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The association of serum Kallikrein-8 with cognitive function in vascular dementia

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Abstract. – OBJECTIVE: Kallikrein-8 (KLK8) is a secreted serine protease related to learning and memory. Evidence has confirmed the important role of KLK8 in neuroplasticity. However, the role of KLK8 in vascular dementia (VaD) is unclear.

PATIENTS AND METHODS: The study recruited 88 VaD patients and 72 normal controls. All subjects were tested for cognitive function by Mini-Mental State Examination (MMSE) upon admission, and their demographic and biochemical data were collected. A sandwich Enzyme-Linked Immunosorbent Assay (ELISA) test was used to detect serum KLK8 levels. The demographic and biochemical data of the two groups of subjects were compared. Spearman's correlation and multivariate regression analysis were used to determine whether serum KLK8 in VaD patients is a risk factor for cognitive function.

RESULTS: A total of 88 VaD patients and 72 controls with normal cognitive function were recruited and divided into VaD group and control group. Except for TT3 (p=0.002), there was no statistically significant difference in other demographic and biochemical data between the two groups (p>0.05). The results of ELISA indicated that the serum KLK8 in VaD patients was significantly higher than that of the control population (p<0.001). Spearman correlation analysis indicated that the serum KLK8 in VaD was significantly inversely correlated with the MMSE score. The results of Spearman's correlation analysis showed that the serum KLK8 level of VaD was significantly inversely correlated with the MMSE (r=-0.305, p=0.017). After correcting for interference factors, the correlation between the two is still significant (β=0.398, p=0.024).

CONCLUSIONS: Serum KLK8 may be an independent risk factor affecting the cognitive function of VaD, which is worthy of further research.

Key Words:
Kallikrein-8, KLK8, Vascular dementia, Biomarker, Mini-Mental State Examination, MMSE.

Introduction

Vascular dementia (VaD) is a kind of brain dysfunction caused by cerebrovascular factors, mainly manifested as memory decline[1-3]. According to statistics, there were 35.6 million dementia patients worldwide in 2010, and this number will double and quadruple by 2030 and 2050, respectively[4,5]. VaD is the main cause of dementia in the elderly, second only to Alzheimer's disease (AD)[6]. VaD has a high prevalence in developing countries and is reported to account for 30% of all dementias in Asia. However, this figure is only 15%-20% in developed countries[7]. Like AD, there is currently no effective cure for VaD[8]. Therefore, it is particularly urgent to find a minimally invasive and highly effective VaD biomarker.

Kallikrein-8 (KLK8), also known as neuropsin, is a secreted serine protease, first reported by Zu-Lin Chen in 1995[9]. The human kininase gene is located on chromosome 19q13.4, and its family includes 15 members, which are widely distributed in the body. Among these, KLK8 is mainly expressed in brain tissue and is believed to be involved in the regulation of neurodevelopment, maturation, aging, and neuroplasticity[10]. In recent years, KLK8 has been proven to be an indicator for the diagnosis or prognosis of many diseases[11]. However, the underlying pathogenic mechanism of KLK8's involvement in the disease is still unclear.

The results of animal experiments show that KLK8 is highly expressed in the limbic system, especially the hippocampus, suggesting that it may be related to learning and memory[12]. Intriguingly, the role of KLK8 in AD has also been reported, which further confirms that KLK8 is involved in the maintenance of cognitive function. The purpose of our current study is to determine

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whether KLK8 is involved in the pathogenesis of VaD, and their correlation has not been reported so far.

Patients and Methods

Study Population

This study was approved by the Ethics Committee of The First People's Hospital of Lianyungang (LW-20180611001). According to the inclusion and exclusion criteria, the patients who were treated at The First People's Hospital of Lianyungang from August 2018 to August 2020 were detailed evaluated. A total of 88 VaD patients and 72 normal cognitive function controls were included in this study. Inclusion criteria are based on the diagnostic criteria of VaD in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[13]. Exclusion criteria include the following: (1) other types of dementia; (2) Serious organ dysfunction or failure; (3) History of mental illness or antipsychotic drugs; (4) History of tumor or autoimmune disease; (5) History of drug abuse or addiction; (6) Unable to complete the necessary testing or evaluation. The study complied with the Declaration of Helsinki and obtained written consent from the research subjects or guardians.

