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Low tongue pressure in peritoneal dialysis patients as a risk factor for malnutrition and sarcopenia: a cross-sectional study

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Abstract

Background: Low tongue pressure contributes to oral frailty. It is a condition in which the pressure between the tongue, palate, and food decreases due to a decrease in tongue muscle function. Low tongue pressure interferes with chewing and swallowing and causes bloating, increasing the risk of malnutrition and aspiration pneumonia due to decreased intake. Its causes include aging, neuromuscular disease, trauma, sequelae, low nutrition, and sarcopenia. Although quantitative evaluation of tongue function has been reported to be useful for prevention of low tongue pressure, there is no report of the evaluation's effectiveness in patients with high-risk renal failure. This study aimed to evaluate the prevalence of low tongue pressure and to clarify the relationship between the tongue pressure value and sarcopenia in peritoneal dialysis (PD) patients.

Methods: A cross-sectional study of 105 PD patients (67 ± 13.5 years old, 73.3% men, 26.7% women; 26.7% with diabetes) was conducted in our hospital. At the start of the study, clinical backgrounds were investigated from the patients' medical records. Sarcopenia was diagnosed by muscle mass, grip strength, and walking speed. Inflammatory, nutritional, and cardiovascular markers were measured. Univariate and multivariate analyses with muscle and nutrition markers were performed to determine the low tongue pressure prevalence rate in PD patients.

Results: Low tongue pressure prevalence rate was 55.2% in patients under 65 and 64.1% in patients 65 and over. Significantly lower tongue pressure (p = 0.019) was observed in the patients in the sarcopenic group. The tongue pressure value was positively correlated with albumin (p = 0.0003), pre-albumin (p = 0.006), body mass index (p = 0.004), grip strength (p < 0.0001), and muscle mass (p = 0.004) and negatively correlated with alge (p < 0.0001). Multiple linear regression analysis indicated that age (p = 0.034) and grip strength (p = 0.007) were the independent factors that affected tongue pressure.

Conclusions: We found low tongue pressure prevalence in PD patients and an association between this condition and sarcopenia. Further studies are needed to support these findings, including intervention studies.

Keywords: Sarcopenia, Oral frailty, Peritoneal dialysis, Malnutrition, Tongue pressure, Swallowing muscles, Aspiration pneumonia

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Background

In Japan, people over the age of 65 years comprised 26. 7% of the population in 2015 [1]. This number is predicted to continue to rise, reaching 39.9% in 2060, which means that one of every 2.5 people in the country will be older than 65 years. In 2013, people older than 65 years accounted for 67% of dialysis patients in Japan [2]. Nutritional disorders and associated complications are extremely frequent in dialysis patients, with a reported occurrence in 18–75% of cases [3–5]. Such disorders pose a particularly serious threat to elderly dialysis patients. Although there is a lack of information concerning the prevalence of these disorders in end-stage renal disease patients, protein-energy wasting (PEW) and sarcopenia seem to be more common in older (> 65 years) than younger maintenance dialysis patients [6].

Previous research reported that malnutrition was strongly associated with a patient's prognosis and quality of life [7]. Thus, the issue of malnutrition should be addressed in aging dialysis patients. According to one study, malnutrition in chronic kidney disease patients progressed over time following deterioration of renal function in early-stage disease until the introduction of renal replacement therapy [8]. Several factors are associated with malnutrition in dialysis patients. These include decreased dietary intake due to physical and social psychological factors; loss of nutrients during dialysis; accumulation of uremic substances (cachexia); acidemia; catabolic stress induced by dialysis; low levels of or resistance to anabolic hormones, such as insulin, growth hormone, and insulin-like growth factor 1; increased levels of catabolic hormones, such as parathyroid hormone and glucagon; oxidative and carbonyl stress; systemic inflammation; and comorbid conditions [9, 10]. Nutritional disorders, muscle mass reduction (i.e., sarcopenia), PEW, and social psychological disorders are part of a syndrome of frailty and known to be associated with malnutrition [8–11]. Sarcopenia refers to progressive, age-related loss of muscle mass, with a decline in muscle function and physical performance. It is common among the geriatric population and can be a precursor to the development of frailty, late-life disability, worsening quality of life, and increased mortality rates in the elderly [11]. One study found that the incidence of sarcopenia was two to three times higher in elderly renal failure patients as compared with that in the general elderly population [12]. Therefore, interventions for patients with renal failure are required.

