Cerebrospinal beta-amyloid peptides(1-40) and (1-42) in severe preeclampsia and HELLP syndrome – a pilot study

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During pregnancy, substantial alterations in cerebral plasticity, vascular remodeling and neuronal growth occur in the maternal brain. We investigated whether concentrations of selected neurodiagnostic biomarkers in the cerebrospinal fluid of women with preeclampsia/HELLP syndrome differ from those in healthy controls using enzyme-linked immunosorbent assay technique. We found that tau protein concentrations (p = 0.016) and phospho-tau/tau ratio (p < 0.001) in cerebrospinal fluid were significantly lower in 39 preeclamptic women compared to 44 healthy controls during third trimester of pregnancy. Beta-amyloid(1-40)/(1-42) ratio was significantly higher in HELLP syndrome than in severe preeclampsia (8.49 ± 2.73 vs. 4.71 ± 1.65; p = 0.007). We conclude that beta-amyloid(1-40)/(1-42) ratio in cerebrospinal fluid can discriminate severe preeclampsia and HELLP syndrome. High beta-amyloid peptide and low tau protein concentrations are associated with impaired development of the materno-feto-placental unit and correlate with placental dysfunction.

During pregnancy, the invasion of trophoblast cells into maternal tissue of the uterus and the conversion of spiral arteries into wide sinuses with low resistance and high flow are paramount for normal placental development1. In preeclampsia (PE) placental development is impaired by defective deep placentation, platelet and thrombin activation, intravascular inflammation, endothelial dysfunction and imbalanced angiogenesis2. Altered expression of proteins comes along with excess of antiangiogenic substances such as soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng) and decreased levels of proangiogenic substances like placental growth factor (PIGF) and vascular endothelial growth factor A (VEGF-A)3. Ciampa et al. observed in 13 patients with PE altered concentrations of proteins related to signaling pathways important for vascular remodeling, inflammation, and neuronal growth4.

Recent studies have shown that PE shares pathophysiologic features with recognized misfolding disorders and aggregation of proteins4–8. There are several dysregulated proteins in PE but it is not clear whether aggregated proteins induce defective trophoblast invasion5. D’Souza et al. reported that neurotrophic factors influence the development of the materno-feto-placental unit during pregnancy6. Altered blood-brain barrier and impaired cerebral autoregulation may affect cerebrovascular flow in the maternal brain7. Aggregated beta-amyloid peptides were observed in PE as well as in Alzheimer’s disease8. The presence of beta-amyloid aggregates in placentas of women with PE and intrauterine growth restriction (IUGR) further supports the notion that this condition goes with protein conformational disorders9,10. Moreover, it was observed that short peptides occupying the self-recognition sites of beta-amyloid inhibit beta-amyloid aggregation11.

The aim of this study was to determine whether CSF concentrations of beta-amyloid peptides and tau protein differ between women with PE and women with HELLP syndrome as compared to healthy pregnant women during the third trimester of pregnancy.

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Results

Patient characteristics. A total of 105 pregnant women who underwent spinal anesthesia participated; 39 women with PE/HELLP were consecutively assigned to a prospective research cohort. Forty-four of 66 pregnant women served as controls (Fig. 1). Demographic data including age, height, current weight and BMI were comparable between study group and controls. The number of previous pregnancies, miscarriages and parities did not differ between the two groups, but weeks of gestation were significantly fewer in women with PE/HELLP syndrome than in controls (33.4 ± 3.4 vs. 38.1 ± 1.1; p < 0.001).

Cardiorespiratory parameters and blood chemistry. In the study group we distinguished mild PE (n = 18), severe PE (n = 13) and HELLP syndrome (n = 8). According to the underlying pathomechanism systolic blood pressure (167.1 ± 16.9 vs. 121.4 ± 8.4; p < 0.001), diastolic blood pressure (103.9 ± 20.4 vs. 77.8 ± 8.4; p < 0.001) and pulse rate (82.5 ± 17.0 vs. 89.8 ± 13.4; p = 0.023) differed significantly between PE and HELLP syndrome and controls. In HELLP syndrome platelet count (85.6 × 10^9/L ± 25.5) was significantly lower than for mild PE (171.3 × 10^9/L ± 54.8; p = 0.002) or severe PE (178.0 × 10^9/L ± 47.1; p = 0.001). In addition, sGOT in HELLP syndrome (263.9 U/L ± 470.4) was significantly higher than in mild PE (29.3 U/L ± 16.4; P < 0.001) or severe PE (33.5 U/L ± 15.0; p = 0.005).