Demographic and Biochemical Data

Upon admission, demographic data of all research subjects were collected. The demographic data include age, gender and Body Mass Index (BMI). All blood samples were collected on an empty stomach within 24 hours of admission. At about 7:00 in the morning, a 5 ml blood sample was collected, centrifuged at a speed of 1000 g for 16 min, and the serum was separated and stored in a refrigerator at -80°C. Standardized laboratory methods are used to detect biochemical blood indicators. The enzymatic method is used to determine serum TT3, TT4, TSH, LDH, and HDL. A blood glucose monitor (Bayer HealthCare LLC., Mishawaka, IN, USA) was used to measure fasting blood glucose levels. Using commercial reagents (KLK8, Abcam, Cambridge, MA, USA), the detection of serum KLK8 levels uses the sandwich ELISA method. The specific steps of ELISA refer to previous reports and product manuals.

Evaluation of Cognitive Function

Marshall et al[14] first published the MMSE in 1975, and since then, it has been widely used as a screening tool for cognitive impairment. The total score of MMSE is 30 points, and the entire evaluation process takes about 10 minutes. It is highly maneuverable. Doctors and nurses can complete this short test only after a short period of training. MMSE includes the assessment of seven cognitive domains: time direction, space direction, attention and calculation, three-word retelling and registration, language function, and visual performance. Generally, 24 points are taken as the cutoff value[15]. The assessment of MMSE is carried out by the trained attending physician, who is blind to the subjects' demographic and biochemical.

Statistical Analysis

The Kolmogorov-Smirnov test was used to determine the distribution of the data. For descriptive purposes, data variables are expressed in terms of mean ± standard deviation (SD) or n (%). The comparison of continuous variables and categorical variables used independent sample *t*-test and chi-square test, respectively. Spearman correlation analysis was used to determine the correlation between cognitive dysfunction and demographic and biochemical data, and then, multivariate regression was used to analyze the independent contributions of modeling factors. All statistics were performed using SPSS 23.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Armonk, NY, USA) and were evaluated as two-tailed statistics. $p<0.05$ was considered statistically significant.

Results

Demographic and Biochemical Data

Compared with the control group, there were no significant differences in the age, gender, BMI, TT4, TSH, FBG, LDL, and HDL of VaD patients ($p>0.05$). In addition, the *t*-test showed that VaD patients have higher levels of TT3 ($p=0.002$) and higher serum KLK8 concentrations ($p<0.001$). The demographic and biochemical data of the research subjects are shown in Table I.

Spearman Correlation Analysis

Spearman correlation test was used for correlation analysis, and the results are presented in Table II. The results showed that there is a significant correlation between the MMSE score of VaD

Table I. Demographic and biochemical data.

	Controls (n = 72)	VaD (n = 88)	p
Age (years)	66.3 ± 3.4	66.8 ± 3.1	0.333
Male/female	42/30	54/34	0.697
BMI (kg/m^2)	24.2 ± 1.3	24.5 ± 1.6	0.202
TT3 (μg/L)	1.3 ± 0.2	1.4 ± 0.2	0.002
TT4 (μg/L)	81.6 ± 4.7	80.5 ± 4.1	0.116
TSH (uIU/mL)	1.5 ± 0.4	1.6 ± 0.3	0.073
FBG (mmol/L)	6.8 ± 2.0	7.1 ± 2.3	0.386
LDL (mmol/L)	2.7 ± 0.8	2.5 ± 0.9	0.144
HDL (mmol/L)	1.2 ± 0.3	1.3 ± 0.4	0.081
KLK8 (pg/ml)	226.4 ± 12.6	314.5 ± 17.7	< 0.001
MMSE (points)	28.1 ± 1.5	21.3 ± 2.2	< 0.001

Abbreviations: VaD, vascular dementia; BMI, Body Mass Index; TT3, Serum total triiodothyronine; TT4, Total serum thyroxine; TSH, Thyroid Stimulating Hormone; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high-density lipoprotein; KLK8, Kallikrein-8; MMSE, Mini-Mental State Examination.

patients and serum KLK8 (r=-0.305, p=0.017). However, the MMSE score has no evident correlation with age, gender, BMI, TT3, TT4, TSH, FBG, LDL and HDL (p>0.05).