The present study focused on oral frailty, which contributes to a decrease in dietary intake. Tanaka et al. reported the longitudinal impact of the accumulated poor oral health (oral frailty) on adverse health outcomes, including sarcopenia, frailty, and mortality [13]. They defined oral frailty as co-existing poor status in ≥ 3 of the six measures of poor oral status as determined by the number of natural teeth, chewing ability, articulatory oral motor skill, tongue pressure, and subjective difficulties in eating and swallowing. Swallowing can be divided into the following four stages: (1) mastication and bolus formation, (2) pharyngeal transport, (3) esophageal transport, and (4) esophageal passage [14]. Stages 1 and 2 are voluntary actions, stage 3 is a reflex movement, and stage 4 is mainly a peristaltic action. In the oral phase (stage 1), the muscular system is mainly involved, and the tongue performs major functions (e.g., chewing, taste, salivation, bolus formation, and pharyngeal transport) in food movement. Aging-related deterioration of the swallowing function is mainly related to functional deterioration of stages 1 to 3. In stage 1 (oral phase), weakness in tongue muscles is the main cause of a decline in swallowing function. Thus, rehabilitation focuses primarily on tongue muscles to maintain the swallowing function. Tongue pressure is the pressure between the tongue, palate, and food. Low tongue pressure (i.e., pressure < 30 kPa) is associated with deterioration in tongue muscle function and is regarded as an important element of oral frailty [15].

As shown in previous studies, quantitative evaluations of tongue function can help to identify the risk of malnutrition and aspiration pneumonia due to decreased nutritional intake, which can exacerbate the risk of sarcopenia [16-19]. There has been no research in the field of internal medicine on tongue function and oral frailty, particularly in patients with renal failure, which are a high-risk group. Therefore, the aim of the present study was to examine the potential role of oral frailty as a risk factor for malnutrition and sarcopenia in peritoneal dialysis (PD) patients. With this aim in mind, our group quantitatively measured tongue pressure in PD patients and stratified the patients into a sarcopenic group and non-sarcopenic group. We compared these groups and examined the correlation between tongue pressure and various nutritional, muscle, and inflammatory markers using univariate and multivariate analyses.

Methods

Study design, setting, and participants

This was a cross-sectional study conducted in the peritoneal dialysis unit of the Institute of the Japanese Red Cross Medical Center. The inclusion criteria were patients who had commenced PD at least 1 month prior to the commencement of the study and patients who were older than 20 years. Patients who had acute disorders or infectious diseases, patients who has been hospitalized, and patients who had recently undergone major surgery were excluded.

The primary endpoint was the prevalence of low tongue pressure based on a quantitative assessment of

tongue function in PD patients. The secondary endpoint was the association between low tongue pressure and sarcopenia. The prevalence of low tongue pressure in these groups and its correlation with nutritional, muscle, and inflammatory markers were examined.

Tongue pressure measurement

A tongue pressure measurement device (TPM-01; JMS Co., Hiroshima, Japan) was used to measure tongue pressure [16]. The device has a small balloon-type disposable oral probe with a plastic pipe (Fig. 1). The probe was placed on the upper surface of the patient's tongue. Tongue pressure was measured while the patient was in a relaxed sitting position. The patients were asked to close their lips around the rigid ring part of the probe, while holding the ring lightly between their front teeth and then compress the small balloon on the palate as hard as they could, using their tongue. The maximum tongue pressure value displayed on the device was recorded. The measurement was performed twice, and the maximum tongue pressure was used in the analysis.

