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Serum 1, 25-Hydroxyvitamin D: A Useful Index of Cognitive and Physical Functional Impairment in Healthy Older Adults in Japan: A Pilot Study

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Abstract

We enrolled 23 Japanese men (age: 76.0 ± 8.7) and 17 women (age: 78.3 ± 9.3) in this study. The physical function of even a person getting on a wheelchair could be tested in all subjects. Blood was collected by venipuncture and the serum 1, 25-hydroxy vitamin D (1, 25OHD) concentration was measured. The Mini-Mental State Examination (MMSE) and the Montreal Cognitive Assessment-Japanese version (MoCA-J) was used for the cognitive function test. Physical function was measured objectively using the Timed UP and Go (TUG) and 4-m walking test (4MWS). A significant positive correlation was found between serum 1, 25OHD and MMSE or MoCA-J. It is expected that an elderly person can maintain a mean serum 1, 25OHD level of about 100 pg/mL for preventing early cognitive disorder. In the present study, a significant positive correlation was found between urinary 25-hydroxy vitamin D (25OHD)/creatinine and MMSE or MoCA-J. Our results showed that urinary 25OHD might be a useful biomarker for predicting cognitive disorder. There was a significant negative correlation between serum 1, 25OHD and TUG or 4MWS. These findings suggest that serum 1, 25OHD levels might serve as a useful index to improve cognitive and physical functional impairment.

Keywords

Serum 1, 25-Hydroxyvitamin D, Cognitive Function, Physical Function, Aging, Urinary 25-Hydroxyvitamin D

1. Introduction

The proportion of older individuals in the population has been rapidly increasing in developed countries. In 2015, the average life expectancy of Japanese male and female was reportedly 80.5 and 86.8 years, respectively [1]. Japan has the highest proportion of older adults and so-called "Super-aged society" in the world. These results suggested that the prevalence of both cognitive and physical functional impairment increases with age. They experience difficulty in cognitive function, walking, housework and shopping in daily life. These are the main reasons for becoming bedridden or requiring nursing care.

Vitamin D is a secosteroid associated with peripheral calcium homeostasis [2]. Vitamin D is available in vitamin D2 of plants and D3 of animals. Both vitamin D2 and D3 are biologically inert and require activation through two hydroxylation processes involving 25-hydrooxylase and 1α-hydroxylase, located in the liver and kidney, respectively [3]. 1, 25-Dihydroxyvitamin D (1, 25OHD) is a biologically active metabolite produced by two steps of hydroxylation reactions [4].

Recent evidence has identified a much broader physiological role for vitamin D including its neuroprotective effect [4]. The Mini-Mental State Examination (MMSE) was used for the cognitive function test developed by Folstein *et al.* in 1975 which is commonly used for dementia screening [5]. Vitamin D supplementation caused significant improvement in the cognitive performance by MMSE score in Alzheimer's disease [6]. The Montreal Cognitive Assessment (MoCA) may be better at detecting early cognitive dysfunction [7]. The low 25-hydroxy vitamin D (25OHD) level has been recently associated with greater risk of cognitive impairment in older as well as younger adults using the MoCA Arabic version [8]. These results suggested that serum vitamin D is the index of cognitive function. 1, 25OHD enters and acts on vitamin D target cells at the level of gene transcription [9]. Therefore, we focused on the serum 1, 25OHD levels.

Physical performance tests included balance, lower limbs muscular strength and walking speed. Vitamin D3 supplementation improves muscle function and physical performance in the elderly population using 4-meter walking test (4MWS) [10]. The Timed UP and Go (TUG) test of functional mobility is assessed by asking the participant to stand up from a standard chair, walk a 3 meter, turn, walk back to the chair and sit down again [11]. Lower serum 25OHD is associated with poorer functional mobility using walking speed, TUG, and cognitive function using MMSE [12]. However, the effectiveness of serum 1, 25OHD concentration on physical performance has not yet been tested.

Therefore, the present study was designed to investigate the effect of 1, 25OHD in cognitive and physical functional impairment in healthy older adults in Japan. Our goal was to estimate the serum 1, 25OHD cut-off value from the results of the cognitive tests (MMSE and MoCA) and physical performance measurement (4MWS and TUG test), and to predict the cognitive and physical function levels from the urine 25OHD concentration.

