Assessment of blood-brain barrier integrity and neuroinflammation in preeclampsia

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BACKGROUND: Although blood-brain barrier integrity is intact under normal pregnancy conditions, animal studies suggest that blood-brain barrier impairment occurs in preeclampsia. Yet, human data are limited, and the integrity of the blood-brain barrier has not been assessed in women with preeclampsia.

OBJECTIVE: We sought to test the hypothesis that the integrity of the blood-brain barrier is impaired and that neuroinflammation is increased in women with preeclampsia.

STUDY DESIGN: We performed an observational case-control study in pregnant women >24 weeks gestation who underwent spinal anesthesia for elective cesarean delivery or combined spinal epidural analgesia for labor. Cases were women with preeclampsia, and control subjects were women with either healthy pregnancy, chronic hypertension, or gestational hypertension. Paired samples of blood, urine, and cerebrospinal fluid were collected from each subject before delivery. We measured albumin, C5a, C5b-9, tumor necrosis factor-α, and interleukin-6 concentrations in plasma and cerebrospinal fluid, and albumin, C5a, and C5b-9 concentrations in urine, using colorimetric or enzyme-linked immunosorbent assays. The ratio of albumin in cerebrospinal fluid to plasma (Q_{alb}) was used as a surrogate for maternal blood-brain barrier integrity. Cerebrospinal fluid concentrations of C5a, C5b-9, tumor necrosis factor-α, and interleukin-6 were used as surrogate markers of neuroinflammation. Differences in Q_{alb} and cerebrospinal fluid protein concentrations between groups were assessed by nonparametric test of medians.

RESULTS: Forty-eight subjects were enrolled, which included 16 cases with preeclampsia, 16 control subjects with healthy pregnancy, and 16 control subjects with either chronic or gestational hypertension. Q_{alb} values were not increased in preeclampsia cases compared with healthy or hypertensive control subjects (Q_{alb} median, 3.5 [interquartile range, 2.9–5.1] vs 3.9 [interquartile range, 3.0–4.8] vs 3.9 [interquartile range, 3.0–4.8]; P=.78]. Moreover, Q_{alb} values were not increased in the subset of women with preeclampsia with severe features (n=8) compared with those without severe features (n=8; Q_{alb} median, 3.5 [interquartile range, 3.3–4.9] vs 3.7 [interquartile range, 2.3–5.5]; P=.62]. Cerebrospinal fluid concentrations of C5a, C5b-9, tumor necrosis factor-α and interleukin-6 were not increased in cases of preeclampsia, compared with control subjects with either healthy pregnancy, chronic hypertension, or gestational hypertension (P>.05, all comparisons). In contrast to the negative findings in cerebrospinal fluid, plasma concentrations of both C5b-9 and interleukin-6 and urine concentrations of C5a and C5b-9 were increased in cases of preeclampsia.

CONCLUSION: Through measurements of albumin, complement proteins, and cytokines in paired samples of blood and cerebrospinal fluid at the time of delivery, we found no evidence of blood-brain barrier impairment or neuroinflammation in preeclampsia. Larger studies that will investigate a wider range of proteins are suggested to validate our findings.

Key words: albumin, brain, central nervous system, cerebrospinal fluid, complement proteins, cytokines, hypertension, neuroinflammation, preeclampsia, pregnancy

Preeclampsia is a common pregnancy disorder characterized by hypertension and proteinuria or end-organ injury.[1] Fulminant disease, termed preeclampsia with severe features (or severe preeclampsia), is a leading cause of maternal and neonatal death around the world.[2,3] Severe preeclampsia is challenging to manage because of its heterogeneous nature, with variable damage to maternal end-organs, such as the brain, kidney, and liver, among others. Central nervous system (CNS) manifestations of disease, such as severe headache, visual scotomata, brain edema, and seizure, contribute disproportionately to maternal harm and premature delivery.[4,5] Yet, our understanding of the factors that predispose to CNS disturbances in preeclampsia is limited.