Health status of the newly born. Mean baby weight differed significantly between mild PE (2,362 g ± 546.9) and severe PE (1,095 g ± 359.2; p < 0.001), as well as between severe PE and HELLP syndrome (1,993 g ± 543.7; p = 0.018). In a post hoc subgroup analysis placental weight (p = 0.021) and baby weight (p = 0.001) were significantly lower in cases with beta-amyloid(1-40) exceeding 5000 pg/mL compared to beta amyloid(1-40) beyond 5000 pg/mL.

Biomarkers. CSF concentrations of tau protein were significantly lower in 39 women with PE and HELLP syndrome than in controls (p = 0.016). Phospho-tau-181/tau ratio was significantly higher in women with PE/HELLP (p < 0.001). In the study group median concentrations of beta-amyloid peptides(1-40) were higher in HELLP syndrome (9,683 pg/mL ± 5,643; p = 0.023) than for severe PE (4,836 pg/mL ± 2,004), and the beta-amyloid(1-40)/(1-42) ratio differed significantly (8.49 ± 2.73 vs. 4.71 ± 1.65; p = 0.007) (Table 1).
Beta-amyloid(1-40) correlated with serum markers sFlt-1 ($r_s = -0.536; p = 0.001$) and PlGF ($r_s = 0.357; p = 0.042$). Tau protein correlated with serum markers sFlt-1 ($r_s = -0.330; p = 0.040$) and PlGF ($r_s = 0.356; p = 0.023$). Beta-amyloid(1-40)/(1-42) ratio was significantly higher in HELLP syndrome than in severe PE ($8.49 \pm 2.73$ vs. $4.71 \pm 1.65$; $p = 0.007$) (Table 1). Using post-hoc analysis with the Dunn-Bonferroni test, the beta-amyloid(1-40)/(1-42) ratio differed significantly between HELLP syndrome and severe PE ($p = 0.007$) and between HELLP syndrome and controls ($p = 0.049$) (Table 2).

**Table 1.** CSF beta-amyloid peptides (Aβ(1-42) and Aβ(1-40)), tau and phospho-tau-181 (p-tau-181) protein levels in 39 women in the study group with either mild preeclampsia (PE) or severe PE or HELLP syndrome, compared to 44 healthy controls at delivery. Values are expressed as median and standard deviation.

| CSF biomarkers          | Mild PE (n = 18) | Severe PE (n = 13) | HELLP syndrome (n = 8) | controls (n = 44) |
|-------------------------|------------------|-------------------|------------------------|-------------------|
| Aβ(1-42) [pg/ml]        | 1.158 ± 444.7    | 1.041 ± 143.3     | 1.071 ± 391.8          | 1.090 ± 166.3     |
| Aβ(1-40) [pg/ml]        | 7.691 ± 3.837    | 4.836 ± 2.004     | 9.683 ± 5.463          | 6.329 ± 2.114     |
| Aβ(1-40)/(1-42) ratio   | 6.40 ± 1.80      | 4.71 ± 1.65       | 8.49 ± 2.73            | 5.81 ± 1.97       |
| Tau [pg/ml]             | 246.7 ± 136.0    | 271.8 ± 127.8     | 240.1 ± 109.7          | 312.9 ± 127.9     |
| P-tau-181 [pg/ml]       | 42.9 ± 18.8      | 47.7 ± 19.1       | 44.9 ± 18.1            | 50.5 ± 18.1       |
| P-tau/tau ratio         | 0.19 ± 0.03      | 0.18 ± 0.02       | 0.19 ± 0.01            | 0.16 ± 0.01       |

**Table 2.** CSF beta-amyloid peptide(1-42)/(1-40) ratio among 39 women in the study group with either mild preeclampsia (PE) or severe PE or HELLP syndrome and 44 healthy controls at delivery. Statistical comparison was made using the Dunn-Bonferroni test. Significant $p$ values are indicated in bold.

