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RESEARCH ARTICLE

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Comparison of dipstick and quantitative tests for proteinuria and hematuria in middle-aged, male Japanese employees: A single-center study

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Abstract

Background and Aims: The early and reliable detection of chronic kidney disease is important. In the present study, we aimed to compare the diagnostic results for proteinuria and hematuria between the dipstick test used in primary occupational health examinations and the quantitative tests used in more thorough examinations in clinics.

Methods: We conducted a single-center observational study of male staff (N = 573) at Kagoshima University who underwent a health examination in 2017. Both dipsticks and biochemical methods were used to assess proteinuria and hematuria.

Results: For the dipstick test, the sensitivity, specificity, and positive predictive value were 55.6%, 92.4% and 10.4% for proteinuria, and 64.3%, 98.3% and 66.7% for hematuria, respectively. Four participants for whom false-negative results were obtained using dipsticks for proteinuria, and two of these had 3+ urinary glucose.

Conclusion: Qualitative tests for proteinuria and hematuria had low sensitivities and positive predictive values. Therefore, for the early and reliable detection of chronic kidney disease, the use of quantitative urine tests should be considered during occupational health examinations.

KEYWORDS
chronic kidney disease (CKD), diabetic nephropathy, flow cytometry, health examination, urinary creatinine

1 | INTRODUCTION

The prevalence of chronic kidney disease (CKD) has increased worldwide in recent decades, including in Japan.[1] To protect against the development of CKD, the diagnosis of CKD at an early stage is important. Useful treatments and preventive interventions for lifestyle diseases and chronic glomerulonephritis, which cause CKD, have been developed.[2] In Japan, health examinations are performed regularly for the national and employees' health insurance schemes. During these health examinations, urinary protein concentrations are estimated qualitatively, as an indicator of potential CKD (Figure 1).[3,4] In addition, the presence of red blood cells (RBCs) in the urine is tested for during some of these examinations. Both urine tests (protein and RBCs) use dipstick methods, which yield qualitative, rather than quantitative outputs.

Dipstick tests for proteinuria and hematuria are useful for screening for CKD, and these test results have been adopted as part of the

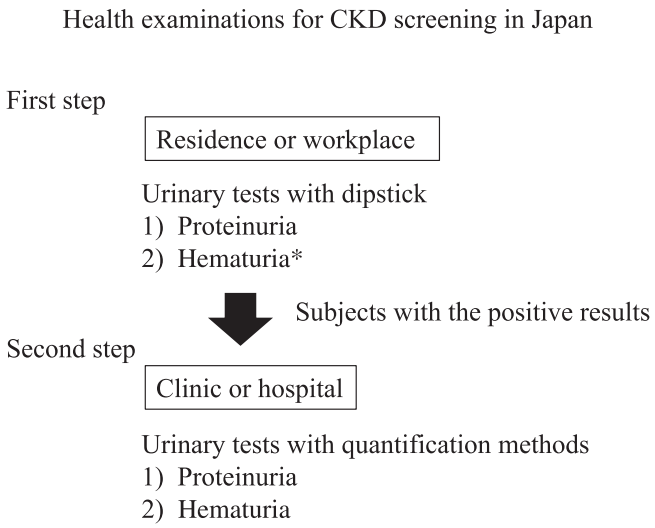

FIGURE 1 Health examinations for chronic kidney disease screening in Japan. *Some heath examinations involve the measurement of urine red blood cell (RBC) count to test for hematuria at the first step

clinical diagnostic criteria for CKD.[5,6] However, to increase the accuracy of the urine tests performed during health examinations, methods for the measurement of the severity of proteinuria using the urine creatinine (Cr) concentration should be introduced, and quantitative methods for the evaluation of hematuria and proteinuria should be considered.[4] These methods already form part of the more thorough examinations performed in clinics and hospitals in Japan. This is important, because tests with a low positive predictive value (PPV) might yield a high rate of false positive results, leading to a loss of work-hours. Therefore, we evaluated the results of dipstick tests for proteinuria and hematuria in occupational health settings. There have been few similar previous comparisons of urinary dipstick and quantitative tests in the context of the mass screening of the general population.[7] One epidemiological study of the general population in Australia, using a protein/Cr ratio of >150 mg/g Cr, yielded a sensitivity of 81% and a specificity of 94.6%.

