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, which affects 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 1,943 subjects (774 males, 1,169 females, mean age 65.8 years) took part in the investigation, and those participants underwent a health examination 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 the cognitive function was significantly and inversely associated with cystatin C and microalbuminuria, in the relatively younger general population.

Keywords: Cystatin C, Microalbuminuria, Cognitive impairment, epidemiology

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 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 ACCORD study, they reported the significance of cystatin C, although the participants were diabetic people with HbA_{1c}>7.5%, who were at high risk for cardiovascular diseases.

Cystatin C is an inhibitor of cysteine proteinase, and released into the bloodstream, which levels are independent of age, sex, and muscle mass, compared to serum creatinine. Microalbuminuria levels indicate that the feature of glomerular filtration. In addition to the early kidney dysfunction, these two biomarkers are also known as an indicator 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 useful confirmatory parameters for the 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 has begun 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 Tanushimaru study, we have performed epidemiological studies in every 10 years and follow up 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]. In the total population of 4,687 subjects (2,151 men and 2,536 women) aged over 40 years in this district in 2009, 1,943 people (774 men and 1,169 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 in 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 intervals 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 analysis. Hypertensive subjects were defined as those with systolic BP≥140mmHg and/or those with diastolic BP≥90mmHg and/or those receiving antihypertensive medication. Subjects with fasting plasma glucose (FPG) ≥6.99mmol/l (126mg/dl), subjects with glycosylated hemoglobin A_{1c} (HbA_{1c} NGSP) ≥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) ≥3.62mmol/l (140mg/dl) and/or triglycerides ≥1.69mmol/l (150mg/dl) and/or high density lipoprotein cholesterol (HDL-c) <1.03mmol/l (40mg/dl) and/or those taking lipid-lowering drugs.

Fasting blood samples were centrifuged within 1 hour after collection. Serum cystatin C levels were measured using latex nephelometric immunoassay [25]. The blood was submitted to the commercially available

laboratory (SRL Inc. Fukuoka, Japan), and the intra- and inter-assay coefficient of variation of cystatin C at the laboratory that performed the assays was 1.80% and 1.81%, respectively [26]. The homeostasis model assessment (HOMA) index [FPG (mg/dl) × insulin (μU/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 latex method. Estimated glomerular filtration rate (eGFR) was calculated by the following estimation formula that has been recommended by the Japan Society of Nephrology: eGFR (ml/min/1.73^2) = (194×Scr$^{-1.094}$×age$^{-0.287}$) × (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 ≥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 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 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; 0.92mg/L, microalbuminuria; 8.9mg/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 1,943 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. **Figure 1-A** shows the distribution of MMSE scores in males and females. As is apparent from **Figure 1-A**, most of the enrolled subjects had high scores, while lower scores (defined as the range of 0-23) were few in both sexes. **Figure 1-B** shows the distribution of serum levels of cystatin C in males and females. The mean levels of log-transformed cystatin C were 0.95 (range 0.41-7.11) mg/L, which was slightly higher than the normal levels (0.53-0.95mg/L). **Table 2** shows the results of uni- and multivariate analyses adjusted for age and sex for correlates of MMSE scores. In multivariate analysis, systolic BP ($p=0.024$; inversely), cystatin C ($p=0.046$; inversely), microalbuminuria ($p=0.019$; inversely), HDL-c ($p=0.045$) and history of cerebrovascular diseases ($p<0.001$; inversely), but not eGFR ($p=0.197$), were significantly associated with MMSE scores. Using the significant factors detected by univariate analysis in **Table 2**, we performed the multiple stepwise regression analysis.

Eventually, cystatin C was a significant and independent factor for MMSE. Although microalbuminuria was not an independent factor for MMSE in this analysis (**Table 3**), we found that this is the independent factor for MMSE if we analyzed data after excluding cystatin C (**supplemental Table 1**). This hierarchical model in **Figure 2** showed the greater effect on cystatin C and microalbuminuria on MMSE score as these parameters increased ($p<0.001$ for trend).