Although blood-brain barrier integrity is intact under normal pregnancy conditions, animal studies suggest that blood-brain barrier impairment occurs in preeclampsia. The permeability of rodent cerebral vessels increases after exposure to plasma of women with either preeclampsia[6] or hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome.[7] This is relevant because cerebral veins are a primary site of blood-brain barrier disruption in acute hypertension,[8,9] which may allow passage of serum factors into the brain, triggering neuroinflammation and propagating the CNS disturbances that are seen in preeclampsia. For example, when brain macrophages, termed *microglia*, are exposed to serum from pregnant rats, they release cytokines such as tumor necrosis factor-α (TNF-α) that promote neuronal excitability and seizure activity.[10]

The circulating serum factors in preeclampsia that are responsible for increased blood-brain barrier permeability and neuronal excitability, as seen in animal studies, remain unknown. Several lines of evidence implicate terminal complement proteins, C5a and

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AJOG at a Glance

Why was this study conducted?

This study was conducted to determine whether the integrity of the blood-brain barrier is impaired and whether neuroinflammation is increased in women with preeclampsia.

Key findings

The ratio of albumin in cerebrospinal fluid to plasma, which is a surrogate for maternal blood-brain barrier impairment, was not increased in women with preeclampsia. Moreover, the concentrations of complement proteins and inflammatory cytokines were increased in plasma, but not cerebrospinal fluid, in preeclampsia.

What does this add to what is known?

This is the first study to assess the integrity of the blood-brain barrier in human preeclampsia. Through measurements of albumin, complement proteins, and cytokines in blood and cerebrospinal fluid, we found no evidence of blood-brain barrier impairment or neuroinflammation in preeclampsia.

C5b-9, which are increased in blood and urine in women with severe preeclampsia and HELLP syndrome.[11–14] C5a is a potent inflammatory mediator that polarizes macrophages to the anti-angiogenic phenotype[15,16]; C5b-9 propagates cell lysis and microvascular thrombosis.[17,18] Importantly, complement receptors are expressed fully on brain endothelial cells, neurons, and microglia,[19–21] and both C5a and C5b-9 can activate microglia and increase secretion of inflammatory cytokines, such as TNF-α and interleukin-6 (IL-6).[22–24] Changes in the composition of proteins in the cerebrospinal fluid (CSF) can be detected in several CNS disorders,[25,26] and the expression of numerous proteins are increased in the CSF of women with preeclampsia, including some complement proteins.[27] Yet, it remains unclear if CSF proteins are increased in preeclampsia because of blood-brain barrier impairment and passage of serum proteins or whether CSF proteins are generated locally from brain parenchyma.[28]

Our primary aim was to test the hypothesis that blood-brain barrier impairment is increased in women with preeclampsia, as measured by the CSF/plasma albumin quotient, compared with a control group of women with healthy pregnancy, chronic hypertension, or gestational hypertension. Our secondary aim was to test the hypothesis that terminal complement proteins C5a and C5b-9, or inflammatory cytokines TNF-α and IL-6, are increased in the CSF of women with preeclampsia.

Materials and Methods

We performed an observational case-control study in pregnant women at Oregon Health & Science University between May 2016 and January 2018. Institutional review board approval was obtained before initiation of the study. All subjects provided written informed consent, with all procedures performed in accordance with institutional guidelines and study protocol.