Data collection

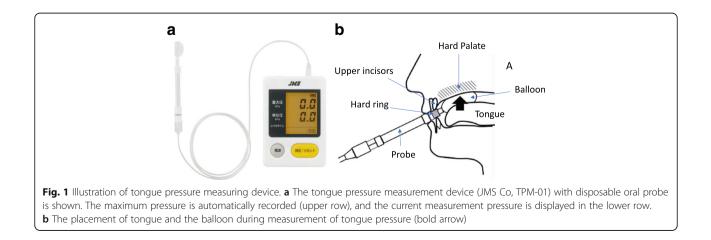
The following basic data on all subjects were recorded at baseline: age, sex, height, weight, body mass index (BMI), comorbidity conditions, and mode and duration of dialysis. Blood levels of hemoglobin, serum albumin (Alb), prealbumin (PRAB), interleukin-6 (IL-6), and C-reactive protein (CRP) were recorded. IL-6 levels were measured using an enzyme-linked immunosorbent assay. Normalized protein equivalent nitrogen appearance (nPNA) was calculated using the method described by Randerson et al. [20] and normalized to actual weight. Dialysis adequacy was measured by total (renal and peritoneal) weekly urea clearance (wKt/V). Muscle mass and body fluid volume were measured using a whole-body multifrequency bioelectrical impedance analysis device (Body Composition Monitor-BCM; Fresenius Medical Care, Bad Homburg, Germany). Low tongue pressure was considered maximum tongue pressure of < 30 kPa [15].

Sarcopenia was examined at the beginning of the follow-up period. Sarcopenia was diagnosed based on the following criteria, according to the diagnostic algorithm developed by the Asian Working Group for Sarcopenia: (1) low muscle mass, (2) weak muscle strength (handgrip strength), and/or (3) poor physical function (slow walking) [21]. The appendicular skeletal muscle mass was calculated as the sum of the skeletal muscle in the arms and legs. The relative skeletal muscle mass index (SMI) was defined as the appendicular skeletal muscle mass divided by the body height in meters squared. Low muscle mass was classified as an SMI score of less than 7.0 and 5.7 kg/m² in men and women, respectively.

Muscle strength was assessed by means of grip strength, as measured using a dynamometer (GRIP-D; Takei Ltd., Niigata, Japan). The participants were asked to exert maximum effort twice using their dominant hand, and the average value was used for the analysis. Low handgrip strength was defined as < 26 and < 18 kg for males and females, respectively.

The patient's usual walking speed (m/s) on a 10-m course was used as an objective measure of physical performance. A slow walking speed was defined as a speed slower than 0.8 m/s.

Various MIA-related factors and tongue pressure were measured at outpatient examination after the start of the study. Alb, PRAB, BMI, and nPNA were measured as nutritional indices, and IL-6 and CRP values were measured as inflammation indices. The Cardio Ankle Vascular Index (CAVI), left ventricular mass index (LVMI), and brain natriuretic peptide (BNP) measurements served as cardiovascular indices, and wKt/V represented dialysis efficiency.



Statistical analysis

The results are expressed as mean ± standard deviation (SD) for continuous variables, as the median and interquartile range for data with a skewed distribution, and as the number of patients and percentages for categorical variables. Differences in the characteristics among study participants between males and females were analyzed using a two-sample t test, χ^2 test, and Mann-Whitney test. The Kolmogorov-Smirnov test was performed to check whether the data had a normal distribution. For comparison of tongue pressure values by age, a one-way analysis of variance, with a multiplecomparison Bonferroni test was used. Spearman's correlation coefficients were calculated to assess the variables correlated with tongue pressure in both groups, including stratification according to sex. To identify independent predictors of tongue pressure, a multiple stepwise backward linear analysis was conducted. Furthermore, a multivariate logistic regression model was used to adjust for factors, including age, Alb, PRAB, BMI, grip strength, and SMI, related to low tongue pressure (< 30 kPa). Twosided provability values < 0.05 were considered to indicate a statistically significant difference. All statistical analyses were conducted using R version 3.2.4 software [22].