4. Discussion

In the present study, we demonstrated for the first time that the cognitive function was significantly and inversely associated with cystatin C and microalbuminuria, but not eGFR, in the Japanese general population, aged over 40 years, suggesting that cognitive dysfunction at early stage was associated with endothelial dysfunction, rather than kidney function, although kidney dysfunction is well known as a risk factor for cognitive dysfunction at advanced stage. Compared to the previous studies [14-22,33], the enrolled subjects in the present study were comparatively younger and healthier, in whom serum cystatin C and microalbuminuria were measured simultaneously.

Cystatin C and cognitive dysfunction

Cystatin C is an inhibitor of cysteine proteinase that is constitutively expressed in all human cells, and released into the bloodstream, after the filtration by the kidney glomerulus and metabolization by the proximal tubule[30]. The serum levels are independent of age, sex, and muscle mass, compared to serum creatinine levels [31]. Although there are a few reports regarding the relationship between cognitive function and cystatin C, they are limited to data in elders. Among community-dwelling older adults (aged 70-79 years), high levels of serum cystatin C (≥1.25mg/L) had worse cognitive function [Modified Mini-Mental State Examination (3MS) score and digit symbol substitution test (DSST) score]. DSST score is a test of visuospatial and motor speed-of-processing [32]. Because it has a considerable executive function component, it is frequently used as a sensitive measurement of only frontal lobe executive function [32]. Participants with high levels of serum cystatin C had worse cognitive function in baseline, and more pronounced decline over the 7-years [14]. Sarnak MJ, et al. [15]. also reported that higher serum cystatin C levels were associated with a reduction in successful life years (free of cardiovascular disease, cancer, chronic obstructive pulmonary disease, having intact

physical and cognitive function) after 6-year follow-up among community-based cohort of older adults (aged≥65years). Although Yaffe K, et al. [16]. reported that higher levels of serum cystatin C were associated with worse cognition [Modified Mini-Mental State Examination (3MS) and some other tools] among patients with CKD.

Microalbuminuria and cognitive dysfunction

As well as serum cystatin C, a marker of early kidney dysfunction, there are some reports regarding to the relationship between microalbuminuria and cognitive function. Among older patients (aged≥60 years) with peripheral arterial disease, higher level of urinary albumin excretion was inversely associated with cognitive function (DSST score) [17,18]. In older patients (aged≥70 years) with type 2 diabetes, microalbuminuria was a risk factor for cognitive decline using MMSE scores and IQCODE scores after a median 1.6-year follow-up [19]. Although Abbatecola AM, et al. [20]. demonstrated similar results, their report was limited to the elderly with impaired glucose tolerance. In addition, the longest follow-up period was found in the ONTARGET/TRANSCEND studies [21], which indicated that in older participants (aged≥60 years) with vascular disease or diabetes mellitus, not only microalbuminuria, but also macroalbuminuria, were more likely to have

a worse cognitive function using MMSE scores compared to participants with no albuminuria. In 5-year follow-up study, participants with baseline albuminuria, who developed new albuminuria, had increased odds ratio of cognitive decline (decrease in MMSE scores≥3points) [21]. As previously reported by Murray AM, et al. [22]. in diabetic patients (HbA_{1c}>7.5%) with high risk of cardiovascular disease, decreased cognitive function was associated with both cystatin C and microalbuminuria. In the entire study population, albuminuria was independently associated with lower information processing speed, whereas eGFR was not associated with cognitive performance [33]. Recently, a systematic review and meta-analysis indicated that renal dysfunction and cognitive impairment was controversial [34]. However, microalbuminuria was still significantly associated with developing cognitive impairment [34].

Cystatin C or microalbuminuria and cerebrovascular diseases

In the present study, history of cerebrovascular diseases was inversely and significantly ($p<0.001$) associated with MMSE scores. Cystatin C is associated with arteriosclerosis and inflammation; on the other hand, microalbuminuria is linked to a state of generalized endothelial dysfunction, suggesting underlying other diverse processes from aging, hypertension,