|       | Mild PE (n = 18) | Severe PE (n = 13) | HELLP syndrome (n = 8) | Controls (n = 44) |
|-------|------------------|-------------------|------------------------|-------------------|
| Mild PE | 0.128            | 0.735             | 1.000                  | 0.006             |
| Severe PE |                   |                   |                        | 0.805             |
| HELLP syndrome |             |                   |                        | 0.049             |

**Discussion**

In our study mean concentrations of beta-amyloid peptides(1-40) were higher in women with HELLP syndrome than in preeclamptic women and healthy controls. Human placenta and thrombocytes abundantly express amyloid precursor protein (APP)\textsuperscript{5,6}. Presumably, thrombocytopenia influences metabolism of beta-amyloid(1-42) and (1-40)\textsuperscript{12}. Although the histopathologic profile and the range of placental lesions differ between PE and HELLP syndrome\textsuperscript{10}, it remains a matter of ongoing debate whether HELLP syndrome is a severe form of PE or a separate disease. We found that beta-amyloid(1-40)/(1-42) ratio can discriminate between severe PE and HELLP syndrome. The findings of our study support the hypothesis that HELLP syndrome is a distinct disease pattern and not simply a variety of severe PE.

In our study, median CSF concentrations of tau protein and the phospho-tau/tau ratio were significantly lower in women with PE and HELLP syndrome than in healthy controls. This is in agreement with recently reported diminished CSF tau and phospho-tau-181 protein concentrations in patients with placental dysfunction\textsuperscript{14,15}. During normal pregnancy there is an increase in tau protein concentrations\textsuperscript{16}. Presumably, diminished CSF concentrations in PE and HELLP syndrome imply fewer maternal brain adaptations. Virtanen et al. observed an inhibitory effect on tubule formation in third trimester preeclamptic women\textsuperscript{17}. Serum concentrations of angiogenic factors sFlt-1 levels were higher and PlGF were lower in third trimester preeclamptic women compared to healthy controls with increasing sFlt-1/PlGF ratio between the first and the third trimester\textsuperscript{18}. Unfortunately, sFlt-1 and PlGF have limited sensitivity for stratification of women with suspected PE\textsuperscript{19}. PE still lacks a reliable, early means of diagnosis or prediction, and a safe and effective therapy\textsuperscript{20}. So far, the diagnosis of PE and HELLP syndrome is mostly based on clinical findings and increasing sFlt-1/PlGF ratio. In particular, the onset threshold of plasma levels of the sFlt-1/PlGF ratio proved to be a valuable screening tool for detecting the imminent onset of PE within four weeks after blood sampling during the second trimester, namely at 19 to 31 weeks\textsuperscript{21}. Potentially, detection of amyloid-targeting fluorophores in the urine may contribute to early identification of PE patients during pregnancy\textsuperscript{22}. Affinity for conformational antibodies raised against aggregated beta-amyloid peptides and dysregulation in the APP proteolytic pathway may offer future diagnostic and therapeutic options\textsuperscript{6,8}.
performed in accordance with the relevant guidelines and regulations. Inclusion criteria for the study group were: pregnant women, 18 years of age or older, with evidence of PE/HELLP syndrome verified by clinical signs of hypertension, proteinuria and edema and, if available, positive sFlt-1/PlGF ratio. Exclusion criteria for the study group were: lacking written consent. Inclusion criteria for the control group were: women suffering from hypertension in pregnancy, thrombocytopenia, proteinuria, evidence of placental dysfunction, IUGR, as well as women with a history of PE or HELLP syndrome, cases lacking written consent. All study findings and documents were handled in strictest confidence. Enrolment of patients, data collection and analysis followed the CONSORT 2010 checklist of information to include when reporting a randomized trial. Recruitment of patients depended on pre-determined sample sizes (quota sampling according to power analysis) stratified by clinical diagnosis and voluntary participation.

Materials and methods

Women with PE/HELLP syndrome during pregnancy who underwent spinal puncture for regional anesthesia were consecutively enrolled during normal work time in a prospective cross-sectional research at the Department of Gynecology, Innsbruck Medical University Hospital. Healthy women with uncomplicated pregnancies who underwent a cesarean section under spinal block served as controls.

Ethical approval & patients consent. The Ethics Committee of Innsbruck Medical University approved this study (AN2017-0073 371/4.25). Written informed consent was obtained from all patients before participation with the understanding that anonymized data would be published in a scientific journal. All methods were performed in accordance with the relevant guidelines and regulations.