2. Methods

2.1. Subjects and Setting

Prior to the study, approval was obtained from the ethics committee of Kyoto Bunkyo Junior College (project registration number in 2016: 7) and Aichi Medical College for Physical and Occupational Therapy (Project registration number in 2016: 468). A total of 40 healthy adults age ≥ 65 years were included in adult day-care center clients in Kyoto (n = 16), Fukui (n = 8) and Aichi Prefectures (n = 16). These areas with varying daylight hours were selected. The annual daylight hours were maximum in Aichi (2255 hrs: the 4th in Japan), and minimum in Fukui (1788 hrs: the 37th in Japan) among the three areas. The researchers attended the adult day-care center and assured the proper management of safety and confidentiality of the study. The manager of the adult day-care center invited participation in the study, and all the subjects whose participation was requested selected from April to Jun in 2016. After obtaining informed consent from a family member belonging to the same household, we enrolled 23 Japanese men (age: 76.0 ± 8.7) and 17 women (age: 78.3 ± 9.3) in this study. The physical function of even a person getting on a wheelchair could be tested among all subjects.

2.2. Cognitive Function Test

MMSE was used for the cognitive function test. It consists of five downstream items of orientation, memory, attentiveness for calculations, speech function, and design capacity. The maximum score for the MMSE is 30 points, and individuals with a score of 24 points were recommended [5]. The Montreal Cognitive Assessment-Japanese version (MoCA-J) was used for the cognitive function test. These tests were performed by verbal questioning of 5- to 10-min duration by skilled occupational and physical therapists. The maximum score for the MoCA-J is 30 points, and individuals with a score of 26 points were the recommended [6].

2.3. Physical Function

Physical function was measured objectively using TUG and 4MWS tests performed by skilled physical therapists. The cut-off values for the predicting the level of risk of falls in community-dwelling elders are 13.5 sec in TUG [13] and 1.0 m/sec in 4MWS [14], respectively.

2.4. Serum 1, 25OHD and Urinary 25OHD Assay

Blood was collected by venipuncture and serum 1, 25OHD concentration was measured by Kyoto Biken Laboratories Inc. (Kyoto, Japan), Nikken Igaku Co. (Fukui, Japan) and Falco Holdings Co. (Kyoto, Japan). Urinary samples were centrifuged at 1500 rpm for 10 min and stored at −30°C for later analysis. On the day of assay, samples were thawed and 25OHD was assayed using an enzyme-linked immunosorbent assay kit (Immundiagnostik AG, USA). Creatinine was assayed using a commercial kit (Bioassay Systems, USA) and 25OHD/creatinine ratios were compared.

2.5. Statistics

Relationships between MoCA-J, MMJSE, TUG, 4 MWT and serum 1, 25OHD or urinary 25OHD/creatinine were evaluated using Pearson's correlation coefficient. A p-value < 0.05 was considered to be statistically significant. Analyses were carried out using SPSS 21 for Windows (IBM, Japan).

3. Results

3.1. Study Subjects

Characteristics of the study subjects are shown in Table 1. Mean age was 76.0 years for males (n = 23) and 78.3 years for females (n = 17). Obesity was defined as a body-mass index (BMI) $\geq$ 25 Kg/m^2. The prevalence of obesity defined from BMI was 26.1% in males and 41.2% in females.

3.2. Serum 1, 25OHD and Cognitive Function Relationship

There was a significant positive correlation between serum 1, 25OHD and MMSE or MoCA-J. Pearson's correlation coefficient (2-tailed) of 0.360, t = 2.31, and p = 0.026 was obtained for MMSE and 0.448, t = 3.010, and p = 0.005 for MoCA-J. The serum 1, 25OHD cut-off value was 26.4 pg/mL for MMSE (24 points) and 97.6 pg/mL for MoCA-J (26 points) (Figure 1, Table 2). These results suggested that serum 1, 25OHD protects cognitive function.

Table 1. Characteristics of the study subjects.