diabetes, hyperlipidemia, to the initiation and progression of cardiovascular disease, cerebrovascular disease and Alzheimer disease (AD). It is interesting to note that higher levels of cystatin C were associated with higher odds ratio of magnetic resonance imaging (MRI)-defined subclinical brain infarction or white matter lesions in a population of community-dwelling older adults (≥60 years) in the cross-sectional studies [35,36]. Moreover, cystatin C localizes with β-amyloid in brains of patients with AD primarily in the hippocampus and entorhinal cortex, and several studies have reported an association between a common polymorphism of the cystatin C gene and risk of AD. In addition, increased levels of urine albumin/creatinine ratio (UACR), which reflect systemic vascular dysfunction, were associated with cerebral small vessel disease, MRI-defined lacunar infarcts and white matter lesions in a population of community-dwelling older adults (≥60 years) in the cross-sectional study [37]. Further, Thomas H, et al. [38]. suggested that UACR was associated with greater brain atrophy and white matter hyper intensities on the brain MR scans in hypertensive sib ships (50-91years).

Recently, attention has been paid to the importance of changes in the cerebral microcirculation contribute to cognitive impairment, which includes MRI-defined subclinical brain infarction, and paid to the changes in silent brain infarcts and the risk of dementia and cognitive decline [39]. Our study

also indicated that the cognitive dysfunction was associated with both of elevated levels of serum cystatin C and microalbuminuria, as consistent with the previous reports.

There are several limitations in our study. First, the study design was a cross-sectional. Thus, nothing conclusive for the association of serum cystatin C and microalbuminuria and cognitive impairment can be stated. Prospective studies are needed to investigate the role of the markers in the subjects with cognitive dysfunction. Second, we used only a single test to evaluate the cognitive function. Although the MMSE is a useful tool for screening such an epidemiological study because of the short time evaluation, several tools may be needed for mild cognitive change in future studies. Third, from the multiple correlation coefficients, these two factors in addition to SBP and history of cerebrovascular disease explained only 8% of the variation of MMSE scores. The final limitation was a relatively small sample size. This could have caused a selection bias in this study.

In conclusion, we demonstrated for the first time that the cognitive dysfunction was significantly associated with both of elevated levels of serum cystatin C and microalbuminuria in a Japanese general population.

5. Conflicts of interest

All authors are admitted disclosing any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, our work.

6. Author contributions

Shoko Kono, primary investigator: performed examination, analysis and interpretation of data. Hisashi Adachi: planned the study. Mika Enomoto, Ako Fukami, Eita Kumagai, Sachiko Nakamura, Yume Nohara, Nagisa Morikawa, Erika Nakao, Akiko Sakaue, Tomoko Tsuru: performed examination with Dr. Kono. Yoshihiro Fukumoto: designed study, head of department, supervisor. All authors were involved in clinical conception and the interpretation of data.

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Figure Legends

Figure 1-A: The distribution of MMSE scores in males and females.

MMSE; mini-mental state examination

Figure 1-B: The distribution of levels of serum cystatin C in males and females.

Figure 2: Impact of the relation between high and low groups of cystatin C and microalbuminuria (MA) on MMSE score.

Table 1. Characteristics of the subjects

	Male (n=774)	Female (n=1,169)	Total (n=1,943)
Age (years)	66.3±10.9	65.5±11.5	65.8±11.3
Body mass index (kg/m^2)	23.8±3.0***	23.2±3.5	23.4±3.3
Waist circumference (cm)	86.1±8.3***	83.2±10.5	84.4±9.8
Systolic blood pressure (mmHg)	135.5±19.3***	132.2±19.3	133.5±19.4
Diastolic blood pressure (mmHg)	83.6±11.5***	80.4±11.0	81.7±11.3
Blood urea nitrogen (mmol/L)	6.1±2.0***	5.7±1.6	5.9±1.7
Creatinine (μmol/L)	77.8±28.3***	57.5±26.5	61.9±26.5
Estimated GFR (ml/min/1.73m^2)	72.9±16.6*	74.4±15.7	73.8±16.1
Uric acid (μmol/L)	356.9±83.3***	273.6±65.4	309.3±83.3
Cystatin C (mg/l) † (min-max)	1.01 (0.51-6.59) ***	0.91 (0.41-7.11)	0.95 (0.41-7.11)
Microalbuminuria (mg/g·cre) †	11.6 (1.1-2600.0) *	10.2 (1.4-1610.0)	10.7 (1.1-2600.0)
Fasting plasma glucose (mmol/L)	5.6±1.4***	5.4±1.5	5.5±1.5
HOMA-IR† (min-max)	1.33 (0.11-105.44)	1.37 (0.14-50.66)	1.36 (0.11-105.44)
Hemoglobin A_{1c} (NGSP%)	5.9±0.8	5.9±0.6	5.9±0.7
LDL- C(mmol/L)	3.1±0.8***	3.4±0.8	3.3±0.8
HDL- C (mmol/L)	1.5±0.4***	1.6±0.4	1.6±0.4
Triglycerides (mmol/L) † (min-max)	1.3 (0.4-9.6) ***	1.1 (0.3-8.0)	1.2 (0.3-9.6)
MMSE	27.4±2.7**	27.8±2.3	27.7±2.5
Smoking (%, yes)	25.9***	2.9	12.1
Alcohol (%, yes)	73.8***	26.0	45.1
Hypertension medication (%, yes)	37.1	33.1	34.7
Diabetes medication (%, yes)	10.1**	6.4	7.9
Hyperlipidemia medication (%, yes)	14.0***	24.5	20.4
History of heart diseases (%, yes)	20.8***	11.4	15.2
History of stroke (%, yes)	6.5***	3.0	4.4