Results

Characteristics of the study participants

Of 130 PD patients treated in our unit in April 2017, 105 patients were included in this study. Twenty-five patients who were hospitalized or who had undergone PD treatment for <1 month were excluded. Of the 105 patients, the mean age \pm standard deviation was 67.0 \pm 13. 5 years. Of these, 77 (73.3%) patients were males and 28 (26.7%) had diabetes. The prevalence of sarcopenia was 13.3% (n = 14) (Table 1).

Tongue pressure values according to age, sex, and the prevalence of low tongue pressure

The tongue pressure distribution by age is shown in Table 2 and Fig. 2. Tongue pressure tended to decrease as age increased. The tongue pressure of females in their 60s and older than 80 years was significantly lower than that of males in the same age groups. However, there were no significant sex-related differences in tongue pressure in the other age groups.

The prevalence rate of low tongue pressure (< 30 kPa) in this study was 55.2%. In the total population and population stratified according to sex, the prevalence of low tongue pressure was high in the sarcopenia group (Table 3).

Correlation of tongue pressure values with MIA-related factors

The results of the univariate analysis and Spearman's correlation coefficients for MIA-related factors and tongue pressure values are shown in Fig. 3 and Table 4. In the stratification of the data according to sex, age, and grip strength showed a significant linear correlation with tongue pressure in females. Age, Alb, BMI, grip strength, PRAB, and SMI showed a significant linear correlation with tongue pressure in males (Table 4). In terms of MIA-related factors, age, Alb, BMI, grip strength, PRAB, and SMI displayed a significant linear correlation with tongue pressure values in all patients (Fig. 3, Table 4). The results of the multiple linear regression analysis, with backward stepwise selection of age, Alb, BMI, grip strength, PRAB, SMI, and tongue pressure indicated that age and grip strength independently predicted low tongue pressure (Table 5).

Table 1	Charac	teristics	of s	study	participants
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	All	Males	Females	<i>p</i> value
Number of patients	105	77 (73.3%)	28 (26.7%)	
Age (years)	67.0 ± 13.5	65.8 ± 13.2	70.0 ± 13.9	0.156
DM	26.7%	23 (29.9%)	5 (17.2%)	0.224
BMI (kg/m²)	23.1 ± 4.23	23.8 ± 4.10	21.5 ± 4.13	0.011*
Prevalence of sarcopenia	13.3%	11.8%	17.2%	0.525
D/P _{cr}	0.57 ± 0.10	0.58 ± 0.10	0.56 ± 0.10	0.402
wtKt/V	1.97 ± 0.67	1.99 ± 0.70	1.92 ± 0.60	0.661
PD duration (months)	56.2 ± 45.8	53.7 ± 44.7	62.9 ± 49.0	0.365
Grip strength (kg)	25.9 ± 9.17	29.1 ± 8.11	17.0 ± 5.31	< 0.001***
SMI (kg/m ²)	7.7 ± 1.41	7.9 ± 1.40	7.1 ± 1.33	0.020*

Values expressed as mean ± SD or number (percent)

*p < 0.05, ***p < 0.001

 Table 2 Tongue pressure measurement value by age and aender

	Male (n)	Female (<i>n</i>)	p value	All (n)
< 60	35.6 ± 8.26 (22)	30.7 ± 6.09 (6)	0.186	34.5 ± 8.01 (28)
60–69	35.2 ± 12.0 (18)	25.4±5.14 (9)	0.029*	31.8 ± 11.1 (27)
70–79	26.9 ± 8.91 (24)	26.1 ± 6.11 (6)	0.848	26.7 ± 8.33 (30)
> 80	28.0 ± 7.77 (13)	17.0±9.38 (7)	0.011*	24.1 ± 9.75 (20)

Values expressed as mean \pm SD (kPa) or number n number of patients

**p* < 0.05

~p < 0.05

Risk factors for low tongue pressure using logistic regression analysis

A stepwise logistic regression model was developed to identify independent risk factors for low tongue pressure. The results revealed that grip strength was an independent risk factor, adjusted for age, Alb, BMI, PRAB, and SMI (odds ratio 1.1; 95% confidence interval [CI] 1.04, 1.16; p < 0.001).