	Mean	S. D.
Age (y)	77.0	9.0
Body height (cm)	156.8	8.2
Body weight (Kg)	59.7	10.3
BMI (Kg/m^2)	24.3	3.8
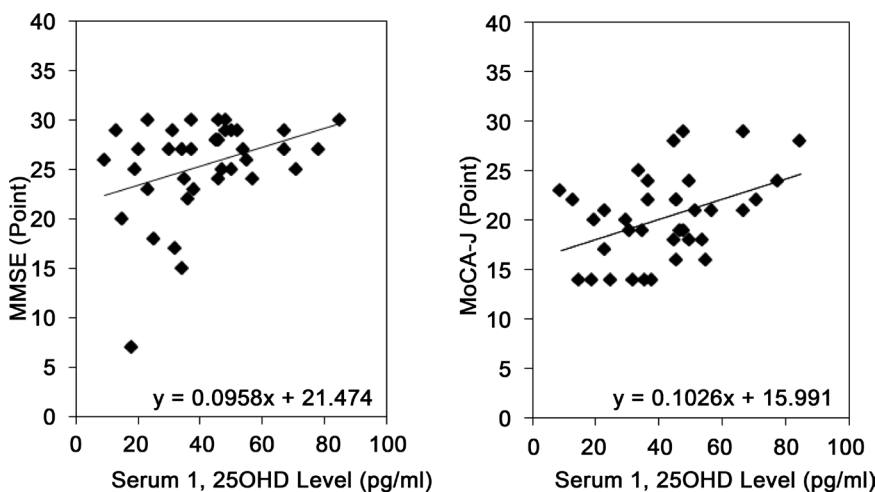
Figure 1. Effects of serum 1, 25OHD on cognitive function.

Table 2. The level of the serum 1, 25OHD and urinary 25OHD cut-off values for cognitive function.

	Cognitive Function	
	MMSE	MoCA-J
Serum 1, 25OHD (pg/mL)	26.4	97.6
Log (Urinary 25OHD/creatinine)	2.6	4.9
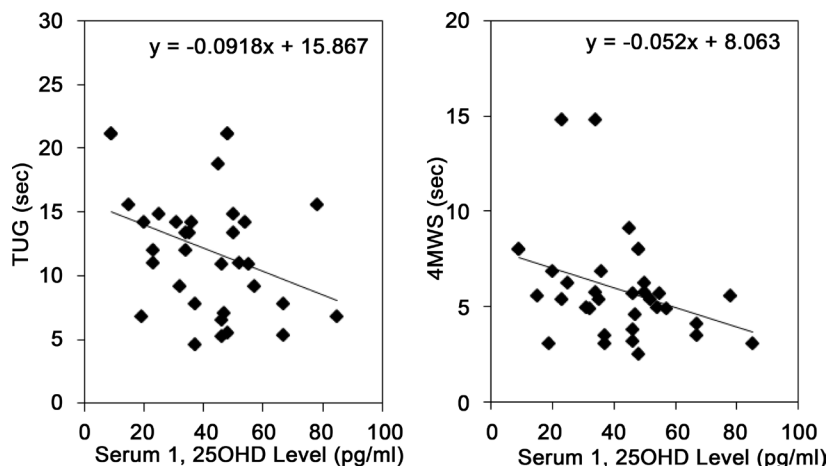
Figure 2. Effects of serum 1, 25OHD on physical function.

Table 3. The level of the serum 1, 25OHD cut-off values for physical functions.

|	TUG	4MWS
Serum 1, 25OHD (pg/mL)	25.8	78.1

3.3. Serum 1, 25OHD and Physical Function Relationship

There was a significant negative correlation between serum 1, 25OHD and TUG or 4MWS. Pearson's correlation coefficient (2-tailed) of −0.338, t = 2.031, and p = 0.050 was obtained for TUG and −0.336, t = 2.022, and p = 0.050 for 4MWS. Serum 1, 25OHD cut-off value was 25.8 pg/mL for TUG (13.5 sec) and 78.1 pg/mL for 4MWS (4 sec) (Figure 2, Table 3). These findings suggest that serum 1, 25OHD levels could contribute to improve physical function.

