medical follow-up, and adherence to treatment.[6,7] For several decades, many projects and studies have been conducted to clarify the factors associated with BP levels,[7–10] based on established treatment guidelines.[3,11,12] Major guidelines include the 2007 Guidelines for the Management of Arterial Hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and the European Society of Cardiology (ESC) (ESH/ESC 2007)[11] from Europe, the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7)[3] from the United States, and the 2003 WHO/International Society of Hypertension (ISH) Statement on Management of Hypertension (WHO/ISH).[12] These reports have produced concise, evidence-based manuals for the most effective and convenient therapy for hypertensive patients, although there are some differences between them regarding recommended first choice drugs and combinations of drugs. These guidelines provide clear and practical treatment algorithms, indicating goal BPs that take into consideration a patient's risk factors: <130/80 mm Hg for patients with diabetes mellitus or chronic kidney disease and 140/90 mm Hg for those without the diseases. Intensive and strict BP control among hypertensive patients with diabetes mellitus and/or chronic kidney disease is emphasized,[3,11,13] because hypertension is a known risk factor for these outcomes.[13] Furthermore, diabetes mellitus often leads to atherosclerotic disorders[14,15] and chronic kidney disease, which is defined as either renal damage or decreased kidney function for 3 months or longer,[16,17] and causes CVD.[18,19]

In Japan, the Japanese Society of Hypertension[20] first published the Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2000) in 2000, which was revised as JSH 2004 in 2004. The guidelines explain the measurement and clinical evaluation of BP as well as basic principals of treatment and lifestyle modification. It also indicates adequate BP goals according to individuals' risk factors, which is similar to previously published guidelines. The average BPs of the Japanese population have decreased between 1961 and 1990 among both men (from 143.2/83.0 mm Hg to 134.3/82.9 mm Hg) and women (from 143.3/82.2 mm Hg to 128.4/77.6 mm Hg) aged 30 to 69 years.[21] During the same period, the incidence of stroke has significantly decreased in response to health promotion activities and introduction of new antihypertensive drugs.[20–22] Nevertheless, there are more than 30 million hypertensive patients in Japan,[23] and it is the second most common disease among those categorized within the metabolic syndrome.[24]

Although a few studies reported robust achievement rates based on 140/90 mm Hg as a goal BP level,[25–27] there have been no reports from Japan assessing the rates toward individualized BP goals, and the present study was the first trial to evaluate these rates in a community. The aim of our study was to assess success rates in achieving treatment goals as defined by JSH 2004 in one prefecture in Japan. We will also explore the factors associated with these success rates, including patients' and physicians' characteristics.

RESEARCH DESIGN AND METHODS

The present study was a prospective cohort study carried out in Fukushima Prefecture, Japan, from July 2006 to May 2007. Fukushima Prefecture is located in the northern region of Japan, with a population of about 2 million. From March to April 2006, we called physician-members of the Fukushima Hypertension Conference to solicit participation in this study. The Fukushima Hypertension Conference was established in 1997 and there were 120 members as of April 2006.

Participants in our study had hypertension and had received antihypertensive medication for at least 3 months and visited a participating physician during the baseline survey period (July 2006). In the baseline survey, the physician enrolled the first 10 consecutive patients who were eligible and willing to participate in our study.[28] The enrollment continued until the total number of registered patients reached 50 for each physician. Even if the number of enrolled patients did not reach 50, the recruitment was stopped on the last day of July 2006. The research date was not announced to patients prior to the survey, and appointments for medical consultation were made according to their requests as usual. Enrolled patients were monitored for 1 year in 3-month intervals.

In the baseline survey, the registered patient's clinical data was copied from medical files to survey sheets. The data included the patient's age, sex, height, weight, waist circumference, family histories (hypertension, diabetes mellitus, dyslipidemia, heart disease, stroke, renal disease, and premature CVD), alcohol consumption, current smoking habits, systolic and diastolic BPs, whether home BP measurement was instructed, duration of hypertension treatment, usage of antihypertensive drugs, and presence of metabolic disorders (diabetes mellitus,

dyslipidemia), end-organ damage, and CVDs (brain, heart, kidney, blood vessel, hypertensive, and diabetic retinopathy). The status of renal disease and diabetes mellitus was obtained from physician reports. In Japan, diabetes mellitus is defined based on the Japan Diabetes Society, Diabetes Treatment Guideline 2008–2009.[29] Renal disease is defined based on the Japanese Clinical Practice Guidebook for Diagnostic and Treatment of Chronic Kidney Disease.[16] As for methods to measure BP, we asked physicians to maintain their usual practices and report BP measurements on each day the patients were surveyed. Follow-up surveys (October 2006, January 2007, and April 2007) collected hypertension-related information. As for physicians' characteristics, the following information was collected in the baseline survey: age, sex, place of employment, main specialty, number of hypertensive patients (per month), and measurer, timing, place, and method of BP measurement. The present report used data from the baseline survey and conducted analyses on achievement toward treatment goals and its associated factors.

All data were entered into a computer and analyzed using SPSS version 14 (SPSS Inc, Chicago, IL). We classified participants into 3 groups according to the JSH 2004: elderly patients 65 years and older without diabetes mellitus or renal disease, young or middle-aged patients without diabetes mellitus or renal disease, and patients with diabetes mellitus or renal disease. The success rates were calculated following treatment goals for each group indicated in JSH 2004: <140/90 mm Hg for elderly patients without diabetes mellitus or renal disease, <130/80 mm Hg for patients with the diseases, and <130/85 mm Hg for young or middle-aged patients without the diseases. For the analysis of factors associated with failure to achieve the treatment goals, we computed odds ratios (ORs) and 95% confidence intervals (CIs) for each item using univariate logistic regression. Significant factors in the univariate analysis ($P<.05$) were then entered into a multivariate logistic regression analysis.

With regard to the analysis of physicians' characteristics and the success rates of their patients, we divided participating physicians into 2 groups using a median split of overall patient success rates (<45% vs ≥45%). The 2 groups were compared using the chi-square test and Fisher exact test for categoric items and Mann–Whitney test for continuous items.

This survey was conducted according to the Ethical Guideline for Epidemiological Studies established by the Japanese government,[30] and work was performed in accordance with the Declaration of Helsinki of 1975 (revised in 2000).[31]

RESULTS

Seventy-two of 120 members of the Fukushima Hypertension Conference enrolled patients into the study. In the baseline survey, 3358 hypertensive patients were initially registered. Of those registered, 38 patients were excluded due to missing data on BPs and nonmedication, and thus 3320 patients were entered into the present analysis. Median age of patients was 71 years (24–99 years) and the percentage of males was 46.1% (Table I). As for anthropometric measurements, median body mass index (BMI) was 24.3 (13.2–45.4), and median waist circumference was 87.6 cm (59.0–126.0 cm) for males and 85.0 cm (53.0–134.0 cm) for females. Among family histories, the prevalence of hypertension was most frequent (55.2%), followed by stroke (27.6%), diabetes mellitus (18.0%), and heart disease (15.3%). The prevalence of alcohol use (daily consumption) was 21.7%, and that of current smoking was 12.1%. The median systolic and diastolic BPs were 134 mm Hg (82–212 mm Hg) and 76 mm Hg (36–124 mm Hg), respectively. Sixty percent of patients were instructed to measure BPs at home, 43.6% of patients were treated by 1 antihypertensive drug, and the median duration of hypertension treatment was 8.0 years (0.5–60.0 years). The proportion of those with diabetes mellitus was 31.7% and that of dyslipidemia was 44.8%. Cardiovascular complications were reported in 21.5% of patients, neurological complications in 13.4%, and renal complications in 11.1%.