Discussion

This study measured tongue pressure values in patients undergoing PD. The results suggested that the tongue pressure values of PD patients were lower than those of healthy subjects. In addition, the prevalence of low tongue pressure was higher in the sarcopenia group than nonsarcopenia group. Furthermore, nutritional and muscle mass markers were associated with tongue pressure values. Age, Alb, PRAB, BMI, grip strength, and SMI were significantly correlated with tongue pressure values, and grip strength was an independent risk factor for low tongue pressure in PD patients. To the best of our knowledge, this is the first study to evaluate tongue

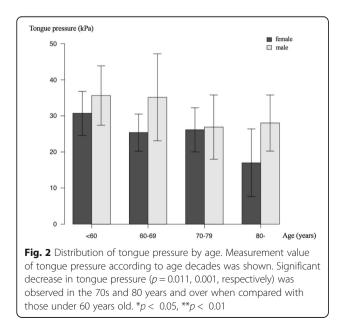


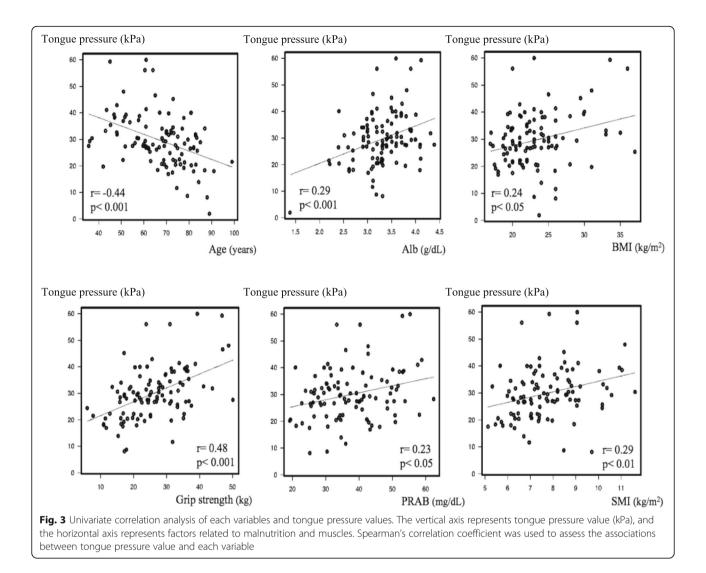
Table 3	Prevalence	of low	tongue	pressure
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	All patients	Elderly patients	Sarcopenic patients
Male	36 (77), 46.8%	26 (45), 57.8%	8 (9), 88.9%
Female	22 (28), 78.6%	15 (19), 78.9%	4 (5), 80.0%
All	58 (105), 55.2%	41 (63), 64.1%	12 (14), 85.7%

Values expressed as number of low tongue pressure patients (all patients), prevalence rate of low tongue pressure in all patients, elderly patients aged 65 years and over, and patients with sarcopenia

pressure quantitatively using a tongue pressure measuring device in renal failure patients and to investigate the association between tongue pressure and malnutrition or sarcopenia in PD patients.

