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# Impact of cystatin C and microalbuminuria on cognitive impairment in the population of community-dwelling Japanese

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ABSTRACT

*Background and aims:* Cognitive impairment is an important element affecting our well-being, and as such, early diagnosis is critical today. We investigated whether serum cystatin C and microalbuminuria are associated with cognitive impairment.

*Methods:* A total of 1943 subjects (774 males, 1169 females, mean age 65.8 years) took part in the investigation, and underwent a health examination in Tanushimaru, Japan, in 2009. The participants' cognitive function was evaluated using of mini-mental state examination (MMSE). We measured the levels of serum cystatin C using latex nephelometric immunoassay. Spot urine samples were used to measure microalbuminuria levels. Multivariate linear regression analyses were used to assess the relationship between MMSE scores and the level of cystatin C or microalbuminuria. All statistical analyses were performed using the SAS system.

*Results:* The mean values of log-transformed serum cystatin C levels and log-transformed microalbuminuria were 0.95 (range 0.41–7.11) mg/L and 10.7 (range 1.1–2600) mg/g·Cr, respectively. The means of MMSE score were 27.7 ± 2.5. In the multivariate linear regression analyses adjusted for age and sex, MMSE was significantly associated with systolic blood pressure ($p = 0.024$, inversely), cystatin C ($p = 0.046$, inversely) and microalbuminuria ($p = 0.019$, inversely), whereas estimated glomerular filtration rate (eGFR) had an insignificant association ($p = 0.197$). In the multiple stepwise linear regression analysis, age, history of stroke, systolic blood pressure, serum cystatin C were independently associated with MMSE levels.

*Conclusions:* We demonstrated for the first time that cognitive function was significantly and inversely associated with cystatin C and microalbuminuria, in the relatively younger general population.



## 1. Introduction

According to the Global Burden of Disease estimates for the 2003 World Health Report (WHO) [1], dementia contributed 11.2% of years lived with disability in people aged 60 years and older, which was more severe than stroke (9.5%), musculoskeletal disorders (8.9%), cardio-vascular diseases (5.0%) and all forms of cancer (2.4%) [2]. Although people with dementia are heavy consumers of health services, direct costs in the developed countries arise mostly from community and residential care [3].

The systematic review and meta-analysis suggested that chronic kidney disease (CKD) is one of the significant and independent somatic risk factors in the development of cognitive decline [9]. Some reports regarding the older adults also revealed that lower kidney function has higher risk of worsening cognitive function [10,11] and incident frailty [12]. Recently, both cystatin C and microalbuminuria have been reported as useful confirmatory markers for early kidney dysfunction [13]. Some studies evaluated the relationship between cystatin C and cognitive function in a community-based cohort of older adults, which indicated that higher levels of cystatin C are associated with worse cognitive function [14–16]. Several other studies also investigated the relationship between microalbuminuria and cognitive function in the elderly with peripheral arterial disease [17,18] and with impaired

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glucose tolerance [19,20]. Although the report from Murray et al. [22]. mentioned the usefulness of both cystatin C and microalbuminuria on the evaluation of cognitive function in the ACCORD study, they reported the significance of cystatin C, although the participants were diabetic people with $HbA_{1c}$>7.5%, at high risk for cardiovascular diseases.

Cystatin C is an inhibitor of cysteine proteinase, released into the bloodstream, which levels are independent of age, sex, and muscle mass, compared to serum creatinine. Microalbuminuria levels indicate the feature of glomerular filtration. In addition to early kidney dysfunction, these two biomarkers are also known as indicators of endothelial dysfunction or atherosclerosis, as one of major risks of cerebrovascular and cardiovascular diseases.

However, serum cystatin C and microalbuminuria have not been simultaneously examined as biomarkers of cognitive impairment. Therefore, we investigated whether the simultaneous measurement of serum cystatin C and microalbuminuria can be a useful confirmatory parameter for cognitive impairment in the general population.

## 2. Patients and methods

### 2.1. Patients

Tanushimaru Study is a cohort of the Seven Countries Study [4], which began in 1958. Tanushimaru is a rural farming community located in southwestern Japan. Although the Seven Countries Study ended in 1989, we continued the epidemiologic study in the same district. In the Tanushimaru Study, we have performed epidemiological studies every 10 years and a follow up of the participants every year. In 2009, a periodic epidemiologic survey was performed in this district [4,6–8,23,24]. As previously reported, the demographic backgrounds of the subjects in this area are similar to those of the Japanese general population [5]. Out of the total population of 4687 subjects (2151 men and 2536 women) aged over 40 years in this district in 2009, 1943 people (774 men and 1169 women, mean age 65.8 years) agreed to receive our health examination, all of whom were enrolled in this study with written informed consent. The baseline data were collected between May and November 2009.