Table II shows various characteristics of the physicians assisting in this study. Seventy of 72 physicians completed the questionnaire. The proportion of males was 93.0% and median years after graduation from medical school was 24 years. The most frequent specialty among participating physicians was general internal medicine (n=35), followed by cardiology (n=17), gastroenterology (n=7), and endocrinology (n=7). The proportion of those working at hospitals was 52.9%, and 60.0% were located in urban areas (defined as cities with >100,000 residents). Median number of hypertensive patients per physician per month was 300. Eighty percent of physicians measured BPs by themselves, 82.9% during medical consultation, 82.9% in a consultation room, and 72.9% using mercury sphygmomanometer.

The median systolic and diastolic BPs were 134 mm Hg (84–190 mm Hg) and 75 mm Hg (36–120

Table I. Characteristics of Hypertensive Patients at Baseline

Variables	Median (Range) or No. (%)
Age, y	71 (24–99)
Male sex	1524 (46.1)
Anthropometric measurements	
Body mass index	24.3 (13.2–45.4)
Waist circumference, cm	
Male	87.6 (59.0–126.0)
Female	85.0 (53.0–134.0)
Family histories	
Hypertension	1805 (55.2)
Stroke	902 (27.6)
Diabetes mellitus	589 (18.0)
Heart disease	499 (15.3)
Dyslipidemia	132 (4.0)
Renal disease	123 (3.8)
Premature cardiovascular disease	47 (1.4)
Alcohol consumption (daily)	705 (21.7)
Current smoking	392 (12.1)
Hypertension-related factors	
Systolic blood pressure, mm Hg	134 (82–212)
Diastolic blood pressure, mm Hg	76 (36–124)
Instruction of home blood pressure measurement (yes)	1969 (59.6)
Duration of hypertension treatment, y	8.0 (0.5–60.0)
No. of antihypertensive drug used	
1	1449 (43.6)
2	1318 (39.7)
≥3	553 (16.7)
Metabolic disorders	
Diabetes mellitus	1050 (31.7)
Dyslipidemia	1484 (44.8)
Organ damage/cardiovascular disease	
Heart	713 (21.5)
Brain	445 (13.4)
Kidney	368 (11.1)
Peripheral vascular disease	249 (7.5)
Hypertensive retinopathy	150 (4.5)
Diabetic retinopathy	176 (5.3)

Table II. Characteristics of Physicians Participating in the Survey

Variables	Median (Range) or No. (%)
Male sex	65 (93.0)
Years after graduation from medical university, y	24 (8–44)
Main specialty	
General internal medicine	34 (48.6)
Cardiology	16 (22.9)
Gastroenterology	7 (10.0)
Endocrinology	7 (10.0)
Others	6 (8.5)
Medical office	
Hospital	37 (52.9)
Clinic	33 (47.1)
Location of medical office (urban[a])	
Urban[a]	42 (60.0)
Rural	28 (40.0)
Number of attending hypertension patients (No. per month)	300 (15–1500)
Measurer of BP	
Physician	56 (80.0)
Nurse	8 (11.4)
Patient	6 (8.6)
Timing of BP measurement	
During medical consultation	58 (82.9)
Waiting time	11 (15.7)
Others	1 (1.4)
Place of BP measurement	
Consultation room	58 (82.9)
Treatment room or waiting space	11 (15.7)
Others	1 (1.4)
Method of BP measure	51 (72.9)
Mercury sphygmomanometer	51 (72.9)
Automated sphygmomanometer	19 (27.1)
No. of registered patients	49.5 (14–51)
Achievement rate toward treatment goals	43.9 (14.3–82.0)

Abbreviation: BP, blood pressure. [a]Urban is defined as a city with a population of ≥100,000.

mm Hg) for elderly without diabetes mellitus or renal disease, 132 mm Hg (100–180 mm Hg) and 80 mm Hg (43–106 mm Hg) for those younger than 65 years without the diseases, and 134 mm Hg (82–212 mm Hg) and 76 mm Hg (39–124 mm Hg) for those with the diseases. Success rates toward treatment goals (defined by JSH 2004) were 66.0% for the elderly without diabetes mellitus or renal disease, 30.4% for those younger than 65 years without the diseases, and 26.7% for those with the diseases (Table III). We conducted an additional analysis among those younger than 65 years without the diseases according to JNC 7[3] (<140/90 mm Hg), whose target BP level differs from JSH 2004, and found the success rate to be 65.9%.

The Figure shows the number of antihypertensive drugs used. Monotherapy was most frequent among the elderly without diabetes mellitus or renal disease (47.7%), while bitherapy was most frequent among the patients with the diseases (40.9%). Median systolic and diastolic BPs were 132 mm Hg (82–190 mm Hg) and 77 mm Hg (36–120 mm Hg) among patients treated with monotherapy, 134 mm Hg (92–190 mm Hg) and

Table III. Median of Blood Pressure (BP) and Success Rates Toward Target BP Levels

	JSH 2004 Target BP Level, mm Hg	Median (Range) of Systolic and Diastolic BP, mm Hg	Success Rates, No. (%)
Elderly patients without diabetes mellitus or renal disease (n=1518)	<140/90	134 (84–190)/75 (36–120)	1002 (66.0)
Young or middle-aged patients without diabetes mellitus or renal disease (n=583)	<130/85	132 (100–180)/80 (43–106)	177 (30.4)
Patients with diabetes mellitus or renal disease (n=1212)	<130/80	134 (82–212)/76 (39–124)	324 (26.7)

Abbreviation: JSH 2004, Japanese Society of Hypertension Guidelines for the Management of Hypertension.

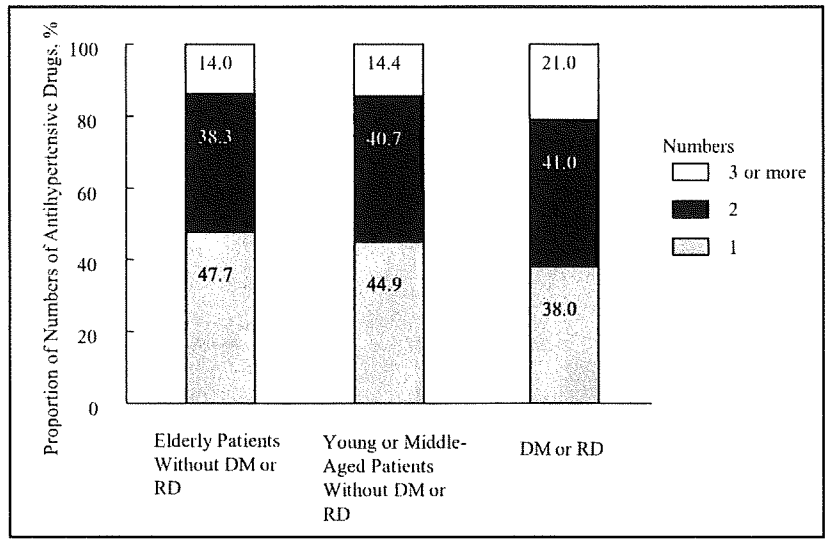

Figure. Numbers of antihypertensive drugs. DM indicates diabetes mellitus; RD, renal disease.

76 mm Hg (39–111 mm Hg) among those treated with bitherapy, and 135 mm Hg (96–212 mm Hg) and 76 mm Hg (41–124 mm Hg) among those treated with ≥3 drugs.