In this study, low tongue pressure was present in 55.2% of the PD patients. As compared with mean tongue pressure values of 40.7 ± 9.9 kPa, 37.6 ± 8.8 kPa, and 31.9 ± 8.9 kPa reported previously by Robbins et al. [18] for healthy patients in their 50s, 60s, and 70s, respectively, the mean tongue pressure of PD patients in the present study tended to be lower [18]. A variety of factors, such as poor dietary intake, inappropriate nutrition management, and uremia, result in an increased prevalence of sarcopenia and PEW in PD patients. These factors likely explain why the prevalence of low tongue pressure in the present study was lower than that reported by Robbins et al. [18]. Regarding sex differences in tongue pressure, in the present study, the average value was significantly lower in females in their 60s and in females aged 80 years and older, whereas there were no significant sex-related differences in tongue pressure in the other age groups. In the 80 years or older age group, the females were significantly older (88.6 ± 5.4 vs. 82.7 ± 1.9, p = 0.002) than the males, which might have affected the results. A previous study of 853 healthy elderly people found no difference in the average tongue pressure values of individuals in an over 60year-old age group [17]. The study reported that tongue pressure decreased with age and that sex differences in tongue pressure according to age groups were apparent in those aged 50 years and younger. In contrast, sex differences in tongue pressure disappeared in those age 60 years and older. In the present study, when the sarcopenia group was stratified according to sex, there was almost no difference in the prevalence of low tongue pressure (Table 3). On the other hand, in the total population, the prevalence of low tongue pressure was higher among females than males. This finding may be because the incidence of sarcopenia tended to be higher in females than in males, with a prevalence rate of 11.7% for males and 17.9% for females. In the age group aged 65 years and older, 20% were males and 26.3% were females. Given the lack of any sex difference in low tongue pressure (cutoff value < 30 kPa), we conclude that the tongue pressure of females with relatively low muscle strength was lower than that of males.



The results of the present study point to a positive correlation between age and tongue pressure. This finding is consistent with that of previous reports, which found that tongue pressure decreased with aging in healthy elderly people [16–19]. Studies of healthy elderly individuals also reported that the opening force and tongue pressure were related to sarcopenia but that only tongue pressure was associated with age [16, 23]. These studies explained this finding as follows: The opening force relies on the condition of the teeth and jaw, whereas tongue pressure depends on the quantity and quality of the muscles of the tongue. Therefore, the influence of age was greater than that of the opening force.

In addition to aging, neuromuscular disorders, trauma, sequelae, malnutrition, and sarcopenia may be risk factors for low tongue pressure [15]. In the present study, tongue pressure values were related not only to age, muscle mass, and muscular strength but also to

nutritional indices, such as Alb, PRAB, and BMI. This result agrees with the findings of an earlier study, which showed that the brachial muscle area, serum Alb, ADL, and BMI increased the risk of loss of tongue pressure in healthy elderly people [19]. A previous study reported that oral function deterioration and tooth loss in the elderly tended to reduce the intake of meat, vegetables, antioxidant vitamins, and dietary fiber [24]. Nakamura et al. [25] reported that weight loss was associated with having fewer teeth and infrequent intake of fruits/vegetables and fish/meat in a sample of 96,794 elderly participants. These studies indicate that a decline in oral function decreases ingestion and causes malnutrition, suggesting that decreases in oral function, including a reduction in tongue pressure, may lead to malnutrition. Malnutrition exacerbates sarcopenia [11]. It is conceivable that by exacerbating sarcopenia, malnutrition causes further reductions in tongue pressure, leading to a cycle of malnutrition and sarcopenia. Several studies established a

Table 4 Spearman's correlation coefficient between tongue

 pressure and MIA associated factors

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	Female (<i>n</i> = 28)		Male (n = 77)		All (n = 105)	
Variables	r	p value	r	p value	r	p value
Age	- 0.48	0.010*	- 0.39	0.0004***	- 0.44	< 0.0001***
Alb	0.30	0.124	0.23	0.043*	0.29	0.002**
BMI	- 0.13	0.504	0.26	0.024*	0.24	0.016*
BNP	- 0.23	0.236	- 0.04	0.717	- 0.16	0.107
CAVI	- 0.17	0.412	- 0.22	0.056	- 0.18	0.075
CRP	- 0.24	0.213	- 0.13	0.245	- 0.14	0.142
IL-6	- 0.12	0.577	- 0.16	0.158	- 0.14	0.175
LVMI	0.02	0.918	0.10	0.384	0.04	0.693
nPNA	0.22	0.28	- 0.04	0.761	- 0.05	0.591
Grip strength	0.43	0.027*	0.38	0.0006***	0.48	< 0.0001***
PRAB	0.19	0.349	0.24	0.039*	0.23	0.018*
SMI	0.12	0.564	0.34	0.003**	0.33	0.0008***

r correlation coefficient

p* < 0.05, *p* < 0.01, ****p* < 0.001

relationship between tongue pressure and swallowing dysfunction. Hirota et al. [26] reported that tongue pressure was significantly lower in dysphagic patients than in nondysphagic patients. Masahiro et al. reported that tongue pressure predicted pneumonia onset in acute stroke patients (hazard ratio, 7.95; 95% CI, 2.09, 52.11; p = 0.0013). In the same study, an improvement in tongue pressure was associated with a significant decrease in the onset of pneumonia [27]. Similar research on the predictive power of tongue pressure in renal failure patients is required.