In the present study, we aimed to compare the findings with respect to proteinuria and hematuria of qualitative examinations using dipstick tests and quantitative examinations using biochemical analyses in the same urine samples obtained from middle-aged male employees. To the best of our knowledge, this is the first field study of occupational health examinations to compare dipstick and quantitative tests for both proteinuria and hematuria in relatively young workers in Japan.

2 | METHODS

2.1 | Participants

Five hundred seventy-six male workers at the Kagoshima University Sakuragaoka campus, who underwent a health examination in November or December 2017, were asked to participate in the present study. Three of these individuals refused to participate; therefore, there were 573 participants. Each health examination was performed in the morning or afternoon of a working day. The study was approved by the Ethics Committee for Epidemiological Studies, Kagoshima University (No. 170189 Epi).

2.2 | Urinary examinations

Each participant provided a spot urine sample during a health examination. Qualitative tests for proteinuria, hematuria, and glucosuria were performed using a dipstick (Uropaper III-5; Eiken Chemical, Co, Ltd, Tokyo, Japan). The chemical principles of these were: proteinuria: the tetrabromophenol blue method; hematuria: the peroxidase-like reaction; and glucosuria: the glucose oxidase reaction. The dipstick results were objectively evaluated using a US-2100R analyser (Eiken Chemical, Co, Ltd, Tokyo, Japan). Urine samples were transported to the Clinical Laboratory, Kagoshima University Medical and Dental Hospital, within 1 hour of collection. The Cr and protein concentrations were measured using a JCA-BM6010 analyser (JEOL Ltd., Tokyo, Japan). The RBC count was determined using a UF-1000i cytometer (Sysmex Co., Hyogo, Japan). According to the manufacturer's manual, a color on the dipstick corresponding to (±), (+), (2+), (3+) or (4+) indicates the presence of proteinuria, and a color on the dipstick corresponding to (+), (2+) or (3+) indicates the presence of hematuria. According to the criteria for the quantitative analysis of urinary protein, ≥ 0.15 g/g Cr is diagnostic for proteinuria,[8] and according to the guidelines for the quantitative analysis of urine RBC count using flow cytometry, ≥20 cells/μL is diagnostic for hematuria.[9] The UF-1000i instrument detects RBCs with high sensitivity, which renders the output clinically relevant.[10] In the present study, we obtained dipstick readings for proteinuria, hematuria and glucosuria, and laboratory data for the urine protein and Cr concentrations and RBC count.

2.3 | Data analysis

Continuous data are summarized as either the mean (SD), median (range) or median (first and third quartiles); and categorical data are summarized as percentages. Significant differences between two groups were identified using the Wilcoxon unpaired two-sample test, and among four groups using the Kruskal-Wallis test, followed by the Steel-Dwass method for multiple comparisons. R version 3.4.3 was used for statistical analyses (www. https://www.r-project.org/). A two-tailed *P*-value of <.05 was considered to indicate statistical significance.

3 | RESULTS

3.1 | Participant characteristics

The study population comprised 576 male staff at Kagoshima University and University Hospital who underwent health examinations. Of

these, 573 provided consent and participated in the present study. Their ages ranged from 23 to 66 years, with mean and median ages of 38.6 and 38 years, respectively (Table 1). The numbers of participants who underwent their health examinations in the morning and afternoon were 345 and 228, respectively (Table 1).