Eligible subjects were enrolled sequentially by research investigators during available work hours. Pregnant women were eligible for enrollment if they had a living nonanomalous gestation of $\geq$24 weeks and were planning to undergo indicated spinal anesthesia for elective cesarean delivery or combined spinal epidural analgesia for labor. Cases were women with preeclampsia; control subjects could be healthy pregnancies or those with chronic or gestational hypertension. Subjects were enrolled from outpatient clinics, labor and delivery ward, or antepartum unit. Diagnoses were made in accordance with 2013 American College of Obstetricians and Gynecologists' criteria for hypertension in pregnancy.[1] Some women were excluded for the following reasons: fetal death, major fetal or chromosomal abnormality, chronic kidney disease, systemic lupus erythematosus or antiphospholipid syndrome, long-term use of corticosteroids (equivalent to prednisone $\geq$10 mg per day for $\geq$3 weeks), active outpatient use of unfractionated or low molecular weight heparin, immune deficiency or use of immunosuppressive agents, febrile illness or untreated bacterial or viral infection, illicit drug use, or contraindication to spinal anesthesia.

The primary endpoint was the ratio of albumin in CSF to plasma (Q_{alb}=CSF albumin [mg/dL]/plasma albumin [g/dL]$\times$1000),[29] which we used as a surrogate for maternal blood-brain barrier impairment. The secondary endpoints were the CSF concentrations of terminal complement proteins (C5a, C5b-9) and the CSF concentrations of inflammatory cytokines (TNF-α, IL-6), which were measured to assess for degree of neuroinflammation. Tertiary endpoints were the concentrations of complement proteins and cytokines in plasma and urine, which gave us an estimation of systemic inflammation. CSF was collected at the time of spinal anesthesia for subjects undergoing elective cesarean delivery or at time of combined spinal-epidural analgesia for subjects in labor. With a 2-mL syringe, up to 1 mL of CSF was aspirated through a 25-gauge Whitacre needle, instilled into a cryovial, and stored at −80°C. Subjects were excluded from the study if CSF could not be obtained because of technical difficulties or because the urgency of delivery necessitated the administration of general anesthesia. Blood and urine were collected on the day of and before delivery. Blood was collected in ethylenediaminetetraacetic acid tubes, and urine was collected via a random clean-catch specimen or indwelling catheter. Blood and urine samples were centrifuged at 4°C; plasma and urine supernatant were aliquoted and stored in cryovials at −80°C. Maternal and

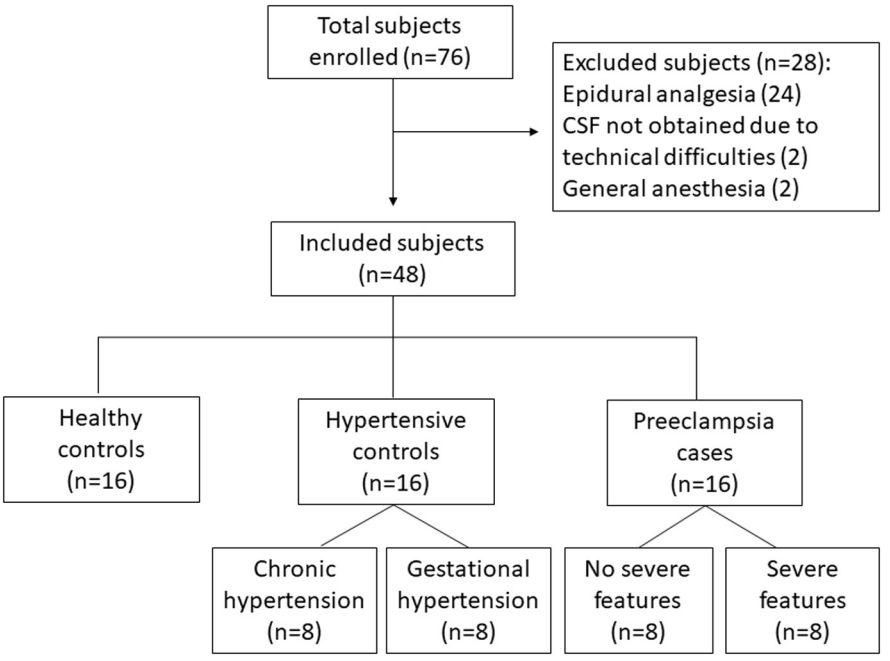
neonatal clinical data were abstracted from the electronic medical record.