The multivariate analysis showed that the factors significantly associated with failure to achieve treatment goals were waist circumference of ≥85 cm for men and ≥90 cm for women (OR, 1.26; 95% CI, 1.01–1.57) and usage of ≥3 antihypertensive drugs (OR, 1.96; 95% CI, 1.42–2.71) for the elderly group without diabetes mellitus or renal disease (Table IVa). For young and middle-aged patients without diabetes mellitus or renal disease, the significant factors were BMI ≥25 (OR, 1.74; 95% CI, 1.19–2.56), family history of hypertension (OR, 1.67; 95% CI, 1.14–2.45), cerebrovascular complication (OR, 0.33; 95% CI, 0.16–0.68), and hypertensive retinopathy (OR, 0.33; 95% CI, 0.12–0.91) (Table IVb). For patients with diabetes mellitus or renal disease, BMI ≥25 (OR, 1.34; 95% CI, 1.03–1.75), family history of diabetes mellitus (OR, 1.40; 95% CI, 1.04–1.87), dyslipidemia (OR, 1.41; 95% CI, 1.08–1.84), and cerebrovascular (OR, 0.62; 95% CI, 0.44–0.87) and vascular complications (OR, 0.48; 95% CI, 0.33–0.70) were significantly associated (Table IVc).

Table V shows the differences in characteristics between 2 groups of physicians categorized by overall success rates of their patients. The proportion of elderly patients without diabetes mellitus or renal disease was higher among physicians with higher success rates.

DISCUSSION

This community-based assessment of hypertension control among our patients in one prefecture in Japan showed excellent results with a median BP <140/90 mm Hg. Now given new target BP levels redefined by recent hypertension management guidelines,[3,11,20] we used the JSH 2004 to calculate the success rates among our patient population. Achievement rates were relatively pessimistic, especially among patients with diabetes mellitus or renal disease and those younger than 65 years without

Table IV. Association of Blood Pressure Achievement Failure

Variables	No. (%)	Odds Ratio	95% Confidence Interval	*P* Value
(a) In elderly patients without diabetes mellitus or renal disease (multivariate logistic regression analyses)				
Waist circumference ≥85 cm for men, ≥90 for women	620 (42.1)	1.26	1.01–1.57	<0.05
No. of antihypertensive drugs used				
1	461 (38.0)	1.00 (Reference)		
2	496 (40.9)	1.15	0.91–1.46	
≥3	255 (21.0)	1.96	1.42–2.71	<0.05
(b) In young and middle-aged patients without diabetes mellitus or renal disease (multivariate logistic regression analyses)				
Body mass index ≥25	241 (42.8)	1.74	1.19–2.56	<0.05
Family history of hypertension (yes)	378 (66.4)	1.67	1.14–2.45	<0.05
Organ damage/cardiovascular disease				
Brain (yes)	33 (5.7)	0.33	0.16–0.68	<0.05
Hypertensive retinopathy (yes)	17 (2.9)	0.33	0.12–0.91	<0.05
(c) In patients with diabetes mellitus or renal disease (multivariate logistic regression analyses)				
Body mass index ≥25	582 (48.5)	1.34	1.03–1.75	<0.05
Family history of diabetes mellitus (yes)	379 (31.5)	1.40	1.04–1.87	<0.05
Dyslipidemia (yes)	656 (54.3)	1.41	1.08–1.84	<0.05
Organ damage/cardiovascular disease				
Brain (yes)	191 (15.8)	0.62	0.44–0.87	<0.05
Blood vessel (yes)	144 (11.9)	0.48	0.33–0.70	<0.05

Table V. Physician Characteristics of Blood Pressure Achievement Rates Toward Treatment Goals

	Achievement Rate Median (Range) or No. (%)[a]		*P* Value
Variables	<45% (n=38)	≤45% (n=32)	
Male sex	35 (92.1)	30 (93.8)	
Years after graduation from medical university	24 (8–40)	25 (11–44)	
Main specialty (internal medicine)	37 (97.4))	29 (90.6)	
Medical office (hospital)	23 (60.5)	14 (43.8)	
Location of medical office (urban[b])	25 (65.8)	17 (53.1)	
Number of attending hypertension patients (for one month)	300 (15–1500)	300 (32–1200)	
Measurer of BP (physician)	31 (81.6)	25 (78.1)	
Timing of BP measurement (during medical consultation)	32 (84.2)	26 (81.3)	
Place of BP measurement (consultation room)	32 (84.2)	26 (81.3)	
Method of BP measure (mercury sphygmomanometer)	28 (73.7)	23 (71.9)	
Proportion of registered patients, %			
Elderly patients without diabetes mellitus or renal disease	42.8 (0.0–74.0)	55.0 (0.0–84.0)	<0.01
Young and middle-aged patients without diabetes	18.6 (0.0–60.0)	13.3 (0.0–61.3)	
Patients with diabetes mellitus or renal disease	29.2 (4.0–100)	27.9 (2.0–100)	

Abbreviation: BP, blood pressure. [a]The chi-square test and Fisher exact test for categoric items and Mann–Whitney test for continuous items were used to assess the significance. [b]Urban is defined as a city with a population of ≥100,000.

diseases based on JSH 2004, although median BPs showed excellent results of <140/90 mm Hg.

Other factors associated with failure to achieve treatment goals included BMI, waist circumference, family histories, dyslipidemia, and usage of ≥3 antihypertensive drugs increased the risk of achievement failure, while presence of complications was paradoxically associated with success rates.

The success rate of elderly patients without diabetes mellitus or renal disease in our study was substantially higher compared with those of young and middle-aged patients. Likewise, a Japanese cross-sectional study reported that achievement rate toward goal BP (defined as <140/90 mm Hg) was 17.0% in patients younger than 60 years while it was 40.6% for patients 60 to 69 years, 54.4% for

patients 70 to 79 years, and 65% for patients 80 years and older.[32] In contrast, previous studies have reported advancing age as an independent predictor of inadequate BP control in the United States.[33,34] The discrepancies in achievement rates between the elderly and nonelderly in Japan and between the elderly in Japan and the United States could be explained in part by differences in health behaviors among the older generations. JNC 7 emphasizes the importance of the following 5 healthy lifestyles: weight reduction, improvement in dietary habits, dietary sodium restriction, increased physical activity, and appropriate alcohol consumption.[3] The proportions of those who exercise regularly, keep healthy weight, and do not smoke were higher in the elderly compared with the nonelderly according to a Japanese national survey.[35] In the United States, on the other hand, these proportions in the elderly are lower compared with the nonelderly.[36,37] Furthermore, BP level is correlated with cardiovascular mortality in the nonelderly,[10] which could result in a survivor effect causing a relatively elevated success rate among the Japanese elderly.

The study indicated that 2 markers of obesity, high BMI and waist circumference, were significantly associated with achievement failure among hypertensive patients with diabetes mellitus or renal disease, and young and middle-aged patients without the diseases. Several studies have reported a high prevalence of hypertension among obese individuals compared with nonobese individuals.[38–40] The sympathetic nervous system, sodium retention/salt sensitivity, and insulin resistance are thought to be involved in the etiology of hypertension accompanied by obesity.[20] As previous research emphasized,[41,42] body weight control is thus considered one of the most important therapeutic strategies in JSH 2004,[20] JNC 7,[3] and ESH/ESC 2007.[12] In other words, obesity is an important risk factor of failure to achieve treatment goals[33] as indicated in our results.

It is well-known that family histories of hypertension,[43] diabetes mellitus,[44] and dyslipidemia[45] are risk factors for hypertension. The number of these risk factors, as well as nonfavorable health behaviors, is associated with an increased incidence of hypertension and overall CVD severity. In Japan, family histories are usually recorded in standard medical files with other basic information and are checked in general clinical practice and routine health check-ups.