### 2.2. Methods

The subjects' medical history, history of cardio-cerebrovascular diseases, use of alcohol and smoking were ascertained by a questionnaire. Alcohol intake and smoking were classified as current habitual use or not. Height and weight were measured, and body mass index (BMI) was calculated as weight (kilograms) divided by the square of height (square meters) as an index of obesity. Waist circumference was measured at the level of the umbilicus in the standing position. Blood pressure (BP) was measured in the supine position twice, at 3-min intervalsm using an upright standard sphygmomanometer. Vigorous physical activity and smoking were avoided for at least 30 min before BP measurement. The second BP with the fifth-phase diastolic pressure was used for the analysis. Hypertensive subjects were defined as those with systolic BP $\geq$ 140 mmHg and/or those with diastolic BP $\geq$ 90 mmHg and/or those receiving antihypertensive medication. Subjects with fasting plasma glucose (FPG) $\geq$6.99 mmol/l (126 mg/dl), subjects with glycosylated hemoglobin $A_{1c}$ ($HbA_{1c}$ NGSP) $\geq$6.5%, and/or subjects taking oral hypoglycemic agents or receiving insulin injection were diabetic. Subjects with dyslipidemia were defined as those with low density lipoprotein cholesterol (LDL-c) $\geq$3.62 mmol/l (140 mg/dl) and/or triglycerides $\geq$1.69 mmol/l (150 mg/dl) and/or high density lipoprotein cholesterol (HDL-c) <1.03 mmol/l (40 mg/dl) and/or those taking lipid-lowering drugs.

Fasting blood samples were centrifuged within 1 h after collection. Serum cystatin C levels were measured using latex nephelometric immunoassay [25]. The blood was sent to the commercially available laboratory (SRL Inc. Fukuoka, Japan), and the intra- and inter-assay coefficient of variation of cystatin C measured at the laboratory that performed the assays was 1.80% and 1.81%, respectively [26]. The homeostasis model assessment (HOMA) index [FPG (mg/dl) $\times$ insulin ($\mu$U/ml)/405] was calculated from fasting glucose and insulin level as a marker of insulin resistance [27]. Serum uric acid concentration was determined by a standard analytical technique. High sensitive CRP (hs-CRP) was measured using the latex method. Estimated glomerular filtration rate (eGFR) was calculated by the following estimation formula recommended by the Japan Society of Nephrology: eGFR (ml/min/$1.73^2$) = (194 $\times$ $Scr^{-1.094}$ $\times$ $age^{-0.287}$) $\times$ (0.739 for females) [28]. In addition, albuminuria was determined as the ratio of urinary albumin to creatinine (UACR) from first-morning void urine. Microalbuminuria was defined as UACR $\geq$30 mg/g·Cr.

The cognitive function of the participants in the study was evaluated by the Mini-Mental State Examination (MMSE) [29]. The MMSE is a worldwide measurement of cognitive function, with components of items assessing orientation, concentration, language, praxis, and memory.

This study was approved by the Tanushimaru branch of the Japan Medical Association and by the local mayor, as well as by the ethics committee of Kurume University School of Medicine. All the participants gave informed consent. The Research Ethics Committee of the Kurume University School of Medicine (Process numbers 9019/2009) approved the study in conformity with the principles embodied in the declaration of Helsinki.

### 2.3. Statistical analysis

Because of skewed distributions, the natural logarithmic transformation was performed for cystatin C, microalbuminuria, FPG, HOMA index and triglycerides. Mean values, upper and lower 95% confidence limits, were exponentiated and presented as geometric mean ± standard deviation (SD), where the SD was approximated as the difference of the exponentiated confidence limits divided by 3.92, the number of SD in a 95% confidence interval for normally distributed data. Chi-square tests were used for evaluation of categorical parameters. Uni- and multiple linear regression analyses adjusted for age and sex were used. Using some significant factors from the univariate linear regression analysis, we performed the multiple stepwise regression analysis to see the strength and independency for MMSE.

In order to investigate the impact of cystatin C and microalbuminuria on MMSE score, we created the hierarchical model stratified by the median values (cystatin C; 0.92 mg/l, microalbuminuria; 8.9 mg/g·cre). $p$-values <0.05 were considered statistically significant. All statistical analyses were performed using SAS version 9.4 (SAS Inc., Cary, NC, USA).

## 3. Results

Characteristics of the 1943 subjects are presented in Table 1. The mean levels of cystatin C ($p < 0.001$) and microalbuminuria ($p < 0.05$) are significantly higher in males than in females. MMSE score was significantly lower ($p < 0.01$) in males than in females. Fig. 1A shows the distribution of MMSE scores in males and females. As it is apparent from Fig. 1A, most of the enrolled subjects had high scores, while lower scores (defined as the range of 0–23) were few in both sexes. Fig. 1B shows the distribution of serum levels of cystatin C in males and females. The