3.2 | Urine Cr concentration

A histogram of the urine Cr concentrations of the participants is shown in Figure 2. The median urinary Cr concentration was 136.5 mg/dL (range: 4.2-466.0 mg/dL). Participants who had their health examination in the morning (median: 129.8; range: 17.6-411.7 mg/dL) and in the afternoon (median: 143.9; range: 4.2-466.0 mg/dL) showed no statistically significant difference in the urine Cr concentration. The participants were allocated to four groups according to their age: 40-49.9 years (N = 160; median: 118.5; range: 8.9-453.1 mg/dL) and those aged ≥50 years (N = 89; median: 114.9; range: 4.2-290.5 mg/dL) had statistically significantly lower urine Cr concentrations than those aged 20-29.9 years (N = 121; median: 154.9; range: 20.5-466.0 mg/dL) and those aged 30-39.9 years (N = 203; median: 141.2; range: 8.9-453.1 mg/dL). The participants were also allocated to four groups according to their height. Those who were ≥180 cm tall (N = 45; median: 165.1; range: 27.5-337.1 mg/dL) had statistically significantly higher urinary Cr concentrations than those who were <160 cm (N = 14; median: 65.5; range: 17.9-241.0 mg/dL), 160-169 cm (N = 209; median: 135.4; range: 13.9-466.0 mg/dL), and 170-179 cm (N = 305; median: 134.4; range: 4.2-453.1 mg/dL) tall.

3.3 | Proteinuria and urinary Cr concentration

We compared the results of the qualitative (dipstick) test and the quantitative test that included the correction of urine protein concentration for Cr (Table 2). The dipstick test for urinary protein generated four false-negative results, yielding a sensitivity of 55.6%, and 43 false-positive results, yielding a specificity of 92.4%. The participants for whom false-negative results were obtained showed statistically significantly lower urinary Cr concentrations than those with negative results for both tests and those with false-positive results. Among the four participants for whom false-negative results were obtained, two showed a (3+) result for urinary glucose on their dipsticks, which might be indicative of diabetes. Moreover, these two participants had high urine protein concentrations (0.71 and 0.83 g/g Cr), indicative of diabetic nephropathy. The other two participants for whom false-negative results were obtained had urine Cr concentrations of 27.1 and 83.2 mg/dL and were in the 40 to 49.9 years age

TABLE 1 Characteristics of the enrolled participants

Characteristic	Mean ± SD	Median (min-max)
Age, years	38.6 ± 9.8	38 (23–66)
Height, cm	171.3 ± 6.0	171.1 (148.7–188.8)
Body mass, kg	69.9 ± 11.6	68.1 (47.0–121.2)
BMI, kg/m^2	23.8 ± 3.6	23.3 (16.1–41.1)
Time of the examination		
a.m. (n)	345 (60)[a]	
p.m. (n)	228 (40)[a]	

Abbreviation: BMI, body mass index.
[a]These values are expressed as %.

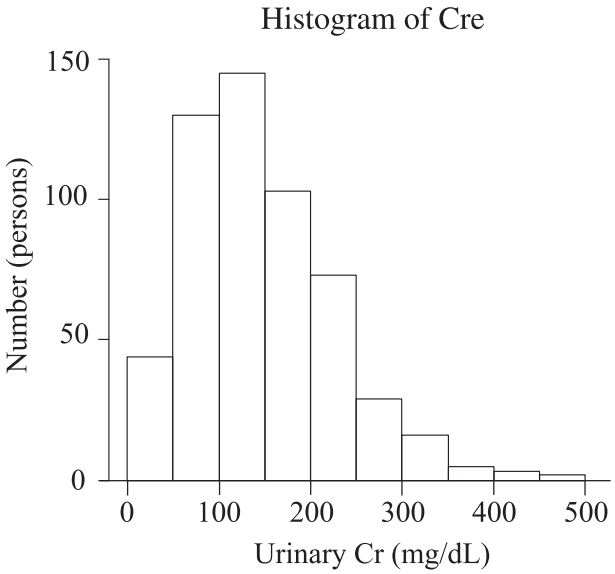

FIGURE 2 Distribution of urinary Creatinine concentration among the participants

TABLE 2 Comparison of the results of the qualitative and quantitative tests of proteinuria and hematuria