Interestingly, we found a positive association between history of organ and vascular complications and achieving treatment goals. It may be possible that both patient and physician become more aware of the need to maintain goal BP levels, once organ and vascular complications present. Previous studies have reported a similar association between history of CVD and improved BP control, explained by increased patient compliance and/or more aggressive treatment.[46,47] Supporting this hypothesis, Street and colleagues[48] also reported that physicians seeing patients with a critical disease paid more attention to their clients than physicians seeing patients with less severe conditions. Furthermore, a lack of disease awareness has been pointed out as a patient-related factor related to poor BP control.[49] Had patients been treated appropriately in the past, such a paradoxical result may not have been found, and presence of complications may have instead become a risk factor of inadequate BP control. Our results suggest the necessity of better management of hypertension prior to the onset of complications.

Using $\geq$2 drugs was a risk factor of achievement failure among our elderly patients without diabetes mellitus or renal disease. A previous report from the United States also showed that a multi-drug regimen was an independent risk factor of poor BP control,[46] and patients whose BP is difficult to control are more likely to be treated with multiple drugs. Additional analyses of our study showed that a multiple antihypertensive drug therapy correlates with higher number of vascular and/or organ damage, longer duration of hypertension treatment, and a family history of hypertension. These findings suggest that resistance to treatment persists among the elderly without diabetes mellitus or renal disease, despite physician adherence to treatment guidelines. Further analysis of follow-up data on change in antihypertensive drugs may provide additional insight on the relationship between different medications and BP control.

LIMITATIONS

Our study has some major limitations. First is a selection bias; the physicians who participated in our study were limited to members of the Fukushima Hypertension Conference, and participants were limited to hypertensive patients who visited these physicians. In addition, some important information on their characteristics and medical practices were not investigated, and the patient characteristics appeared as the only factor that differed significantly between 2 groups of physicians classified by their achievement levels. It is possible that participating members might be more aware about hypertension management compared with nonmembers.

Success rates might become lower showing significant associations with physician's characteristics once nonmembers are included. Secondly, this was a cross-sectional analysis using baseline data, and causal relationships between success rates and the associated factors cannot be fully elucidated. Further analyses of follow-up survey data are needed. Thirdly, other important factors, such as patient's health behaviors, physicians' treatment strategies, and their disease awareness were not obtained in our survey. The inclusion of additional factors in the multivariate analysis model might have modified our results.

CONCLUSIONS

The present study, which was conducted in one prefecture in Japan, revealed low achievement rates toward treatment goals among hypertensive patients regardless of physician characteristics, especially in groups with diabetes mellitus or renal disease and those younger than 65 years without the diseases. Analysis of associated factors indicated the importance of weight control, assessment of family history, and a need for better management before atherosclerotic complications appear.

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ORIGINAL ARTICLE

Prevalence of chronic kidney disease in the Japanese general population

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Abstract

Background We previously estimated the prevalence of chronic kidney disease (CKD) stages 3–5 at 19.1 million based on data from the Japanese annual health check program for 2000–2004 using the Modification of Diet in Renal Disease (MDRD) equation multiplied by the coefficient 0.881 for the Japanese population. However, this equation underestimates the GFR, particularly for glomerular filtration rates (GFRs) of over 60 ml/min/1.73 m^2. We did not classify the participants as CKD stages 1 and 2 because we did not obtain proteinuria data for all of the participants. We re-estimated the prevalence of CKD by measuring proteinuria using a dipstick test and by calculating the GFR using a new equation that estimates GFR based on data from the Japanese annual health check program in 2005.

This work was presented in part at the 50th Annual Meeting of the Japanese Society of Nephrology at Fukuoka in 2008.

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Methods Data were obtained for 574,024 (male 240,594, female 333,430) participants over 20 years old taken from the general adult population, who were from 11 different prefectures in Japan (Hokkaido, Yamagata, Fukushima, Tochigi, Ibaraki, Tokyo, Kanazawa, Osaka, Fukuoka, Miyazaki and Okinawa) and took part in the annual health check program in 2005. The glomerular filtration rate (GFR) of each participant was computed from the serum creatinine value using a new equation: GFR (ml/min/1.73 m^2) = 194 × $Age^{-0.287}$ × $S\text{-}Cr^{-1.094}$ (if female × 0.739). The CKD population nationwide was calculated using census data from 2005. We also recalculated the prevalence of CKD in Japan assuming that the age composition of the population was same as that in the USA.
Results The prevalence of CKD stages 1, 2, 3, and 4 + 5 were 0.6, 1.7, 10.4 and 0.2% in the study population, which resulted in predictions of 0.6, 1.7, 10.7 and 0.2 million patients, respectively, nationwide. The prevalence of low GFR was significantly higher in the hypertensive and proteinuric populations than it was in the populations without proteinuria or hypertension. The prevalence rate of CKD in Japan was similar to that in the USA when the Japanese general population was age adjusted to the US 2005 population estimate.
Conclusion About 13% of the Japanese adult population—approximately 13.3 million people—were predicted to have CKD in 2005.

Keywords Chronic kidney disease · Japanese · eGFR · Serum creatinine

Introduction

The number of chronic dialysis patients has been increasing over the last three decades in Japan, and it reached to 275,119 in 2007 [1]. The number of new dialysis patients has continuously increased, and 36,909 patients developed end-stage kidney disease (ESKD) in 2007 [1]. The latent chronic kidney disease (CKD) population therefore appears to be enormous in Japan. In addition, a growing body of evidence suggests that individuals with CKD are at high risk of cardiovascular disease (CVD) [2–4]. Thus, in order to gain a deeper knowledge of the target CKD population for better public policy making and government administration of medical affairs, it is necessary to estimate the prevalence of CKD in Japan with a nationwide epidemiological study.

In our previous study, we estimated the prevalence of CKD stages 3–5 at 19.1 million [5] based on data from the Japanese annual health check program in 2000–2004 using the MDRD equation multiplied by a coefficient of 0.881 for the Japanese population. However, this underestimates GFR, particularly for GFRs of over 60 ml/min/1.73 m^2 [6]. Therefore, the prevalence of CKD may be overestimated when using this equation. The creatinine was measured by an enzymatic method as well as by the uncompensated Jaffe method during that time period, and we corrected the creatinine to the uncompensated Jaffe method. In addition, we did not classify the participants as CKD stage 1 or 2 because we did not correct the data on proteinuria for all of the participants.

The Japanese Society of Nephrology recently established an equation for estimating GFR from serum creatinine and age for the Japanese general population [7]. The new equation provides reasonably accurate estimated GFR (eGFR) values for clinical practice and epidemiological study.

In this study, we used the new Japanese equation for estimating GFR, and the data were sampled from over half a million members of the general population who participated in an annual health check-up program in 2005 conducted in 11 prefectures of Japan; serum creatinine levels were calibrated against a central laboratory.

Methods

Study population

In this study, serum creatinine values were obtained from 574,024 members of the adult population (male 240,594, female 333,430) who participated in a large-scale annual health check-up program that was conducted in 11 prefectures of Japan (Hokkaido, Yamagata, Fukushima, Tochigi, Ibaraki, Tokyo, Kanazawa, Osaka, Fukuoka, Miyazaki and Okinawa) in 2005. All of the participants remained anonymous and the study was conducted according to Japanese privacy protection laws and ethical guidelines for epidemiological study published by the Ministry of Education, Science and Culture and the Ministry of Health, Labor and Welfare in 2005.

Calibration of serum creatinine values

Serum samples were assayed by an enzymatic method in all participating laboratories. To calibrate the samples, ten laboratories measured the calibration panel of 40 samples that was kindly provided by Dr. Frederic van Lante, Cleveland Clinic (Cleveland, Ohio). The creatinine values obtained in each laboratory were compared with the IDMS-traceable value at Cleveland Clinic (Cleveland, Ohio).