Abbreviations; HOMA-IR; Homeostasis model assessment of insulin resistance,

LDL-C; Low-density lipoprotein cholesterol, HDL-C; High-density lipoprotein cholesterol

MMSE; Mini Mental State Examination

Data are means± standard deviation or percentage, unless otherwise indicated.

†: These variables were represented in the original scale after analysis using log (natural) transformed values.

*: $p<0.05$ **: $p<0.01$ ***: $p<0.001$

Table 2. Association between MMSE scores and parameters

	Univariate			Multivariate (Adjusted for age and sex)		
Parameters	**β**	**SE**	***p*-value**	**β**	**SE**	***p*-value**
Age	-0.074	0.005	<.001			
Sex (Male=0, Female=1)	0.354	0.114	0.002			
Body mass index	-0.009	0.017	0.583	-0.013	0.016	0.400
Waist circumference	-0.013	0.006	0.027	-0.0003	0.005	0.953
Systolic blood pressure	-0.017	0.003	<.001	-0.006	0.003	0.024
Diastolic blood pressure	0.001	0.005	0.804	-0.006	0.005	0.193
Blood urea nitrogen	-0.053	0.012	<.001	0.0001	0.011	0.992
Creatinine	-0.628	0.172	<.001	-0.185	0.173	0.285
Estimated GFR	0.024	0.003	<.001	0.005	0.004	0.197
Uric acid	-0.069	0.040	0.084	0.010	0.042	0.805
Cystatin C †	-2.363	0.239	<.001	-0.550	0.275	0.046
Microalbuminuria †	-0.340	0.049	<.001	-0.115	0.049	0.019
Fasting plasma glucose	-0.005	0.002	0.020	0.0007	0.002	0.736
HOMA-IR†	-0.238	0.061	<.001	-0.046	0.059	0.442
Hemoglobin A_{1c} (NGSP)	-0.132	0.081	0.106	-0.019	0.077	0.809
LDL- C	0.007	0.002	<.001	0.002	0.002	0.159
HDL- C	0.016	0.004	<.001	0.007	0.004	0.045
Triglycerides †	-0.186	0.107	0.083	-0.120	0.101	0.235
Smoking	0.351	0.173	0.043	0.040	0.177	0.822
Alcohol	0.272	0.113	0.016	0.178	0.123	0.149
Hypertension medication	-0.698	0.117	<.001	-0.013	0.120	0.913
Diabetes medication	-0.460	0.209	0.028	-0.146	0.198	0.460
Hyperlipidemia medication medication	-0.158	0.141	0.260	0.122	0.135	0.363
History of heart diseases	-0.479	0.157	0.002	-0.012	0.151	0.939
History of stroke	-1.501	0.274	<.001	-0.917	0.261	<.001

Abbreviations as in Table 1.

†: These variables were represented in the original scale after analysis using log (natural) transformed values.

