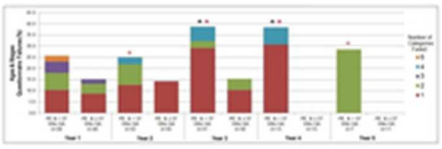

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Page No	Recommendation
Title and abstract	1	1-3	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
			(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	4	Explain the scientific background and rationale for the investigation being reported
Objectives	3	5	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	5	Present key elements of study design early in the paper
Setting	5	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	5-6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	5-6	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*		For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	6	Describe any efforts to address potential sources of bias
Study size	10	6	Explain how the study size was arrived at
Quantitative variables	11	6-7	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	6-7	(*a*) Describe all statistical methods, including those used to control for confounding
			(*b*) Describe any methods used to examine subgroups and interactions
			(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
			(*e*) Describe any sensitivity analyses

Continued on next page

			Results
Participants	13 *	7	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
			(b) Give reasons for non-participation at each stage
			(c) Consider use of a flow diagram
Descriptive data	14 *	7	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
			(b) Indicate number of participants with missing data for each variable of interest
			(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15 *	9	*Cohort study*—Report numbers of outcome events or summary measures over time
			Case-control study—Report numbers in each exposure category, or summary measures of exposure
			Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	9	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
			(*b*) Report category boundaries when continuous variables were categorized
			(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	10-12	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
			Discussion
Key results	18	13-14	Summarise key results with reference to study objectives
Limitations	19	14-15	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	15	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	15	Discuss the generalisability (external validity) of the study results
			Other information
Funding	22	15	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.