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The serum creatinine values measured at each local laboratory (X) were corrected to the IDMS-traceable value obtained at Cleveland Clinic by the following formulae:

Miyazaki: $Y = 1.0617\ X - 0.1128$
Yamagata: $Y = 1.0543\ X - 0.0482$
Tochigi: $Y = 0.9558\ X + 0.0851$
Okinawa: $Y = 1.0176\ X - 0.0644$
Tokyo: $Y = 1.0595\ X - 0.0760$
Ibaraki: $Y = 1.0356\ X + 0.0074$
Hokkaido: $Y = 1.0418\ X + 0.0600$
Fukushima: $Y = 1.0429\ X - 0.0625$

Data from Ishikawa, and Osaka were not corrected because their data were accurate enough to be used without correction. Data from Fukuoka were not corrected on procedural grounds.

Estimation of GFR using the new Japanese equation for estimated GFR from serum creatinine

The GFR of each participant was calculated from their serum creatinine value (SCr) and their age using the new Japanese equation as follows [7]:

$$\text{GFR (ml/min/1.73 m}^2) = 194 \times \text{Age}^{-0.287} \times \text{S-Cr}^{-1.094} \text{ (if female} \times 0.739)$$

Evaluation of renal function and estimation of CKD prevalence

Renal function was evaluated in each participant using the estimated GFR. The prevalence of CKD was calculated for CKD stages 1, 2, 3, and 4 + 5, defined as GFR ≥90, 89–60, 30–59, and <30 ml/min/1.73 m^2, respectively. The age-specific prevalence of CKD stages 3–5 (ages 20–29, 30–39, 40–49, 50–59, 60–69, 70–79, and above 80 years) were calculated for each sex for the study population. The prevalence of CKD was also estimated for the general adult population using data on the Japanese adult population (103.2 million) obtained from a census in 2005 [8].

Comparison of the prevalence of CKD in Japan with that in the USA

The Japanese demographic statistics used were the population estimates from the census in 2005 [8].

The prevalence of CKD in the general population was reported on the basis of the National Health and Nutrition Examination Survey (NHANES 1999–2004, $n = 13233$) in the USA [9]. The demographic statistics for the USA that were used were the population estimates from a census from 2005 conducted by the Population Projections Branch, US Census Bureau (11 May 2004) [10].

Prevalence of CKD among hypertensive, proteinuric and diabetic populations

Proteinuria was defined as a urinary protein excretion of 1+ or more by dipstick test. Hypertension was defined as a blood pressure of 140/90 mmHg or more. The diabetic population was defined as having HbA1c ≥6.0%. The age-specific prevalence of CKD in the hypertensive proteinuric and diabetic populations were compared with those in the populations without hypertension, without proteinuria, and with HbA1c <6.0%, respectively.

Distribution of GFR in diabetic and nondiabetic populations

The distribution of estimated GFR in diabetic patients with HbA1c >6.0% was compared with that in patients with HbA1c <6.0%.

Statistics

Prevalence of proteinuria, hypertension and diabetes are expressed as percentages (%) with respect to the age-specific study population. The prevalences in males and females were compared by chi-square test. A P value of less than 0.05 was considered statistically significant. Age-specific prevalences of CKD stages are expressed as percentages (%) with respect to the age-specific study population with a 95% confidence interval (CI). The prevalences of CKD for subjects with complications such as hypertension, proteinuria and high HbA1c were compared with the prevalences in subjects without these complications by chi-square test.

Results

Prevalence of proteinuria, hypertension and diabetes in the Japanese general population

The prevalence of dipstick proteinuria (1+ or more) is shown in Fig. 1A. The prevalence of proteinuria in males increased from about 1.7 to 8.7% depending on age, while that in females remained approximately 2% (1.6–2.3%) until age reached the 70. Prevalence of hypertension, as defined by a blood pressure of 140/90 mmHg or more, increased from 5.1 to 41.5% as age increased from the 20s to the 80s in male subjects, while the prevalence also increased from 0.7 to 45.0% in females, although to a lesser extent until the 60s (Fig. 1B). Prevalence of diabetes, as defined by HbA1c >6.0%, increased from 0.2 to 12.9% as age increased from the 20s to 70s in males, while that in females also increased with age, although to a lesser extent (Fig. 1C).

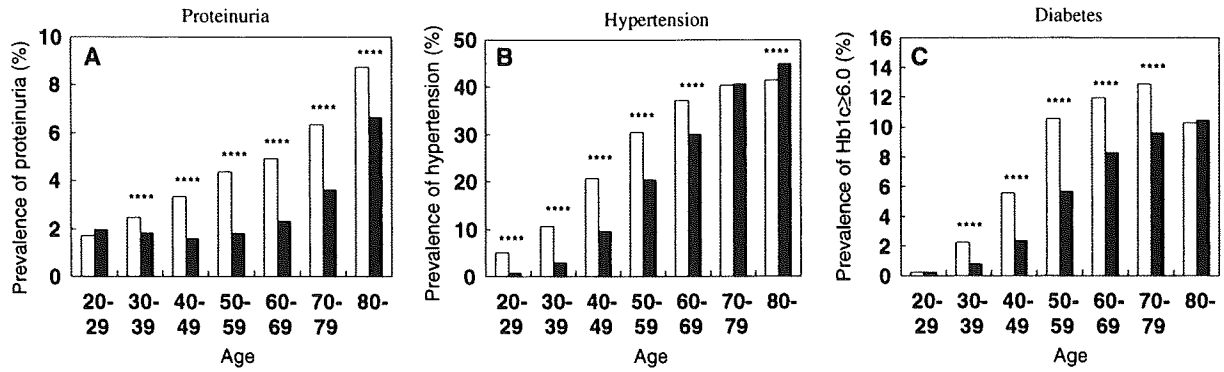

Fig. 1 Prevalence of proteinuria, hypertension, and diabetes in the study population. Proteinuria was defined as 1+ or more by dipstick test (**a**). Hypertension was defined as a systolic blood pressure of ≥40 mmHg, or a diastolic pressure of ≥90 mmHg (**b**). Diabetes was defined as HbA1c ≥6.0% (**c**). *White columns* male, *black columns* female. ****$p < 0.0001$ versus female

Table 1 Age-specific prevalence of chronic kidney disease (CKD) stages in males

	Age						
	20–29	30–39	40–49	50–59	60–69	70–79	80 and over
GFR ≥90 ml/min/1.73 m^2							
N	5166	8684	7325	9056	7714	3374	539
Prevalence (%)	52.7	37.4	20.1	17.1	13.2	6.7	5.0
95% CI	51.7–53.7	36.8–38.1	19.7–20.5	17.4–18.1	12.9–13.4	6.5–6.9	4.6–5.4
GFR 60–89 ml/min/1.73 m^2							
N	4629	14295	27704	38167	41638	33190	5455
Prevalence (%)	47.2	61.6	75.9	74.8	71.0	65.6	50.4
95% CI	46.2–48.2	61.0–62.3	75.5–76.4	74.4–75.1	70.6–71.4	65.2–66.0	49.4–51.3
GFR 50–59 ml/min/1.73 m^2							
N	5	187	1290	3209	7030	9469	2864
Prevalence (%)	0.1	0.8	3.5	6.3	12.0	18.7	26.4
95% CI	0.0–0.1	0.7–0.9	3.4–3.7	6.1–6.5	11.7–12.3	18.4–19.1	25.6–27.3
GFR 40–49 ml/min/1.73 m^2							
N	4	17	107	439	1811	3476	1353
Prevalence (%)	0.0	0.1	0.3	0.9	3.1	6.9	12.5
95% CI	0.0–0.1	0.0–0.1	0.2–0.4	0.8–0.9	3.0–3.2	6.7–7.1	11.9–13.1
GFR 30–39 ml/min/1.73 m^2							
N	0	4	21	84	306	784	456
Prevalence (%)	0.0	0.0	0.1	0.2	0.5	1.5	4.2
95% CI	0.0–0.0	0.0–0.0	0.0–0.1	0.1–0.2	0.5–0.6	1.4–1.7	3.8–4.6
GFR <30 ml/min/1.73 m^2							
N	3	6	31	88	141	312	161
Prevalence (%)	0.0	0.0	0.1	0.2	0.2	0.6	1.5
95% CI	0.0–0.1	0.0–0.1	0.1–0.1	0.1–0.2	0.2–0.3	0.6–0.7	1.3–1.7

Prevalence of CKD in Japan

Age-specific percentages of specific GFR ranges (age <30, 30–39, 40–49, 50–59, 60–79 and ≥80 ml/min/1.73 m^2) in the study population indicated that the prevalence rate of low GFR increased with age (Tables 1, 2). The prevalences of CKD stage 3 and stages 4 + 5 in each age group are shown for each sex in Fig. 2. The prevalence of CKD stage 3 (GFR 40–59 ml/min/1.73 m^2), in particular, increased with age.