Multivariable Regression Analysis

Table III shows the risk of VaD cognitive impairment based on multiple regression. After adjusting for risk factors, such as age, gender, BMI, TT3, TT4, TSH, FBG, LDL and HDL, the MMSE score of VaD patients still has a significant association with serum KLK8 levels (β=0.398, p=0.024). Serum KLK8 level may be an independent predictor of cognitive function in VaD patients.

Table II. PCorrelation between MMSE and demographic and biochemical data in VaD.

	r	p
Age (years)	-0.287	0.612
Male	-0.194	0.501
BMI (kg/m^2)	-0.316	0.397
TT3 (μg/L)	0.328	0.278
TT4 (μg/L)	0.309	0.139
TSH (uIU/mL)	-0.252	0.264
FBG (mmol/L)	-0.430	0.715
LDL (mmol/L)	-0.261	0.683
HDL (mmol/L)	0.273	0.436
KLK8 (pg/ml)	-0.305	0.017

Abbreviations: MMSE, Mini-Mental State Examination; VaD, vascular dementia; BMI, Body Mass Index; TT3, Serum total triiodothyronine; TT4, Total serum thyroxine; TSH, Thyroid Stimulating Hormone; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high-density lipoprotein; KLK8, Kallikrein-8.

Discussion

The positive finding of this study is that the serum KLK8 level of VaD patients is significantly higher than that of the control population with normal cognitive function, and the serum KLK8 level is inversely correlated with the MMSE score of VaD patients. This association is not affected by age, gender, BMI, TT3, TT4, TSH, FBG, LDL, and HDL. As far as I know, there is no research report on the correlation between serum KLK8 level and cognitive function in VaD patients. This finding has certain clinical significance, and it can be extended to patients with the same characteristics.

In 1878, Paul Pierre Broca proposed the concept of the limbic lobes and applied the limbic lobes to the curved edges of the cortex, including the cingulate and hippocampus. The limbic system is composed of the phylogenetic ancient hippocampal marginal lobe and other subcortical structures and their connections[16]. Cognitive function is mainly caused by the function of limbic system hippocampus and amygdala. Emotions have a powerful influence on learning and memory. Together with the prefrontal and medial lobes, the amygdala is involved in consolidating and retrieving emotional memories[17]. However, the hippocampus is related to the storage of new memories, and this process requires the participation of the adjacent cortex, the parahippocampal area, the intraolfactory area and the periolfactory area[18]. The degenerative changes of the limbic system may play a role in the occurrence of neurodegenerative diseases, such as Pick's disease and Alzheimer's disease, mainly manifested as

Table III. Multivariable analyses of demographic and biochemical data with MMSE in VaD.

	Regression coefficient	*p*	95% CI
Age (years)	0.219	0.183	0.192-1.103
Male	0.254	0.471	0.221-1.098
BMI (kg/m^2)	0.307	0.216	0.274-1.124
TT3 (μg/L)	0.425	0.354	0.403-1.265
TT4 (μg/L)	0.383	0.299	0.316-1.271
TSH (uIU/mL)	0.271	0.302	0.215-1.182
FBG (mmol/L)	0.332	0.137	0.311-1.087
LDL (mmol/L)	0.160	0.565	0.109-1.116
HDL (mmol/L)	0.246	0.328	0.188-1.130
KLK8 (pg/ml)	0.398	0.024	0.237-0.859

Abbreviations: MMSE, Mini-Mental State Examination; VaD, vascular dementia; BMI, Body Mass Index; TT3, Serum total triiodothyronine; TT4, Total serum thyroxine; TSH, Thyroid Stimulating Hormone; FBG, fasting blood glucose; LDL, low density lipoprotein; HDL, high-density lipoprotein; KLK8, Kallikrein-8.

atrophy of the limbic system[19]. Therefore, the limbic system plays an important role in behavior and cognition, and the exact mechanism needs to be further clarified.