At completion of study enrollment, the concentrations of albumin, C5a, C5b-9, TNF-α, and IL-6 were measured in CSF and plasma. Levels of albumin, C5a, and C5b-9 were also measured in urine. Measurement of protein levels were determined by colorimetric (albumin) or enzyme-linked immunosorbent assays (ELISA) in the following manner: albumin (Sigma-Aldrich, St. Louis, MO); C5a and C5b-9 ELISA (BD Biosciences, San Jose, CA); TNF-α and IL-6 ELISA (R&D Systems, Minneapolis, MN). Samples were run in duplicate with negative (blank) and positive control subjects. The following assay dilutions were used: CSF (1×[albumin, TNF-α] and 2×[C5a, C5b-9, IL-6]); plasma (1× [TNF-α, IL-6], 2×[albumin], and 100×[C5a, C5b-9]). Urine samples were undiluted. The lower limit of detection for each assay was albumin (0.01 g/dL), C5a (0.05 ng/mL), C5b-9 (0.01 ng/mL), TNF-α (1.6 pg/mL), and IL-6 (0.7 pg/mL). The interassay coefficient of variation for each assay was albumin (3.0%), C5a (3.2%), C5b-9 (14.0%), and IL-6 (8.8%). The interassay coefficient of variation for TNF-α could not be determined because the concentration in the control sample was below the limit of detection (<1.6 pg/mL).

The study was designed and powered to test the primary hypothesis that maternal blood-brain barrier impairment (Q_{alb} ratio) is increased in cases of preeclampsia compared with pregnant control subjects without preeclampsia. The ratio of albumin in CSF to plasma (Q_{alb}) has not been investigated during pregnancy, but among adults who undergo lumbar puncture for Alzheimer's disease, the mean±standard deviation value for Q_{alb} was 6.0±1.8.[29] This Q_{alb} value is similar to that of adults who underwent diagnostic lumbar puncture but had normal results.[30] In a study of CSF samples from women with preeclampsia and control subjects, proteomic analysis revealed that differentially abundant proteins may be increased ≥27% in preeclampsia.[31] Using the data from these studies, we estimated mean Q_{alb} 6.0±1.8 in control subjects and powered our study to detect a 27% difference (or greater) in Q_{alb} values among cases of preeclampsia, using β=.80 and α=.05. These power calculations resulted in a targeted sample size of 16 subjects per group.

For anticipated a priori subgroup comparisons, we targeted enrollment of 8 preeclampsia cases with severe features and 8 without severe features. Similarly, among hypertensive control subjects, we targeted enrollment of 8 subjects with chronic hypertension and 8 subjects with gestational hypertension. We assessed differences in Q_{alb} and biomarker concentrations between groups using a 2-tailed *t*-test or analysis of variance for normal data and nonparametric equality of medians test for nonnormal data. Chi-squared testing was used for comparison of categoric data between groups, and Spearman's rank correlation coefficient for correlative studies, with significance determined by $P<.05$.

FIGURE 1
Study flow diagram



After exclusions, 48 subjects were enrolled into one of three study arms: i) healthy controls; ii) hypertensive controls (chronic or gestational hypertension) and; iii) preeclampsia cases (with or without severe features).

CSF, cerebrospinal fluid.