The prevalence rates of CKD stages 1, 2, 3, 4 + 5 in the Japanese population in 2005 were 0.6, 1.7, 10.4, and 0.2%, respectively (Table 3). The total predicted number of cases

Table 2 Age-specific prevalence of chronic kidney disease (CKD) stages in females

	Age						
	20–29	30–39	40–49	50–59	60–69	70–79	80 and over
GFR ≥90 ml/min/1.73 m^2							
N	7032	11161	17685	17819	7514	4633	681
Prevalence (%)	67.2	53.3	34.1	22.1	8.6	6.9	4.7
95% CI	66.3–68.1	52.7–54.0	33.7–34.5	21.8–22.4	8.4–8.8	6.7–7.1	4.4–5.0
GFR 60–89 ml/min/1.73 m^2							
N	3402	9575	32834	57354	67071	41410	7139
Prevalence (%)	32.5	45.8	63.3	71.1	76.6	61.4	49.2
95% CI	31.6–33.4	45.1–46.4	62.9–63.7	70.8–71.4	76.4–76.9	61.0–61.7	48.4–50.0
GFR 50–59 ml/min/1.73 m^2							
N	19	168	1206	4822	10601	15859	3385
Prevalence (%)	0.2	0.8	2.3	6.0	12.1	23.5	23.3
95% CI	0.0–0.3	0.7–0.9	2.2–2.5	5.8–6.1	11.9–12.3	23.2–23.8	22.6–24.0
GFR 40–49 ml/min/1.73 m^2							
N	2	13	98	521	1939	4333	2367
Prevalence (%)	0.0	0.1	0.2	0.6	2.2	6.4	16.3
95% CI	0.0–0.1	0.0–0.1	0.2–0.2	0.6–0.7	2.1–2.3	6.2–6.6	15.7–16.9
GFR 30–39 ml/min/1.73 m^2							
N	1	2	20	80	263	927	710
Prevalence (%)	0.0	0.0	0.0	0.1	0.3	1.4	4.9
95% CI	0.0–0.0	0.0–0.0	0.0–0.1	0.1–0.2	0.5–0.6	1.4–1.7	3.8–4.6
GFR <30 ml/min/1.73 m^2							
N	1	5	16	79	133	318	232
Prevalence (%)	0.0	0.0	0.0	0.1	0.2	0.5	1.6
95% CI	0.0–0.0	0.0–0.1	0.1–0.1	0.1–0.1	0.1–0.2	0.4–0.5	1.4–1.8

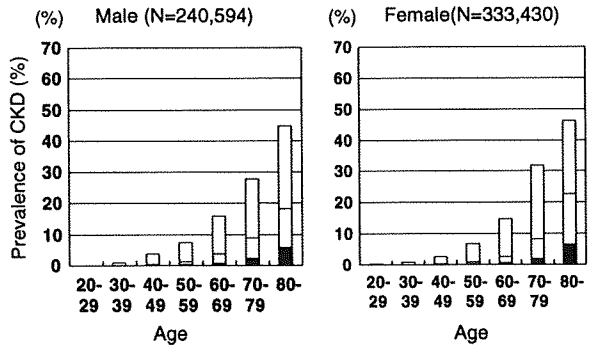

Fig. 2 Prevalence rates for CKD stages 3 to 5 for each age group in males and females in the study population. The prevalence of CKD (%) (as defined by <60 ml/min/1.73 m^2) for each age group was calculated separately for males and females in the study population. *White column* GFR 50–59 ml/min/1.73 m^2, *striped column* GFR 40–49 ml/min/1.73 m^2, *black column* GFR 40 or less ml/min/1.73 m^2

of CKD stages 1, 2, 3, 4 + 5 in the Japanese adult population in 2005 were 0.61, 1.71, 10.74, and 0.23 million, respectively (Table 3).

Prevalence of CKD stages 3–5 in proteinuric and hypertensive populations

The prevalence of CKD stages 3–5 was examined in proteinuric and hypertensive populations (Fig. 3A, B). The prevalence of CKD stages 3–5 was significantly higher in subjects with proteinuria ($P < 0.0001$) in all age groups, and in subjects with hypertension ($p < 0.01$ to $p < 0.0001$) in all age groups except for 80 years or older and in females in their 20s.

Prevalence of CKD stages 3–5 in the diabetic population

The prevalence of CKD stages 3–5 was examined in subjects with HbA1c ≥6.0 (Fig. 3C). The prevalence of CKD (defined as GFR <60 ml/min/1.73 m^2) was significantly lower in the diabetic population in some age groups (Fig. 3C), while its prevalence in subjects with reduced renal function (GFR <40 ml/min/1.73 m^2) was significantly higher in diabetic individuals in their 50s and 60s (Fig. 3D).

Table 3 Prevalence rates of CKD stages in Japanese adults (20 years or older), and estimated number of CKD cases per CKD stage based on the 2005 census

GFR (ml/min/1.73 m^2)	Total	Proteinuria (+)	Proteinuria (−)
Prevalence rate (%)			
GFR ≥90	27.8	0.6	27.2
60–89	61.6	1.7	60.0
30–59	10.4	0.8	9.6
<30	0.2	0.1	0.1
Stage 3			
50–59	7.6	0.4	7.2
40–49	2.3	0.3	2.0
30–39	0.6	0.1	0.4
Estimated number of Japanese adults in 2005			
GFR ≥90	28639274	605313	28033961
60–89	63576938	1708870	61868068
30–59	10743236	8238881	9919355
<30	236569	125190	111379
Stage 3			
50–59	7809261	425146	7384116
40–49	2363987	267158	2096828
30–39	569988	131577	438411

Prevalence of hyperfiltration in the diabetic population

The prevalence of subjects with GFR ≥120 ml/min/1.73 m^2 was significantly higher in the diabetic population ($p < 0.05$ to $p < 0.0001$) at ages 30–79 (Fig. 4). The distribution of GFR in the diabetic population was shifted to higher values than for the population with HbA1c <6.0%. A representative figurefor ages 50–59 is shown in Fig. 5. The prevalence of hypertension with GFR ≥120 ml/min/1.73 m^2 was significantly higher in the diabetic population ($p < 0.0001$) compared with the nondiabetic population (Fig. 5).

Comparison of GFR in the general population between Japan and the USA

The distribution of GFR across the whole Japanese population, calculated on the basis of the census from 2005, is shown in Fig. 6. Japan is an aging society, and the age pyramid for the population is shifted towards the elderly. An aging population tends to have low GFR, and this aging affects the distribution of GFR in the country. We recalculated the distribution of GFR by age adjusting the Japanese population to the 2005 US population estimate. As shown in Fig. 6, the distribution of GFR in the Japanese population is shifted to higher values after the correction for aging affects.