KLK8 is a serine protease that acts in the central nervous system[20]. It is expressed in the hippocampus, the lateral nucleus of the amygdala, and other areas of the limbic system, which are all involved in learning and memory[21]. KLK8 is divided into type 1 with 260 amino acids and type 2 with 305 amino acids according to different splicing methods[22]. Scientists have compared and analyzed the gene sequences of different primate species and found that KLK8 (type 2) is preferentially expressed in the human brain, which may help explain the origin of human cognition[23]. Li et al[24] reported that KLK8 is involved in the pathophysiological process of cognitive function. The mechanism by which KLK8 participates in learning and memory is related to N-Methyl-D-aspartate (NMDA) receptor and bispecific mitogen-activated kinase 1 (MEK1)[25]. It has also been pointed out[26] that activated KLK8 participates in NMDA receptor-dependent synaptic plasticity by cleaving synaptic adhesion molecule L1 (L1CAM or NCAML1) in the presynaptic zone, thereby improving LTP. Tamura et al[27] pointed out that KLK8 regulated the neurotransmission of aminobutyric acid (GABA) through neurotonin 1 (NRG-1) and its receptor and regulates neuroplasticity. Konar et al[28] pointed out that KLK8-mediated MAP2c deletion inactivates PKA, and downstream transcription factors phosphorylate CREB (pCREB), leading to impaired memory consolidation.

Recently, KLK8's involvement in the pathogenesis of AD has been widely reported. Sarah Teuber-Hanselmann et al[29] found that the levels of cerebrospinal fluid and blood KLK8 in AD patients were significantly higher than normal controls, and its predictive value for AD was equivalent to the traditional biomarker Aβ42 of AD. Similar to the results of the above study, a Yousef et al[30] also detected that the significant increase in KLK8 expression in AD may impair the plasticity of the hippocampus by promoting the over-regulation of the extracellular matrix. The problem that women seem to be more likely to suffer from AD has plagued the scientific community for a long time. Recently, Keyvani et al[31] systematically compared the effects of gender on various aspects of AD-related pathology and found that KLK8 overexpression may be a potential cause of the prevalence of Alzheimer's disease in women[31]. Interestingly, the same research team further confirmed that downregulation of KLK8 can improve the neuroplasticity of AD mice[32]. Although reports of KLK8's involvement in AD continue to emerge, its underlying mechanism should be still investigated.

The kallikrein-kinin system (KKS) was first discovered in a study of human urine 70 years ago because it has the effect of lowering blood pressure[33,34]. KKS is considered to be the main mechanism against the harmful effects of the overactive renin-angiotensin system (RAS)[35]. The interaction of KKS and RAS maintains blood pressure balance. In our study, VaD patients have higher serum KLK8 levels, but the MoCA score is low, which seems strange. There

are two reasons to partially explain this problem. One reason is that KLK8 can relax blood vessels and lower blood pressure, further aggravating the cerebral ischemia and cognitive decline in VaD patients, which is manifested as a decrease in the MoCA score. Another reason is that based on the inconsistent conclusions of different studies, we speculate that the effect of KLK8 on memory function may depend on the upper and lower limits at the same time[27].

So far, KLK8's involvement in VaD research has not been reported. We indicated for the first time that elevated serum KLK8 is an independent predictor of cognitive impairment in VaD patients, which is the strength of our study. However, our research also has some limitations: first of all, our sample size is small, it is a single-center cross-sectional study, and its conclusions may not be universal. Secondly, we did not detect the KLK8 level in the CSF of VaD patients, so we cannot compare the difference between serum and CSF in predicting the cognitive impairment of VaD. Finally, we did not consider the effect of gender on KLK8 predicting differences in cognitive impairment in VaD patients. However, there is no significant difference in gender between the VaD group and the control group in this study, which reduces the bias of gender on the conclusion.

Conclusions

To our knowledge, our study reported for the first time that KLK8 is involved in the pathophysiological process of VaD. Therefore, KLK8 may be a novel biomarker for VaD, which provides new options for clinical treatment of VaD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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Data Available Statement

The data used to support the findings of this study are available from the corresponding author upon request.

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