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Results

Seventy-six pregnant women were enrolled, and 48 of them had paired collection of blood, urine, and CSF on the day of delivery. The study flow diagram is shown in Figure 1. The final study group included 16 cases of preeclampsia (8 cases without severe features and 8 cases with severe features), 16 healthy control subjects without hypertension, and 16 hypertensive control subjects with either chronic (n=8) or gestational (n=8) hypertension. Of the 76 subjects initially enrolled, 28 women were excluded because CSF could not be obtained for the following reasons: received epidural analgesia (n=24), provider was unable to collect CSF at time of spinal anesthesia because of technical difficulties (n=2), or because the urgency of delivery necessitated administration of general anesthesia (n=2). Summary demographics and clinical characteristics of the study groups are shown in Table 1. Body mass index was highest in women with chronic or

TABLE 1
Participants' baseline characteristics stratified by pregnancy-related hypertensive disorder

Characteristic	Healthy (n=16)	Chronic or gestational hypertension (n=16)	Preeclampsia with or without severe features (n=16)	*P* value[a]
Maternal age, y[b]	32.8±5.5	35.1±5.0	30.6±6.7	.09
Body mass index, kg/m^2[b]	31.9±5.6	42.8±7.6	36.3±10.0	.002
Gestational age at delivery, wk[b]	38.4±1.1	37.5±2.2	35.1±3.8	.002
White, non-Hispanic, %	81.3	68.8	62.5	.49
Hispanic, %	18.8	25.0	31.2	.72
Nulliparous, %	18.8	18.8	37.5	.37
Cesarean delivery, %	100	100	75	.01

[a] Analysis of variance for continuous data, Chi-squared test for dichotomous data; [b] Data are given as mean±standard deviation.
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gestational hypertension; gestational age at delivery was earliest in women with preeclampsia.

Among hypertensive control subjects without preeclampsia, peak systolic and diastolic blood pressure measurements on the day of delivery were similar in subjects with chronic vs gestational hypertension (mean systolic blood pressure, 156±10 vs 150±7.0 mm Hg [*P*=.21]; diastolic blood pressure, 101±14 vs 92±8.8 mm Hg (*P*=.18]). Among preeclampsia cases, systolic blood pressure, but not diastolic blood pressure, was significantly higher in those with severe features compared with those without severe features [systolic blood pressure, 168±16 vs 151±7.5 mm Hg [*P*=.016]; diastolic blood pressure, 106±11 vs 100±7.3 mm Hg [*P*=.17]). Among 8 cases of preeclampsia with severe features, the following criteria were present: severe blood pressure (≥160/110 mm Hg) with headache or visual disturbances (n=3); severe blood pressure with headache and thrombocytopenia (n=1); severe blood pressure with acute kidney injury (n=2); pulmonary edema (n=1); and liver enzyme elevation (n=1).

Q_{alb} value, the surrogate measure of blood-brain barrier integrity, was no different in cases of preeclampsia compared with control subjects (Table 2). In pairwise comparisons, Q_{alb} was no different between preeclampsia cases and either healthy or hypertensive pregnant control subjects, respectively (Q_{alb} median [interquartile range]: preeclampsia, 3.5 [2.9−5.1] vs healthy control subjects 3.9 [3.0−4.8]; *P*=.72; or vs hypertensive control subjects, 3.9 [3.2−5.8]; *P*=1.0). Among preeclampsia subjects, Q_{alb} values were no different in those with severe features vs those without severe features (Q_{alb} median [interquartile range], 3.5 [3.3−4.9] vs 3.7 [2.3−5.5], *P*=.62), those with CNS features vs those without CNS features (Q_{alb} median [interquartile range], 3.5 (3.3−4.1) vs 4.3 (2.8−5.4); *P*=.48], or those with CSF collected at time of labor vs cesarean delivery (Q_{alb} median [interquartile range], 2.9 9 [2.3−3.3] vs 4.3 [3.4−5.4]; *P*=.08]. Moreover, albumin was detectable in 100% of CSF samples, but absolute concentrations were no different between groups (Table 2), including subgroup comparison of preeclampsia with severe features to preeclampsia without severe features (CSF albumin: median [interquartile range], 16.0 [12.5−17.0] vs 15.0 [9.0−22.0]; *P*=1.0].