Study design and study population. Anesthetists from obstetrical anesthesia screened patients by medical history before participation. Blood chemistry (blood sugar, hemoglobin, serum protein) and kidney function parameters (blood urea nitrogen, creatinine, glomerular filtration rate, and urinary protein) were obtained from medical records. Circulatory and respiratory status (systolic blood pressure, pulse rate and oxygen saturation) were recorded before and during anesthesia. Perceived stress and physical activity were inquired, as they may affect incidence and severity of PE. We used a 5-point Likert scale with (1, very much; 2, much; 3, moderate; 4, poor; 5, very poor) for individual grading of conceptualization of estimated stress. Demographics, clinical characteristics and laboratory findings for participating women were recorded on a working chart. Inclusion criteria for the study group were: pregnant women, 18 years of age or older, with evidence of PE/HELLP syndrome verified by clinical signs of hypertension, proteinuria and edema and, if available, positive sFlt-1/PlGF ratio. Exclusion criteria for the study group were: lacking written consent. Inclusion criteria for the control group were: pregnant women, 18 years of age or older, in good general health. Participants were on no chronic medication other than multivitamins, magnesium and iron supplementation. Subjects were in particular free from past or present major psychiatric disorders other than mild depression, major neurological disease, gestational diabetes, and chronic renal and hepatic disease. Exclusion criteria for the control group were: women suffering from hypertension in pregnancy, thrombocytopenia, proteinuria, evidence of placental dysfunction, IUGR, as well as women with a history of PE or HELLP syndrome, cases lacking written consent. All study findings and documents were handled in strictest confidence. Enrolment of patients, data collection and analysis followed the CONSORT 2010 checklist of information to include when reporting a randomized trial. Recruitment of patients depended on pre-determined sample sizes (quota sampling according to power analysis) stratified by clinical diagnosis and voluntary participation.

Sample collection. All pregnant women undergoing a spinal block with direct access to CSF and meeting the inclusion criteria were eligible as study subjects. Spinal anesthesia was performed according to standard operating procedures of the Department of Anesthesiology and Critical Care Medicine. Following skin disinfection, a 20-gauge introducer needle (B Braun, 34209 Melsungen, Germany) was inserted into the mid-line lumbar region and directed towards the interspinous ligament. Then, a 25-gauge needle (Spinocan® pencil-point spinal
Thrombocytopenia in pregnancy, defined as a platelet count <100,000/μL, was observed in 10% of the studied population. Correlations were found between thrombocytopenia and physical activity, BMI, and blood pressure. The statistical analysis revealed significant differences in platelet counts between women with PE/HELLP syndrome and women with normal pregnancy using nQueryAdvisor v.7.0 software. Calculations were conducted with SPSS 25 (IBM SPSS Statistics Standard) using the Mann-Whitney U test as an indicator of multiple pairwise comparisons between more than two subgroups. The Kruskal-Wallis test was applied with Bonferroni correction. A post hoc subgroup analysis was performed for beta-amyloid(1-40) <5000 pg/mL and for beta amyloid(1-40) >5000 pg/mL. Group comparisons of the beta-amyloid(1-42)/(1-40) ratio were assessed by analysis of variance followed by the Dunn-Bonferroni test. Pearson's correlations (two-tailed, bivariate) were calculated for CSF marker levels and the post hoc test was used to detect differences in CSF biomarkers beyond two subgroups.

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Author contributions
W.L. conceived the idea, designed the study, prepared proposal and working charts, discussed and wrote the manuscript. H.S. conceived the clinical investigation and sample collection, wrote and discussed the manuscript. C.A.D. conceived the clinical investigation and sample collection, prepared the working charts, wrote and discussed the manuscript. J.T. and R.F. conceived the clinical investigation and sample collection, wrote and discussed the manuscript. L.D. performed statistical analysis, wrote and discussed the manuscript. G.P. conceived the clinical investigation and sample collection, wrote and discussed the manuscript. C.H. conceived the method for this study, supervised processing, wrote and discussed the manuscript. All authors were involved in the interpretation of the results and all authors approved the revised manuscript before its submission.

Competing interests
The authors declare that they have no conflict of financial and non-financial interests including personal relationships with other persons or organizations that could inappropriately influence, or be perceived to influence, the work.

Additional information
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