Discussion

In this study, we examined the prevalence of CKD for participants in a nationwide annual health check program in 11 prefectures of Japan using a new equation for estimating GFR from serum creatinine in the Japanese population [7]. The prevalence rates of CKD stages 1, 2, 3, and 4 + 5 in the study population of 574,024 were 0.6, 1.7, 10.4 and 0.2%, which resulted in predictions of 0.6, 1.7, 10.7 and 0.2 million patients, respectively, nationwide based on the census from 2005. Proteinuria resulted in a preponderance of declining GFR. The prevalence of concurrent CKD was significantly higher in the hypertensive population than in the population without hypertension, particularly in males. The diabetic population showed a preponderance of hyperfiltration, defined as GFR ≥120 ml/min/1.73 m^2.

The prevalence of CKD stages 1–5 has been reported for several countries (Fig. 7). According to the reliable and unbiased NHANES III surveys conducted from 1988 to 1994, from 1999 to 2000 [11], and from 1999 to 2004 [9], the prevalence of CKD remained the same between the first two surveys but increased for the third screening. For CKD stages 3 and stage 4, the prevalences were 4.2 and 0.19% in the first survey and 3.7 and 0.13% in the second survey, respectively [11]. In the third survey, the prevalences of CKD stages 1, 2, 3, 4 were 1.78, 3.24, 7.69, 0.35%, respectively (Fig. 7) [9], suggesting that the prevalence rates of CKD stages 3 and 4 increased in the USA. In Nord-Trondelag, a county in Norway, the prevalences were 4.2% for CKD stage 3 and 0.2% for CKD stages 4 + 5 [12]. The reported prevalence of CKD varies among countries in Asia. In Taiwan, about half a million participants were examined, and the MDRD equation was applied without correction using an ethnic coefficient; here, the prevalence rate of CKD was 11.9%, and those for CKD stages 1, 2, 3, 4, and 5 were 1.0, 3.8, 6.8, 0.2, 0.1%, respectively [13]. In Beijing, China, the prevalence of CKD was obtained using the original Chinese equation for estimating GFR, and the prevalences of CKD stages 1, 2, 3, 4 and 5 were 5.5, 3.3, 1.3, 0.0010 and 0.0003%, respectively [14]. Overall, about 10–13% of the population exhibited CKD in these countries. The different prevalences of CKD stages 1 and 2 among the countries appears to be mainly due to how proteinuria is defined. The definition of albuminuria differed considerably between countries. China defined albuminuria as 17 mg/g Cr [14], while the USA defined it as 30 mg/g Cr [9]. Taiwan defined proteinuria as (±) on dipstick test [13], while Japan defined a dipstick of (1+) as proteinuria. This difference in definition must affect the the prevalences of CKD stages 1 and 2 considerably. In addition, the methods used for creatinine measurement varied considerably among countries. We advocate the use of the

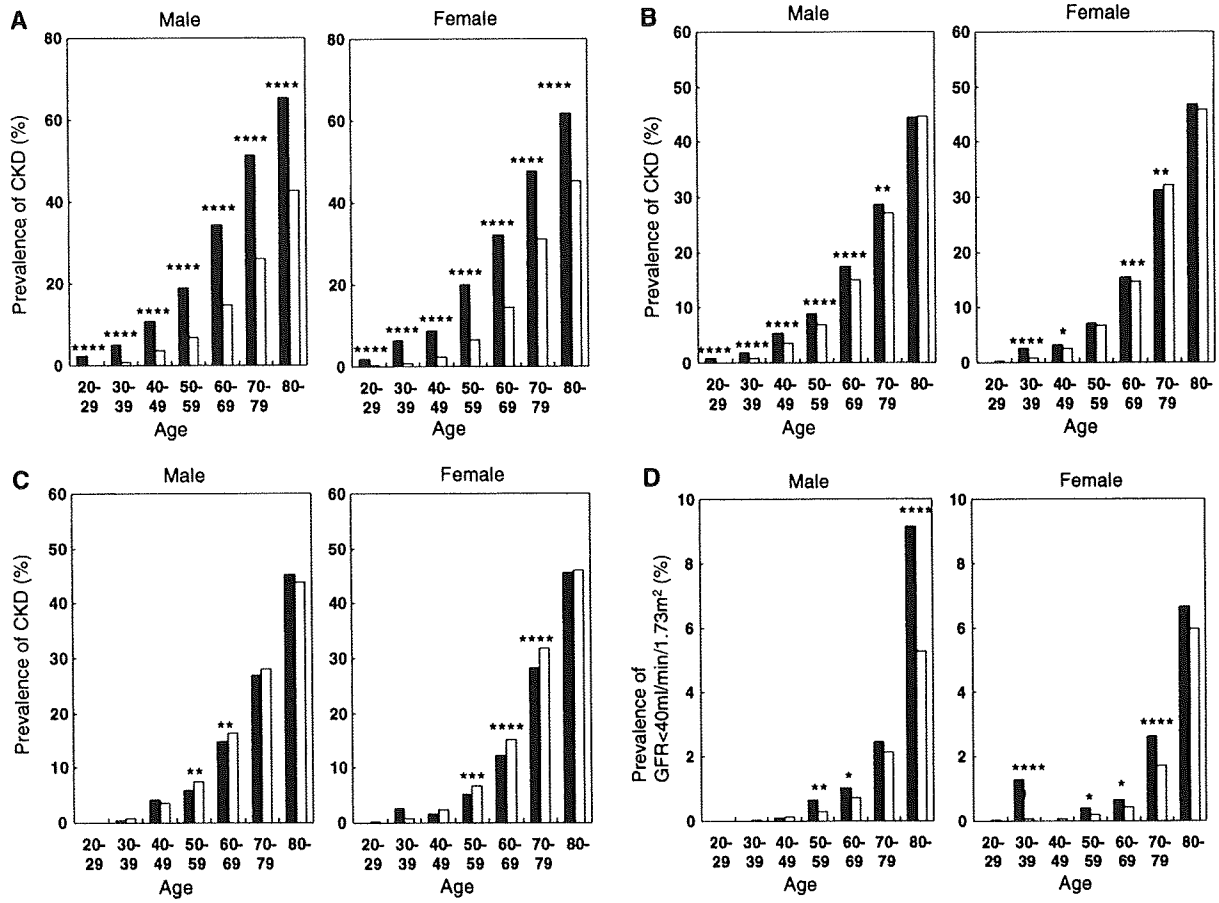

Fig. 3 Prevalence of CKD in proteinuria, hypertensive and diabetic populations. The prevalence of CKD (defined by GFR <60 ml/min/1.73 m^2) in the proteinuric population, shown by the *black column*, was compared with that in the population without proteinuria, shown by the *white column*, for each generation (**a**). Proteinuria was defined as 1+ or more by dipstick test. Prevalence of CKD (defined as GFR <60 ml/min/1.73 m^2) in the hypertensive population, shown by the *black column*, was compared with that in the population without hypertension, shown by the *white column*, for each generation (**b**). Hypertension was defined as a blood pressure of 140/90 mmHg or over. Prevalences of GFR <60 ml/min/1.73 m^2 and of GFR <40 ml/min/1.73 m^2 in the diabetic population (*black columns*) are compared with that in the nondiabetic population (*white columns*) (**c**, **d**). Diabetes was defined as HbA1c ≥6.0%. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$ versus individuals without cormobidity of proteinuria (**a**), hypertension (**b**), or diabetes (**c**, **d**)

following in order to compare the prevalence of CKD among different countries. First, the definition and method of measuring proteinuria must be unified across countries. Albuminuria or albuminuria-to creatinine ratio, which is scientifically more reliable than the dipstick test, should be used for proteinuria. Repeated measurements are recommended. Second, the serum creatinine that is used to estimate GFR should be measured by isotope diluted mass spectrometry (IDMS)-traceable creatinine assay. Third, the equation used to estimate GFR for each ethnic group must be established. Another alternative is to establish an IDMS-traceable MDRD equation [15] with an ethnic coefficient. The measurement of proteinuria by dipstick test and serum creatinine is accurate enough for daily practice and screening, but international comparisons of the prevalence of CKD should be done by a unified standard method involving the measurement of albuminuria and serum creatinine with an IDMS-traceable creatinine assay.