We then assessed neuroinflammation by measuring CSF levels of C5a, C5b-9, TNF-α, and IL-6, in preeclampsia cases and control subjects; the results are summarized in Table 3. We detected measurable concentrations of C5a in 73%, C5b-9 in 17%, TNF-α in 15%, and IL-6 in 100% of CSF samples. However, median CSF concentrations of C5a, C5b-9, TNF-α, and IL-6 were no different between preeclampsia cases and control subjects, including pairwise comparisons between preeclampsia cases and either healthy or hypertensive pregnant control subjects, respectively. Because C5b-9 and TNF-α concentrations were detected infrequently in CSF, we evaluated whether absolute detection rates were greater in preeclampsia cases vs control subjects, but there was no significant difference (detection of C5b-9 in CSF: 25.0% vs 12.5% [P=.27]; detection of TNF-α in CSF: 31.3% vs 9.4% [*P*=.06]). Arguing against a permissive blood-brain barrier, plasma and CSF concentrations of C5b-9, TNF-α, and IL-6 did not correlate with each other (Spearman's correlation: C5b-9 [rho=0.21; *P*=.16]; TNF-α [rho=−0.14; *P*=.35]; and IL-6 [rho=0.25; *P*=.08]). The exception was C5a, which showed a modest positive correlation between plasma and CSF concentrations (Spearman's rho=0.39; *P*=.007).

Finally, we measured concentrations of C5a, C5b-9, TNF-α, and IL-6 in plasma and concentrations of C5a and C5b-9 in urine (Table 3). We found that concentrations of C5b-9 and IL-6, but not C5a and TNF-α, were increased in plasma in preeclampsia cases vs control subjects. In pairwise comparisons, plasma C5b-9 and IL-6 concentrations were increased specifically in preeclampsia cases vs healthy, but not hypertensive, control subjects. Among preeclampsia cases, plasma C5b-9 and IL-6 concentrations were no different between subjects with or without severe

TABLE 2
Primary study endpoint, stratified by pregnancy-related hypertensive disorder

Laboratory measure	Healthy (n=16)[a]	Chronic or gestational hypertension (n=16)[a]	Preeclampsia with or without severe features (n=16)[a]	*P* value[b]
Cerebrospinal fluid albumin, mg/dL	15.0 (13.0–19.0)	16.0 (13.0–24.0)	15.0 (11.0–18.0)	.92
Plasma albumin, g/dL	4.2 (4.0–4.4)	4.2 (3.8–4.4)	3.7 (3.5–4.3)	.17
Q_{alb}[c]	3.9 (3.0–4.8)	3.9 (3.2–5.8)	3.5 (2.9–5.1)	.78

[a] Data are given as median (interquartile range); [b] By nonparametric test of medians; [c] Cerebrospinal fluid albumin (mg/dL)/plasma albumin (g/dL)×1000.
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features. In urine, both C5a and C5b-9 were increased in preeclampsia cases vs control subjects. In pairwise comparisons, both urine C5a and C5b-9 were increased significantly in preeclampsia cases vs hypertensive control subjects ($P \leq .005$).

Comment

Principal findings

Through measurements of albumin, complement proteins, and cytokines in blood and CSF, we found no evidence of blood-brain barrier impairment or neuroinflammation in cases of preeclampsia, compared with control subjects with healthy pregnancy, chronic hypertension, or gestational hypertension.

Results in context

Arguing against blood-brain barrier impairment, neither the ratio of CSF-to-plasma albumin (Q_{alb}) or the absolute concentration of CSF albumin were increased in preeclampsia. In addition, neither the concentrations of terminal complement proteins C5a and C5b-9 or inflammatory cytokines TNF-α and IL-6 were increased in the CSF of women with preeclampsia. Negative findings in CSF were noted, despite systemic activation of the terminal complement pathway (increased C5b-9 in both plasma and urine) and systemic inflammation (increased IL-6 in plasma). Thus, our results suggest that the blood-brain barrier is not permissive to albumin or C5b-9 in preeclampsia or even smaller inflammatory factors such as C5a, TNF-α, and IL-6. In addition, the systemic increase in C5b-9 and IL-6 concentrations in preeclampsia is not mimicked by a central, locally generated, neuroinflammatory response.

