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# Changes of ubiquitin C-terminal hydrolase-L1 levels in serum and urine of patients with white matter lesions

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ABSTRACT

*Objectives:* Ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1) has been established as a potential biomarker of neuronal damage. There is not much information about the effects of white matter lesions (WMLs) on serum and urine UCH-L1 levels in white matter disease patients. This study was aimed to assess whether serum or urine UCH-L1 levels are a reliable marker of brain damage in patients with WMLs.
*Design and methods:* Serum and urine levels of UCH-L1 were assessed in 125 patients with dizziness, hypertension, type 2 diabetes mellitus, or dyslipidemia. Of these 125 patient cases, 41 showed periventricular WMLs (P-WMLs), 46 showed subcortical WMLs (S-WMLs), and 38 displayed no well-defined WMLs (controls).
*Results:* Serum UCH-L1 levels were significantly different between the WML group and controls ($p < 0.05$). Further subgroup analysis proved that serum UCH-L1 levels in participants with S-WMLs were significantly increased when compared with controls ($p < 0.001$), but there was no significant differences between controls and patients with P-WMLs ($p > 0.05$). However, urine levels of UCH-L1 were similar between these three groups ($p > 0.05$). In addition, multivariate analysis showed that increased serum UCH-L1 levels were independently associated with the severity of WMLs using Fazekas scale ($\beta = 0.432$, $p < 0.001$).
*Conclusions:* These findings suggest that serum UCH-L1 levels may serve as a novel biomarker for neuronal damage from WMLs, especially S-WMLs.



## 1. Introduction

Brain damage markers released in cerebrospinal fluid (CSF), blood and urine may provide valuable information about diagnosis and prognosis in a determined altered physiological condition or disease status [1–3]. One putative marker is the neuronal-specific protein, ubiquitin C-terminal hydrolase-L1 (UCH-L1), present in almost all neuronal tissue and represents between 1 and 5% of total soluble brain protein [4]. It is mainly involved in regulating ubiquitin protein modification and has been reported to be indispensable for brain function, and required for normal cognitive function and synaptic plasticity [5,6]. UCH-L1 has been implicated in the pathogenesis of Parkinson's disease (PD) [7,8] as well as Alzheimer's disease (AD) [9,10]. In addition to PD and AD, UCH-L1 has been shown to be associated with other neurological disorders such as Huntington's disease [11] and epileptic seizures [12]. Recent studies have demonstrated that CSF and serum UCH-L1 levels were significantly elevated after acute neurologic insults such as ischemic stroke, subarachnoid hemorrhage, and traumatic brain injury [13–15]. These empirical findings have led to the suggestion that UCH-L1 may be a candidate brain injury biomarker.

Cerebral white matter lesions (WMLs), synonymous with leukoaraiosis, are a common finding on magnetic resonance imaging (MRI) of the brain. Lesions can be located periventricularly, subcorticaly or both [16]. The periventricular (P-WMLs) and subcortical WMLs (S-WMLs) might have different pathogenesis and may lead to different cognitive or motor performances. There is evidence that P-WMLs are peculiarly correlated to cognitive decline, whereas S-WMLs may be associated with late-onset depression [17]. Accumulating evidence suggests that WMLs are potentially related to increased risk of several neurological diseases including PD [18], AD [19], epileptic seizures [20], ischemic stroke [21] and TBI [22], which are all associated with UCH-L1. However, the molecular connection between UCH-L1 and WMLs was not fully established.

Thus, in the present study, we aimed to assess whether UCH-L1 was elevated in serum and urine from white matter disease patients

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compared to control subjects. This could deepen our understanding of the pathophysiology underlying this highly prevalent cerebral vascular disease.

## 2. Methods

### 2.1. Subjects

125 patients were enrolled into study from March 1st, 2014 to May 1st, 2015 at the neurology department of the First and Second Hospital affiliated to Dalian Medical University.

Inclusion criteria were: (1) patient underwent cerebral MRI and (2) available clinical data. Exclusion criteria were: (1) patients with leukoencephalopathy of nonvascular origin (immunological-demyelinating, toxic, infectious, other); (2) patients with brain injury, including brain trauma, acute cerebral ischemia, acute cerebral hemorrhage; (3) patients with brain tumors, dementia, psychoses; (4) patients with neurodegenerative disease; (5) patients with epilepsy; and (6) patients with liver or kidney dysfunction.

The present study was approved by the Institutional Ethic Committee of both Dalian Medical University, and followed the tenets of the Declaration of Helsinki. All participants provided an informed written consent.

### 2.2. MRI

All participants underwent a MRI examination using a Sonata 1.5-T MR scanner (Siemens, Erlangen, Germany) with a standard head coil. The MRI protocol included an axial 3D gradient-echo T1-weighted acquisition (TR, 20 ms; TE, 5.6 ms; flip angle, 25°; and voxel size, $1.0 \times 0.5 \times 2.0$ mm$^3$), an axial T2-weighted turbo spin-echo acquisition (TR, 5020 ms; TE, 106 ms; voxel size, $1.0 \times 1.0 \times 6.0$ mm$^3$), and a fluid attenuated inversion recovery (FLAIR) sequence (TR, 8000 ms; TE, 105 ms; TI [inversion time], 2300 ms).