	Dipstick			
	(–)	(±)	(+)	(2+)
Proteinuria				
<0.15 g/L	525	32	3	0
≥0.15 g/L	0	6	6	1
<0.15 g/g Cr	521	37[a]	6[a]	0
≥0.15 g/g Cr	4[b]	1	3	1
Hematuria				
<20 cells/μL	508	28	7[a]	2[a]
≥20 cells/μL	2[b]	8[b]	10	8

[a]False-positive results for proteinuria or hematuria, using thresholds of 0.15 g/g Cr and 20 cells/μL, respectively, according to the Japanese criteria.
[b]False-negative results for proteinuria or hematuria, using thresholds of 0.15 g/g Cr and 20 cells/μL, respectively, according to the Japanese criteria. Proteinuria and hematuria were evaluated quantitatively using a biochemical method, with and without Cr correction, and flow cytometry, respectively (see Methods section). The dipstick test did not yield (3+) or (4+) results for proteinuria or (3+) for hematuria for any of the participants.

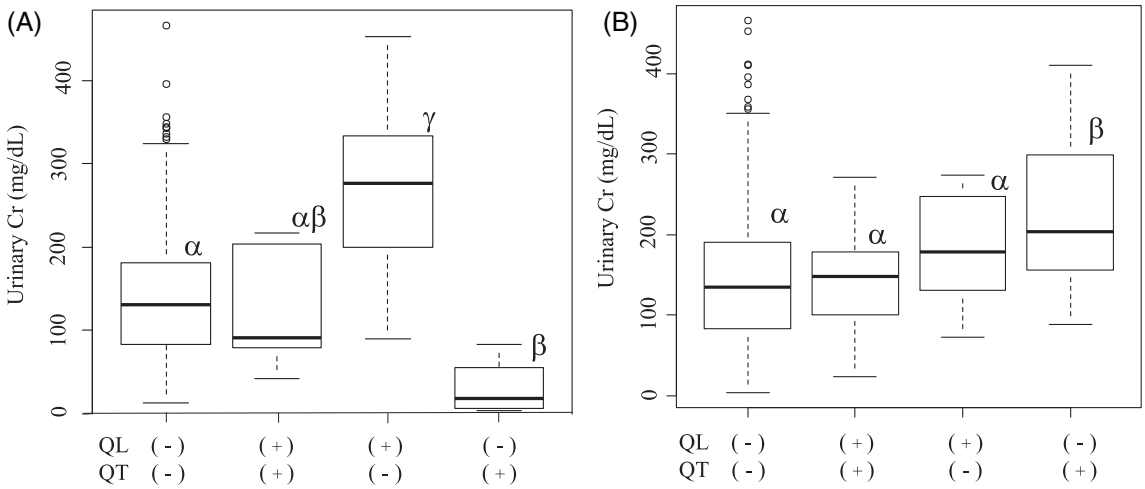

FIGURE 3 Urinary Creatinine (Cr) concentration, according to the qualitative (QL) and quantitative (QT) tests. The results for proteinuria (A) and hematuria (B) are shown, and (–) and (+) denote negative and positive results, respectively. Box plots show the distribution of urinary Cr concentration (mg/dL) for the four groups. The median values are shown as thick horizontal lines and outliers are shown as open circles. Groups labeled with the same letter were not significantly different

group and the 170 to 179 cm height group, which indicates that they were neither of advanced age nor small stature, which are generally associated with low urine Cr concentration. The participants for whom false-positive results were obtained showed statistically significantly higher urine Cr concentrations than the other three groups (Figure 3A). In addition, the PPV for the assessment of proteinuria using the dipstick test was 10.4%. The identification of a proteinuria of >0.15 g/g Cr in those participants for whom the urine dipstick test was negative for proteinuria may be explained by the correction for the urine concentration of Cr or by the urine dipstick principally reacting to albumin, whereas other proteins can also be quantified using the laboratory assay.