Table 3. Multiple stepwise linear regression analysis between MMSE score and parameters

	β	S.E.	*p*-value
Age	-0.07	0.005	<0.001
History of stroke	-0.94	0.27	<0.001
Systolic blood pressure	-0.001	0.003	0.004
Cystatin C †	-0.68	0.28	0.014

R^2=0.13

†: These variables were represented in the original scale after analysis using log (natural) transformed values.

Figure1-A

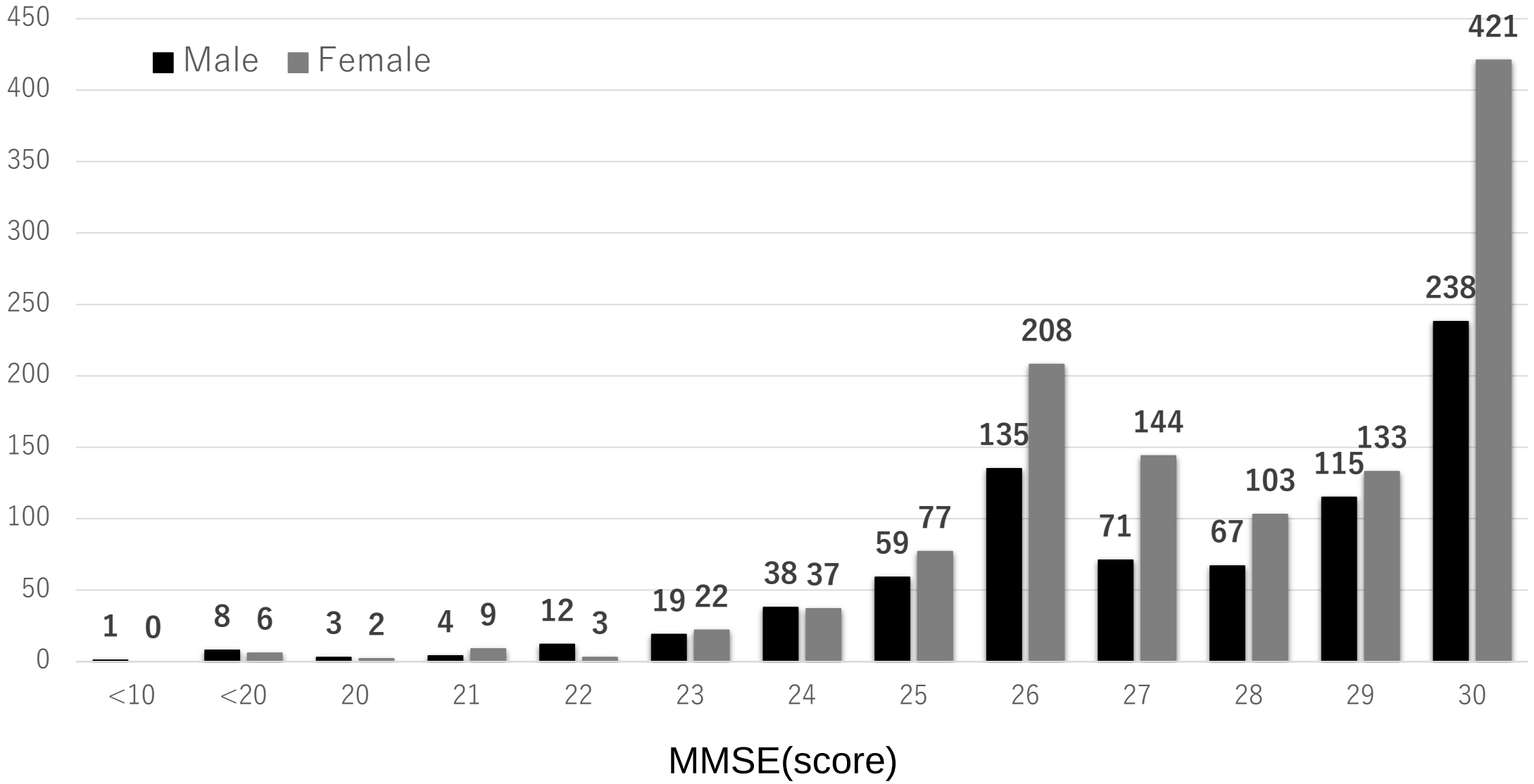

Male
Female
450
400
350
300
250
200
150
100
50
0
1 0
8 6
3 2
4 9
12 3
19 22
38 37
59 77
135 208
71 144
67 103
115 133
238 421
<10
<20
20
21
22
23
24
25
26
27
28
29
30
MMSE(score)