WMLs were identified as hyperintense regions on T2-weighted images, without corresponding prominent hypointensity on T1-weighted images. When the largest WML diameter was directly adjacent to the ventricle, the lesion was defined as P-WML, otherwise as S-WML [23]. The degree of WMLs severity was rated by two experienced neuroradiologists who were blind to all clinical data, using the semiquantitative visual rating scale devised by Fazekas et al. [24]. Disagreements of imaging analysis were resolved by consensus. P-WMLs were scored on a four-point scale of increasing severity, according to the following: 0, normal; 1, 'caps' or pencil-thin lining; 2, smooth 'halo'; or 3, irregular periventricular hyperintensities extending into the deep white matter. Grade 0–1 was defined as P-WML (−), and grade 2–3 was defined as P-WML (+). S-WMLs were scored as follows: 0, normal; 1, punctuate foci; 2, beginning confluence of foci; or 3, large confluent areas. Grade 0–1 was defined as S-WML (−), and grade 2–3 was defined as S-WML (+). The reliability of the Fazekas scale is excellent for white matter lesions, with an intraoperator correlation coefficient of 0.85 and interrater agreement Cohen's kappa = 0.78 [25].

### 2.3. Clinical data

Each participant underwent a standardized clinical examination and completed a health history questionnaire survey to obtain demographic data, past medical history, and the use of antihypertensive medications, lipid-lowering medications, and oral hypoglycemic agents or insulin.

Sitting brachial blood pressure was measured using a routine procedure, and the mean of two measurements was used for analyses. Patients with systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg, or currently using antihypertensive medication were considered to be hypertensive.

### 2.4. Standard biochemistry

Blood and urine samples were collected in the morning after an overnight fast, and sent to the central clinical laboratory. Serum were obtained by centrifugation of blood samples at 3200 rpm/min for 10 min at 4 °C, and stored immediately at −80 °C. Fresh urine samples were centrifuged at 2000 rpm/min for 10 min at 4 °C, and supernatants were stored at −80 °C.

The levels of total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), fasting blood glucose (FBG), and homocysteine were measured using standard enzymatic techniques. Dyslipidemia was defined as TC ≥240 mg/dL; TG ≥200 mg/dL; LDL-C ≥160 mg/dL; and/or HDL-C <40 mg/dL. Diabetes mellitus was defined as FBG ≥7.0 mmol/L or current treatment with hypoglycemic agents or insulin. Patients were managed according to the standard of care.

### 2.5. Measurement of UCH-L1

All samples were analyzed within 15 months of collection. Levels of UCH-L1 in serum and urine samples were assessed in duplicate using commercially available ELISA kits (Cloud-Clone Corp., Houston, TX, USA), according to the manufacturer's instructions. The technician who performed the assays was blind to the clinical and radiological findings.

### 2.6. Statistical analysis

Statistical analysis was performed using SPSS 18.0 (IBM, Armonk, NY, USA). Results were expressed in percentages for categorical variables, expressed as mean ± standard deviation (SD) for normally distributed data, or expressed as median (interquartile range) for factors that were not normally distributed. The Mann–Whitney U-test was used to compare 2 groups; while the Kruskal–Wallis H-test was used to compare three groups. Linear regressions were used to examine the association between serum UCH-L1 concentration (dependent variable) and the grade of WMLs (independent variable). Correlations between serum UCH-L1 concentration and other variables were evaluated with Spearman's correlation coefficients. Two-tailed $p$ values $< 0.05$ were considered statistically significant.

## 3. Results

The mean age of the study population was 63.8 ± 11.5 years; 44% were men. Among the 125 participants, 41 showed P-WMLs (aged 69.0 ± 9.6, ranging from 46 to 93 years; male:female ratio, 21:20), 46 had S-WMLs (aged 62.7 ± 10.5, ranging from 39 to 82 years; male:female ratio, 18:28), and 38 showed no or marginal lesions (aged 59.4 ± 12.5, ranging from 37 to 80 years; male:female ratio, 16:22). Representative MRI scans are presented in Fig. 1. The demographic and clinical characteristics of these patients are presented in Table 1. Mean grade of P-WMLs was 2.2 ± 0.4, mean S-WMLs was 2.4 ± 0.5, and mean control was 0.3 ± 0.5 on the Fazekas scale (/3) ($p < 0.001$). Patients with P-WMLs were older in comparison with those with S-WMLs ($p < 0.01$) and without WMLs ($p < 0.001$). Moreover, patients with P-WMLs and S-WMLs were more likely to be hypertensive or have an antihypertensive medication ($p < 0.001$) than controls.

Serum and urine UCH-L1 levels in WML cases and controls are presented in Table 2 and Fig. 2. Serum levels of UCH-L1 were significantly higher in WML cases (n = 81) compared with controls (n = 35) [2.83 (1.90–6.44) vs. 2.06 (1.66–3.16) ng/ml; $p = 0.013$]. However, in urine, there were no significant differences found between these two groups [3.15 (2.98–3.51) (n = 51) vs. 3.20 (3.01–3.70) (n = 27) ng/ml; $p > 0.05$].