3.4 | Hematuria

We next compared the qualitative (dipstick) and quantitative (flow cytometry) tests of the urine RBC count (Table 2). The dipstick generated 10 false-negative results, yielding a sensitivity of 64.3%, and nine false-positive results, yielding a specificity of 98.3%. The participants for whom false-negative results were obtained had significantly higher urine RBC counts than participants in any of the other groups (Figure 3B). Among the participants for whom false-negative results were obtained, all were negative for urine glucose by dipstick. The PPV for hematuria using the dipstick test was 66.7%.

4 | DISCUSSION

In the present study, we compared qualitative tests (dipstick), which were performed during an occupational health examination, with quantitative tests performed at clinics and hospitals, for the analysis of urine protein and RBC count. This field study was performed in relatively young workers, with a median age of 38 years. Their Cr concentrations ranged from 4.2 to 466.0 mg/dL, which implies that their urine protein concentration should be corrected for Cr to improve the accuracy of the analyses in this population. In addition, a comparison between the qualitative and quantitative results for the urine protein concentration and RBC count showed that the qualitative tests had low sensitivity and PPV. These findings suggest that caution should be used when using qualitative analyses for the diagnosis of CKD during general health examinations. The examinations were performed in a physician's office and the same urine samples were analysed using each method, which should improve the accuracy of the comparison and limit the degradation of the samples prior to the measurements being made. As shown in Table 2, 35 participants who had a urine protein concentration of <0.15 g protein/L were proteinuria-positive on the dipstick test. However, none of the participants who had a urine protein concentration >0.15 g protein/L were proteinuria-negative on the dipstick test. These findings imply that the difference between the results of the urine protein measurement with correction for Cr concentration and the dipstick test can be explained by the technical quality of each technique, the threshold value chosen, and whether the values obtained are adjusted for Cr concentration.

The urinary Cr concentrations in spot urine samples showed a great deal of variation, but were significantly associated with age and height. The finding that advanced age and small stature each show statistically significant associations with low Cr concentration may be explained by the relationship between urine Cr concentration and the skeletal muscle mass, as previously described.[11] In addition, the urine Cr concentration may be affected by the time of sample collection, which affects the degrees of urinary condensation and dilution. In the present study, there were no statistically significant differences in urine Cr concentration between the participants who underwent a health examination in the morning and those who underwent one in the afternoon. With respect to the urine protein measurements made

in the study, the false-negative and false-positive groups had statistically significantly lower and higher Cr concentrations, respectively, than the reference group. These false-negative and false-positive results may be explained by the dilution and condensation of the urine samples, as indicated by the statistically significant differences in Cr concentration among the groups (Figure 3A). The finding that two of the four participants for whom false-negative results for proteinuria were obtained had (3+) results for urine glucose suggests the importance of making quantitative measurements with Cr correction, especially for the diagnosis of diabetic nephropathy, and this finding is consistent with that of a previous study.[12] In patients with type 2 diabetes who did not have proteinuria, according to dipstick testing, >10% were found to have microalbuminuria when their urine protein concentrations were measured using a quantitative method with correction for their urine Cr concentration. Furthermore, Nagai and Yamagata reported that urinary protein concentration highly correlates with urinary albumin concentration (r = .99).[4] Therefore, Cr correction of total urinary protein concentration could be used to improve the accuracy of the measurements. Furthermore, for the assessment of the urine RBC count, the false-negative group had significantly higher Cr concentrations than the other groups. Therefore, the lack of detection of hematuria using the qualitative test cannot be explained by a low urinary Cr concentration. Instead, condensation secondary to the high Cr concentration may affect the results of the peroxidase activity test on the dipstick.