TABLE 3
Secondary and tertiary study endpoints stratified by pregnancy-related hypertensive disorder

Analyte	Healthy (n=16)[a]	Chronic or gestational hypertension (n=16)[a]	Preeclampsia with or without severe features (n=16)[a]	*P* value[b]
CSF				
C5a, ng/mL	0.03 (0.0–0.06)	0.02 (0.0–0.03)	0.02 (0.0–0.05)	.92
C5b-9, ng/mL	0 (0–0)	0 (0–0)	0 (0–0.01)	.35
TNF-α, pg/mL	0 (0–0)	0 (0–0)	0 (0–0.42)	.27
IL-6, pg/mL	2.4 (1.8–2.9)	2.7 (2.1–3.2)	2.4 (2.0–3.2)	.78
Plasma				
C5a, ng/mL	34.2 (28.9–38.6)	40.6 (30.6–49.1)	41.0 (26.9–51.0)	.47
C5b-9, ng/mL	131 (95–158)	159 (136–264)	187[c] (134–234)	.05
TNF-α, pg/mL	0.47 (0–1.7)	1.54 (0.4–2.9)	0.69 (0–2.6)	.17
IL-6, pg/mL	3.2 (2.5–4.1)	4.0 (3.6–9.1)	8.7[c] (2.8–17.6)	.04
Urine				
Albumin, mg/dL	11.0 (6.0–17.0)	12.0 (5.0–19.0)	27.0 (10.5–43.5)	.07
C5a, ng/mL	0.27 (0.11–0.42)	0.21 (0.08–0.28)	0.44[d] (0.22–1.2)	.02
C5b-9, ng/mL	0.26 (0.13–0.91)	0.29 (0.14–0.82)	3.7[e] (1.6–14.6)	<.001

[a] Data are given as median (interquartile range); [b] Nonparametric equality of medians test, with values indicating results of testing between all 3 study groups; values for pairwise comparisons are not significant ($P > .05$), unless otherwise noted; [c] $P = .03$, preeclampsia vs healthy control subjects; [d] $P = .005$ vs control subjects with chronic or gestational hypertension; [e] $P = .001$, preeclampsia vs healthy control subjects, or control subjects with chronic or gestational hypertension.
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Clinical implications

Neurologic features of preeclampsia, such as headache and hyperreflexia, and more rare complications, such as seizure and posterior reversible encephalopathy syndrome, suggest that the blood-brain barrier is disrupted in preeclampsia and that neuroinflammatory factors may contribute directly or indirectly to neuronal excitation, brain edema, and seizure susceptibility. Preeclampsia has systemic inflammatory features,[32] and animal studies have shown that CNS excitability may be increased in response to peripheral inflammation. In nonpregnant rats, induction of intestinal inflammation leads to increased activation of brain microglia in the hippocampus, with increased seizure susceptibility that is reduced by inhibition of either TNF-α or microglial activation.[33] Similarly, when hippocampal slices from nonpregnant rats are exposed to pregnant serum, there is a TNF-α–dependent increase in neuronal excitability and seizure activity.[10] However, under normal pregnancy conditions, the brain and resident microglia within the brain are not exposed to maternal serum because of a restrictive blood-brain barrier.

In a pregnant rodent model of severe preeclampsia, which was characterized by reduced uteroplacental perfusion and a high-cholesterol diet, investigators found that blood-brain barrier permeability, neuroinflammation, and seizure susceptibility were increased.[34] Specifically, blood-brain barrier permeability was increased for small solutes (sodium fluorescein, 470 Da), but not larger compounds (Texas red dextran, 70 kDa); neuroinflammation was determined by an increase in microglial activation. Administration of magnesium sulfate lowered the seizure threshold in that model, mimicking the protective effect of magnesium sulfate that is seen in human preeclampsia.[5,34] Yet, to our knowledge, blood-brain barrier permeability has not been investigated in human preeclampsia, and we were unable to detect any degree of blood-brain barrier impairment in a well-defined group of preeclampsia subjects. Neither albumin (69 kDa) nor smaller compounds, such as IL-6 (21 kDa) or C5a (11 kDa), were increased in CSF of women with preeclampsia.

