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学位論文題目 Development of a high-sensitivity method for the measurement of human nasal A β 42, tau, and phosphorylated tau

(ヒト鼻腔におけるA β 42、タウ、リン酸化タウの高感度測定法の開発)

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論文内容要旨

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学位論文題目	Development of a high-sensitivity method for the measurement of human nasal A β 42, tau, and phosphorylated tau ヒト鼻腔における A β 42、タウ、リン酸化タウの高感度測定法の開発		
<p><u>Purpose</u></p> <p>Alzheimer's disease (AD) is the most common form of dementia, which accounts for 60-70% of dementia cases. It is important to develop cost-effective, convenient and feasible methods for early diagnosis of AD. Previous studies have suggested that nasal mucosa might be one of the target tissues to measure AD biomarkers, such as Aβ42, Aβ40, tau and phosphorylated tau. Therefore, we aimed to develop novel methods to measure AD biomarkers in one nasal smear simultaneously and compare their levels between AD patients and normal people.</p> <p><u>Methods</u></p> <p>A total of 25 cases of Alzheimer's disease patients and 25 control individuals (22 men and 28 women) aged 63-85 years old were recruited in 2015-2016 for this case-control study. All participants provided written informed consent. This study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Shiga University of Medical Science (Approval number = 25-218). Nasal smears of the common nasal meatus, inferior concha, middle nasal meatus, and olfactory cleft were collected from all participants and proteins in samples were analyzed by two methods: PGD (pre-treatment with guanidine-n-dodecyl-beta-D-maltoside solution) method 1 (PGD-I) and method 2 (PGD-II). Measured protein concentrations and ratios were compared between patients with AD and control subjects in both PGD-I and PGD-II methods. Wilcoxon's rank sum test was used to compare median protein levels between the two groups. Receiver operating characteristics (ROC) curves and</p>			

(備考) 1. 論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、2千字程度でタイプ等を用いて印字すること。

2. ※印の欄には記入しないこと。

areas under the curve (AUC) were used to assess the ability of nasal protein levels in predicting AD. Youden's Index (sensitivity + specificity—1) was used to determine the optimal nasal protein level cut-offs. All statistical analyses were performed using SAS version 9.4. Two-tailed p-values of <0.05 were considered significant.

Results

The PGD-I method measured total tau and amyloid- β (A β)₄₂, but no differences in median levels of total tau and A β ₄₂ between AD cases and controls were found in any of the nasal locations. The PGD-II method measured A β ₄₂, total tau, and phosphorylated tau, but levels of A β ₄₀ in all nasal locations of both groups were near zero. Median levels of phosphorylated tau to total tau (p-tau/t-tau) ratios in the middle nasal meatus and in the olfactory cleft were significantly higher in AD cases than in controls, and could significantly predict AD. To assess diagnostic reliability, areas under the ROC curve were 0.74 (95% CL=0.52-0.95, p=0.030) for the middle nasal meatus and 0.72 (95% CL=0.52-0.92, p=0.029) for the olfactory cleft.

Considerations and Conclusion

PGD-II method could detect A β ₄₂, A β ₄₀, p-tau and t-tau from nasal smears, but levels of A β ₄₀ in all nasal locations of both groups were near zero. This method may be useful in distinguishing between AD patients and cognitively healthy individuals by assessing the p-tau/t-tau ratio in the olfactory cleft and middle nasal meatus.

学位論文審査の結果の要旨

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<p>(学位論文審査の結果の要旨) (明朝体 11ポイント、600字以内で作成のこと。)</p> <p>アルツハイマー病 (AD) 患者の脳に蓄積するアミロイドβ ($A\beta$) とタウ蛋白質 (総タウ、リン酸化タウ) を鼻腔スメアより高感度に測定する方法を開発し、診断における有用性を検討した研究である。スメアを2つの方法によってグアニジン HCl 溶液で溶解させた成分 (PDG-I, II) を ELISA によって解析した。その結果、以下の点が明らかとなった。</p> <ol style="list-style-type: none"> 1. AD 患者 25 名と対照健常人 25 名の嗅裂、中鼻道、下鼻甲介、総鼻道から採取したスメアにて、$A\beta$、$A\beta 42$、t-Tau、p-Tau は検出されたが、中間値の平均では両者の間に優位な差は認めなかった。 2. 一方、p-tau/t-tau 比の中間値を算出したところ、中鼻道と嗅裂において AD 群で優位に上昇していた。 3. 嗅裂と中鼻道における p-tau/t-tau 比の ROC 曲線における AUC は各々 0.738 と 0.722 であり、カットオフ値を 0.02 と 0.15 にすることで良好な感度特異度が得られた。 <p>本論文は、先制治療の開発が進むアルツハイマー病において、鼻腔スメアという非侵襲的な試料から、高感度にバイオマーカーを検出することを可能にした、画期的な診断手法であり、アルツハイマー病患者の診断と治療の発展に高く寄与する。最終試験として論文内容に関連した試問を受け合格したので、博士 (医学) の学位論文に値するものと認められた。</p> <p style="text-align: right;">(総字数 571 字)</p> <p style="text-align: right;">(平成 30 年 8 月 27 日)</p>			