In the present study, the dipstick test was shown to have low sensitivity (55.6%) and PPV (10.4%) for proteinuria and hematuria (64.3% and 66.7%, respectively). These results for proteinuria are consistent with those of a previous study of Japanese employees.[13] The sensitivity and PPV are derived from the number of false-negative and false-positive results, respectively. The false-negative and false-positive results have been obtained because of the different methods used by the qualitative and quantitative tests for the quantification of protein concentration and RBC count, in addition to the urine Cr concentration. The qualitative measurement of urine protein concentration can be affected by the pH of the sample, leading to over- or underestimation compared with the results of the quantitative test, which is based on the biuret method and is performed in solution.[14] For the urine RBC assessment, the qualitative method is based on peroxidase activity, which can be affected by contamination with white blood cells and tissue, high concentrations of antioxidants, such as ascorbic acid, and low urine pH. These can also lead to over- or underestimation of the RBC count compared with quantitative methods, typically flow cytometry or microscopic examination.[15,16] The qualitative tests had PPVs of 10.4% for proteinuria and 66.7% for hematuria. These relatively low values indicate the importance of performing quantitative analyses of samples obtained during occupational health examinations. However, such employees are not always able to visit hospitals or clinics for more thorough examinations.

Regarding the direct costs of the tests, the dipstick method for the analysis of urine protein and RBC count costs approximately 45 yen per test, whereas the quantitative test with Cr correction costs 150 yen, which comprises 80 yen for the Cr measurement, 10 yen for the protein measurement and 60 yen for the RBC count by flow cytometry. Although the quantitative method costs more than the qualitative method, false-positive results are not only associated with direct costs, but also indirect costs related to the loss of work-hours, owing to the necessity for hospital visits for more thorough examinations. Regarding the practicality of the tests, the qualitative dipstick method is more convenient than the quantitative method. However, efforts should be made to avoid false-negative results when screening for CKD as part of a general health examination.

There were several limitations to the present study. First, it was performed in a single workplace, albeit that this was fairly representative of workplaces in this area of Japan. Second, the cut-off value of urine protein used to define proteinuria, which was 0.15 g/g Cr in the present study, affects the identified false negative and positive rates. For patients with diabetes, ≥30 mg albumin/g Cr is regarded as indicating proteinuria.[7] There is a strong correlation between the amounts of protein and albumin in urine[4] because urine protein is principally albumin. Therefore, the value of urinary protein in the evaluation of CKD may be lower in certain circumstances, and especially in the presence of diabetes.[17] This issue should be evaluated further in future studies. Third, with respect to hematuria, positivity in the quantitative test is defined as ≥20 cells/mL, which is not corrected for urine Cr concentration according to the criteria.[8] Thus, false-negative and false-positive results are not explained by variation in Cr concentration (Figure 3B), but Cr correction may be applicable to the diagnosis of hematuria. Fourth, we used urine Cr to correct urine protein concentrations, but urinary Cr concentration was highly variable in the present study. Urinary Cr concentration is believed to remain relatively constant; however, the amount of Cr released by muscles is likely to be related to the amount of muscle present. Therefore, the severity of proteinuria may be overestimated using Cr correction more frequently in older or shorter individuals, who tend to have lower muscle mass, and underestimated in younger or taller people, who tend to have higher muscle mass. Therefore, a general health examination can only provide a rough guide to an individual's health status, unless more thorough and frequent (eg, annual) examinations are performed.

5 | CONCLUSION

The qualitative dipstick tests that are used for the diagnosis of proteinuria and hematuria have low sensitivity and PPV, according to the results of quantitative tests performed in clinics. This low sensitivity is particularly concerning, given that dipsticks are routinely used as screening tests for CKD during general health examinations. In addition, the low PPVs for proteinuria and hematuria indicate the importance of the use of quantitative tests in occupational health screens because employees are not always able to visit hospitals or clinics for more thorough assessments. The results may depend on the method used to measure urine protein concentration and RBC count; therefore, the conclusion is specific to this type of dipstick. Further detailed

studies should be performed to confirm the findings of the present field study.

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CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Kanako Ikeda, Masahisa Horiuchi

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All authors have read and approved the final version of the manuscript.

Masahisa Horiuchi had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The corresponding author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

DATA AVAILABILITY STATEMENT

Data are available on request due to privacy/ethical restrictions.

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