Of note, in the present study, the logistic regression analysis results indicated that grip strength rather than muscle mass was an independent risk factor for low tongue pressure. Several studies reported that grip strength rather than muscle mass was associated with patients' prognoses [28–30]. These studies showed that muscle strength as a marker of muscle quality was more important than absolute muscle mass, which was consistent with our results. In the present study, the prevalence of low tongue pressure was higher than the prevalence of sarcopenia, suggesting that low tongue pressure preceded sarcopenia. Interventions to address reductions in oral function could help to prevent sarcopenia caused by malnutrition and offset the development

Table 5 Independent variables associated with tongue pressure by multiple linear regression analysis

Independent Variables	β	SE	t	p value
Age	-0.16	0.07	- 2.14	0.034*
Grip strength	0.39	0.11	3.46	0.007**

 β regression coefficient, SE standard error, t t-statistic

*p < 0.05, **p < 0.01

of muscle weakness throughout the body. Clinically, viewpoints on oral frailty may influence interventions targeting malnutrition and sarcopenia in PD patients. Thus, cross-sectoral health promotion measures are necessary.

The present study has a number of strengths. It is the first study to evaluate tongue pressure quantitatively, in addition to investigating the association between tongue pressure and malnutrition or sarcopenia among patients with renal failure. Nevertheless, the study has some limitations. First, as this study used a cross-sectional design, we were unable to show causal relationships, and unknown or unmeasured confounders might exist. However, as malnutrition and oral function, including tongue pressure and muscle strength, are correlated, it is reasonable to assume that these variables together compound the risk of oral frailty rather than acting as single risk factors. Oral frailty, malnutrition, and sarcopenia are age-related disorders. Future prospective observational studies are needed to clarify the role of tongue pressure as a prognostic factor in the incidence of aspiration pneumonia and its effect on mortality rates. In addition, intervention studies, such as rehabilitation of the lingual muscle, should be conducted to clarify the causal relationship of tongue pressure with sarcopenia. Second, we stratified the population according to sex. However, as the study included more male PD than female PD patients, the sex-related differences identified may not be statistically significant. A further study that includes a larger number of participants is required. Third, the appropriateness of the criterion of low tongue pressure (< 30 kPa) needs to be considered. A value of < 30 kPa was accepted as low tongue pressure in the present study based on an observational study, which showed that elderly people with tongue pressure of 30 kPa or less found it difficult to consume a normal diet [14]. As there is no established definition of low tongue pressure, and the topic has not been discussed thus far, the present study utilized a definition of < 30 kPa. The results of longitudinal studies of the relationship between tongue pressure values and prognoses are needed to determine appropriate cut-off values for tongue pressure.

Conclusions

To conclude, this study showed that the prevalence of low tongue pressure was correlated with malnutrition and sarcopenia. In the multivariate analysis, grip strength was related to tongue pressure in PD patients. Comprehensive care, including oral rehabilitation, nutrition, and exercise, may help to prevent sarcopenia in this patient population. Further research is needed to elucidate the ability of a comprehensive care program to combat sarcopenia.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YK contributed to the research idea and study design; KO, KM, AY, RT, MY, and YI contributed to the data acquisition; YK and EK contributed to the data analysis/interpretation. MY is the research director. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was fully approved by the Ethics Committee of Japanese Red Cross Medical Center (No.751), according to the provisions of the Declaration of Helsinki (as revised in Tokyo 2004). Informed consent was obtained from all individual participants included in the study.

Competing interests

The authors declare that they have no competing interests.

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