We investigated terminal complement proteins C5a and C5b-9 because human data have shown that these factors are increased specifically in preeclampsia.[13,14] Both C5a and C5b-9 have been associated with increased generation of inflammatory cytokines TNF-α and IL-6.[24,35,36] C5b-9 has been detected in CSF of adults with other CNS disorders[37-40]; the C5b-9 complex is capable of directly activating microglia to secrete TNF- α and other inflammatory mediators.[23] Although we confirmed systemic activation of the terminal complement pathway in preeclampsia, we did not detect increased CSF concentrations of either C5a or C5b-9, which suggests that terminal complement proteins do not access CSF in preeclampsia, neither through a leaky blood-brain barrier nor locally via brain parenchyma. We also found no evidence that TNF- α or IL-6 concentrations are increased in CSF in preeclampsia, decreasing the likelihood that terminal complement proteins mediate CNS effects indirectly through release of inflammatory cytokines. Our findings are supported by recent data that shows that brain microvascular endothelial cells have increased expression of complement regulators, which effectively reduce complement activation at the blood-brain barrier, even under TNF-α–stimulated conditions.[41]

Research implications

It remains possible that other serum factors cross the blood-brain barrier in preeclampsia. Recent studies have shown that CSF concentrations of alpha-1-microglobulin/bikunin precursor, alpha-1-microglobulin, activin A, and follistatin-related gene are increased in women with preeclampsia.[27,31] Meanwhile, others have shown that plasma concentrations of neuronal proteins, which include neurofilament light chain, tau, S100B, and neuron-specific enolase, are increased in women who experience preeclampsia.[42] However, in these studies, protein concentrations were not assessed in both plasma and CSF to determine if CSF proteins cross a permissive blood-brain barrier. Larger studies that will investigate a wider range of proteins in paired blood and CSF samples of women with preeclampsia, particularly those with more advanced disease, are suggested to validate our findings.

Strengths and limitations

The strengths of our study include its case-control design, with inclusion of both healthy and hypertensive control subjects, and paired collection of blood and CSF at time of delivery, which allowed us to investigate blood-brain barrier permeability formally in human preeclampsia. In addition, this was the first study to specifically examine albumin, C5a, C5b-9, TNF- α, and IL-6 in CSF of women with preeclampsia and control subjects. However, our study was not without limitations. First, the specific proteins that we chose to measure may not be fully indicative of blood-brain barrier integrity or neuroinflammation in preeclampsia. Second, we were unable to enroll subjects with eclampsia or posterior reversible encephalopathy syndrome; blood-brain barrier impairment is more likely in those circumstances. Finally, our results may be limited by small sample size. We were underpowered to detect small differences in protein concentrations, and Q_{alb} values were lower than expected, possibly because of low serum albumin and even lower CSF albumin concentrations in pregnant women. Yet, among the biomarkers that were studied in our analysis, there were no apparent trends between groups to suggest that a larger sample size would generate significant results.

Conclusions

We found no evidence of blood-brain barrier impairment in preeclampsia, using the CSF/plasma albumin quotient (Q_{alb}) and CSF measurements of C5a, C5b-9 TNF- α, and IL-6. Despite evidence of increased systemic inflammation and terminal complement activation, we were unable to detect an increase in neuroinflammatory markers in preeclampsia. Considering the prominence and morbidity associated

with neurologic features and CNS disturbances in severe forms of preeclampsia, more human studies are needed to investigate potential causative factors underlying these disease processes. ■

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