Figure1-B

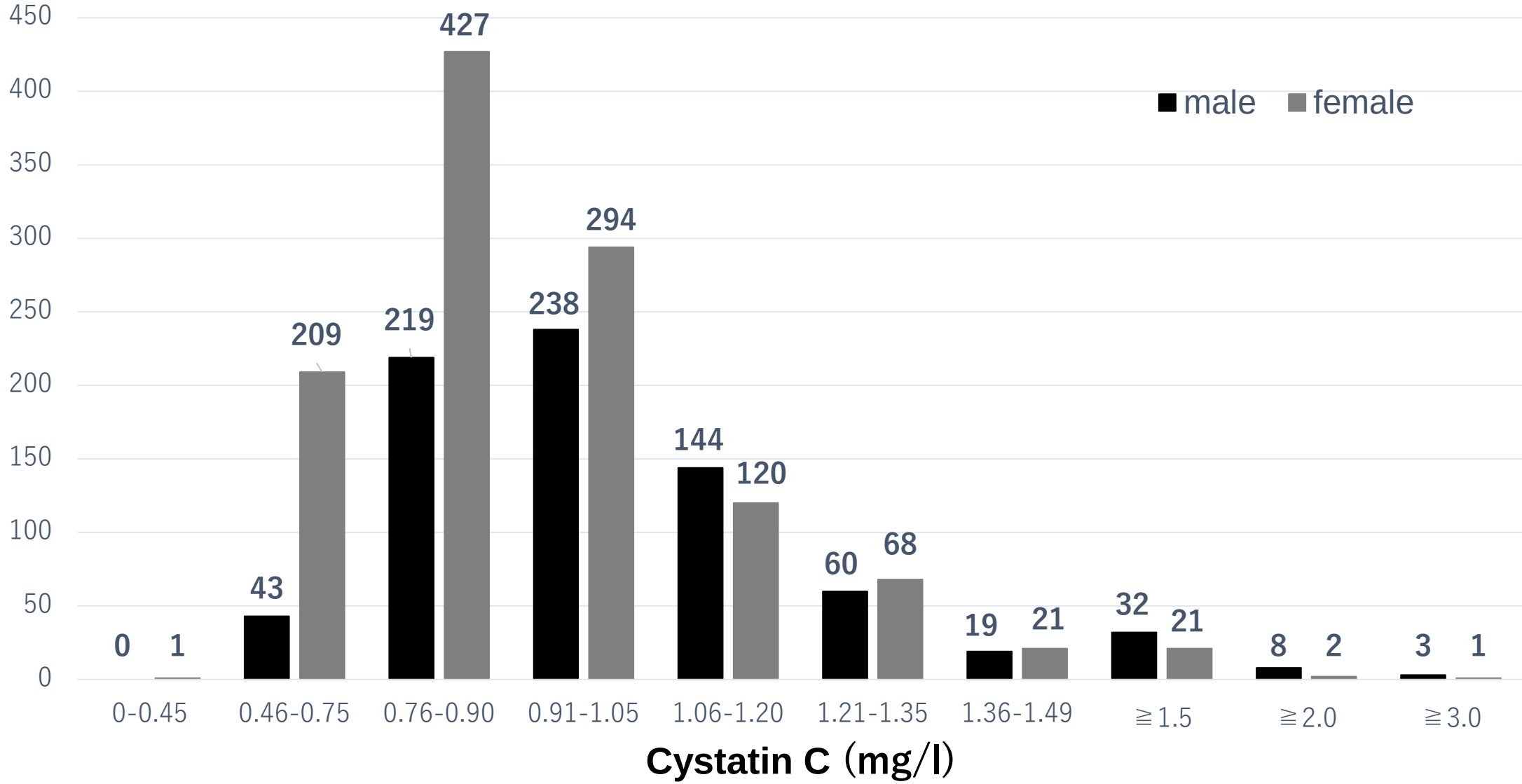
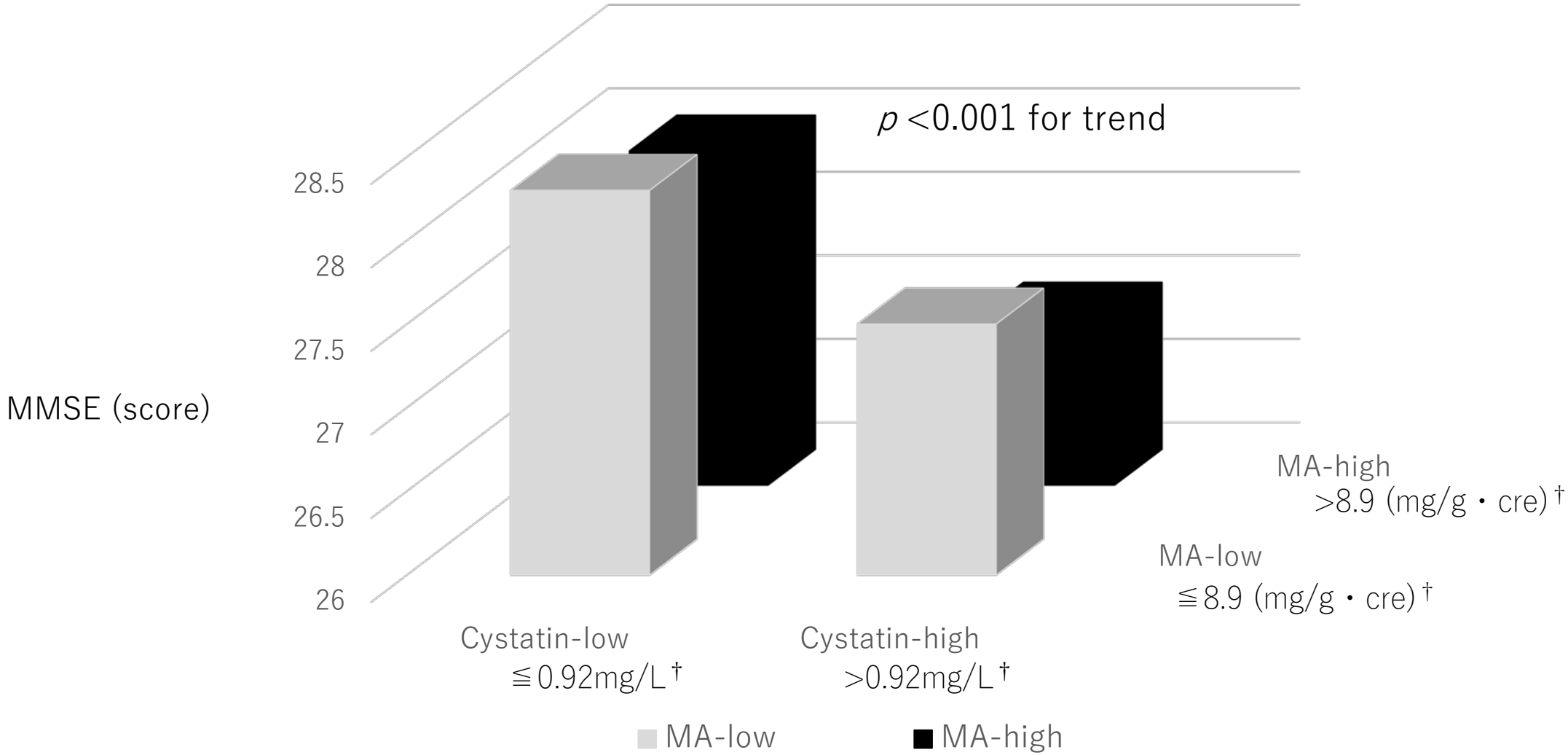

450
400
350
300
250
200
150
100
50
0
male
female
0
1
43
209
219
427
238
294
144
120
60
68
19
21
32
21
8
2
3
1
0-0.45
0.46-0.75
0.76-0.90
0.91-1.05
1.06-1.20
1.21-1.35
1.36-1.49
≧1.5
≧2.0
≧3.0
Cystatin C（mg/l）

Figure2



†: These variables were represented in the original scale after analysis using log (natural) transformed values.