3.4. Urinary 25OHD and Cognitive Function Relationship

There was a significant positive correlation between urinary Log (25OHD/creatinine) and MMSE or MoCA-J. Pearson's correlation coefficient (2-tailed) of 0.400, t = 2.62, and p = 0.012 was obtained for MMSE and 0.405, t = 2.657, and p = 0.012 for MoCA-J (Figure 3, Table 2). These findings suggest that urinary 25OHD might be a useful biomarker for predicting cognitive disorder.

4. Discussion

In the present study, there was a significant positive correlation between serum 1, 25
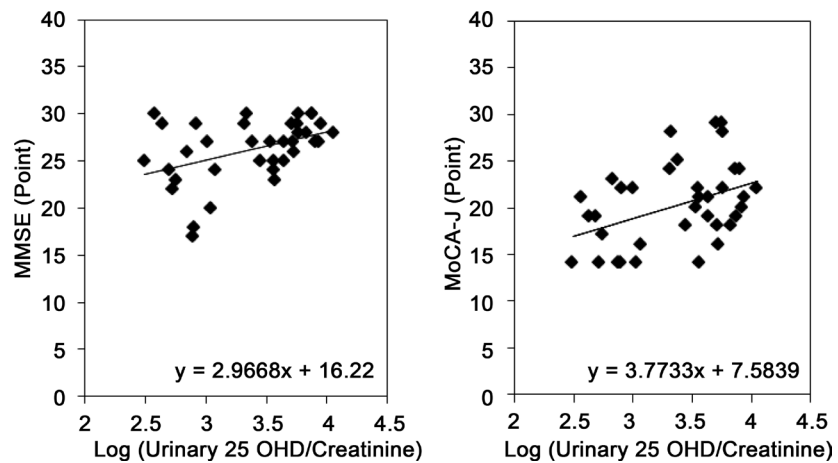
Figure 3. Effects of urinary 25OHD on cognitive function.

OHD and MMSE or MoCA-J. The MoCA may be a more challenging test for cognitively intact individuals. The total scores on the MoCA were lower than those on the MMSE [15]. In our results, the cut-off value of 1, 25OHD in MoCA-J was about ten-fold higher than that in MMSE. 1, 25OHD plays a neuroprotective role in human brain pericytes in culture [16]. Serum 25OHD was positively associated with cognitive performance [7] [8]. The circulating 1, 25OHD is produced from 25OHD in the kidney [4]. These results suggested that serum 1, 25OHD protects cognitive function. It is expected that an elderly person maintains a mean serum 1, 25OHD level of about 100 pg/mL for preventing early cognitive disorder. This level is higher than the normal circulation level (10 - 80 pg/mL) [9]. One must be careful about balanced diets and nutrition as well as excess avoidance of ultraviolet light.

The present study demonstrated a significant positive correlation between urinary 25OHD/creatinine and MMSE or MoCA-J. To our knowledge, ours is the first study to examine the effect of urinary 25OHD on cognitive function. Urine collection is non-invasive and readily available. In conclusion, our results show that urinary 25OHD might be a useful biomarker for predicting cognitive disorder.

The serum 25OHD concentration is known to contribute to physical performance [10] [12]. Our results suggested that there was a significant negative correlation between serum 1, 25OHD and TUG or 4MWS. These findings suggest that serum 1, 25OHD levels could contribute to improving physical function. Japan has the world's highest proportion of older adults. Locomotive syndrome means being restricted in one's ability to walk or lead a normal life owing to dysfunction in one or more of the parts including muscles [17]. These findings show that rehabilitation associated with serum 1, 25OHD as an index may be beneficial.

Over expression of reactive oxygen species (ROS) stimulated by the disruption of cerebral blood flow is one of the main causes of vascular dementia-induced cognitive deficits and behavioral dysfunction in the rat model [18]. Skeletal muscle mitochondrial function declines and oxidative stress increases with advancing age [19], and these changes have been implicated in the etiology of sarcopenia [20]. 1, 25OHD was re-

ported to have a significant physiological antioxidant activity [21]. Thus 1, 25OHD passively acts by activation of the antioxidant pathway. In conclusion, antioxidant 1, 25OHD may be recommended as a supplement to maintain cognitive and physical function.

The limitation of this study includes small sample size and possible selection bias. Further subjects are needed.

Acknowledgements

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