Our aging society results in a decline in the average GFR in this country. More than 20% of the Japanese population is over 60 years old, and the elderly population (over 75 years old) is much higher than in other countries. Because of this increased average age, the prevalence of CKD is higher in Japan. In fact, the distribution of the age-adjusted eGFR was shown to be similar for Japan and the USA (Fig. 6).

The prevalence of proteinuria increased as GFR decreased (Table 3) in this study. However, the prevalences of proteinuria in CKD stages 3 and 4 + 5 were 7.7 and 52.9%, respectively. In data from a mass health

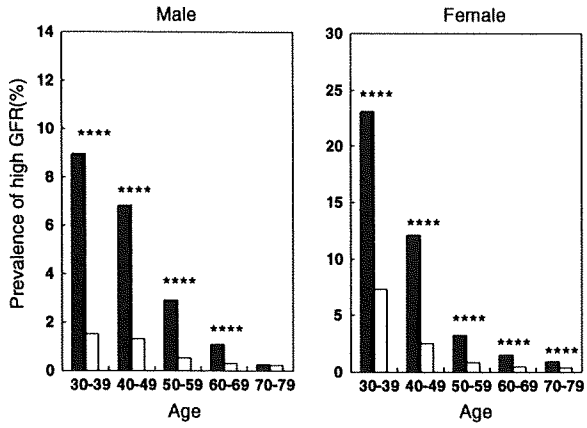

Fig. 4 Prevalence of GFR ≥120 ml/min/1.73 m^2 in the diabetic population. Individuals with diabetes as defined by HbA1c ≥6.0% are represented by the *black column*. Individuals with HbA1c <6.0% are represented by the *white column*. ****$p < 0.0001$ versus individuals with HbA1c <6.0%

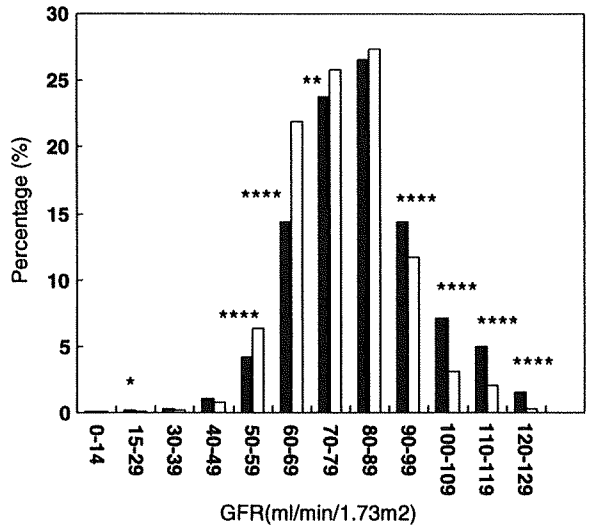

Fig. 5 Distribution of estimated GFR in populations with HbA1c ≥6.0% and HbA1c <6.0%. Distributions of estimated GFR are shown separately for diabetic individuals (defined as HbA1c ≥6.0%) and for individuals with HbA1c <6.0%. The population with diabetes is represented by the *black column*, and individuals with HbA1c <6.0% are represented by the *white column*. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ versus individuals with HbA1c <6.0%

screening in Okinawa, proteinuria (defined as a dipstick urinalysis result of 1+ or more) was a strong predictor of ESKD [16]. The rate of decline of GFR in individuals with proteinuria was more than twofold faster than that in individuals without proteinuria [17]. This may suggest that most of CKD stage 3 and half of Japanese stage 4 + 5 CKD patients without proteinuria may progress slowly to ESKD and may not even reach ESRD during their

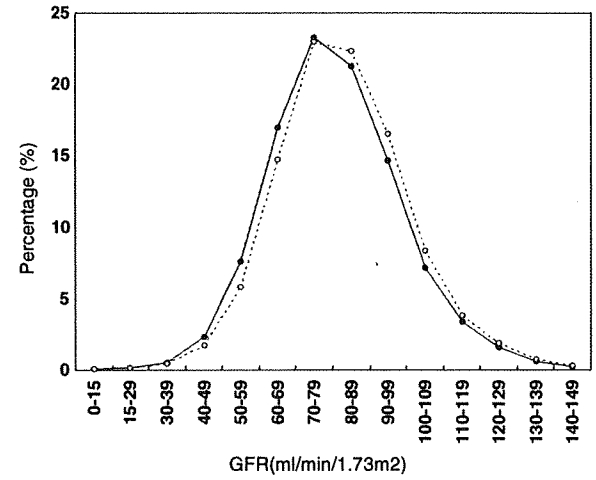

Fig. 6 Distribution of GFR in the Japanese general population. The distribution of estimated GFR for Japanese is shown by the *solid line*. We then recalculated the distribution of the GFR by age adjusting the Japanese population to the US population, as shown by the *dotted line*

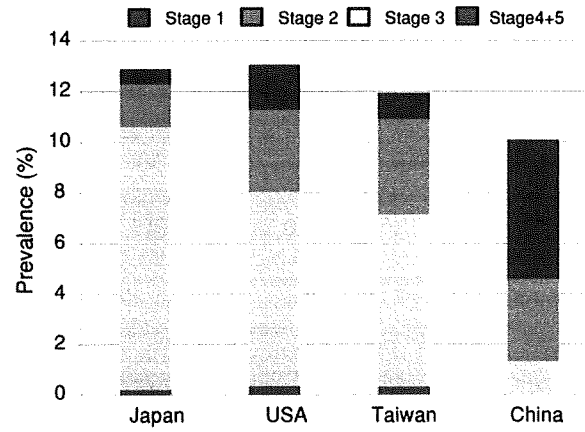

Fig. 7 Prevalences of CKD stages 1, 2, 3, and 4 + 5 in Japan, USA, Taiwan and China. The prevalence of each stage of CKD was obtained from previous publications. In Japan, the prevalence of CKD was estimated from accumulated data on 570,244 individuals aged 20 and over in the annual health check program in 2005. Proteinuria was evaluated by dipstick test, where 1+ and over was defined as proteinuria. In the USA, the prevalence of each stage of CKD was studied using data on nationally representative samples from 13,233 adults aged 20 and over taken from 1999 to 2004 [9]. The presence of albuminuria was estimated from the albumin-to-creatinine ratio, and microalbuminuria was defined as 30 mg/g creatinine. In Taiwan, the prevalence of each stage of CKD was estimated based on data from a private firm on 462,293 individuals aged 20 and over, obtained from 1994 to 2007 [13]. Proteinuria was evaluated by dipstick test, and (± or 1+) was defined as minimal proteinuria and (2+ and over) as overt proteinuria. In China, representative samples from 13,925 individuals aged 18 and older were analyzed [14]. Albuminuria was measured, and microalbuminuria was determined as ranging from 17 to 250 mg/g creatinine for males and from 25 to 355 mg/g creatinine for females

lifetimes. Further study is required to obtain risk stratifications for the stage 3 and 4 populations.

In the diabetic population, the prevalences of high GFR (GFR $\geq$120 ml/min/1.73 m^2) and low GFR (<40 ml/min/1.73 m^2) were higher than those in the population with HbA1c <6.0%, suggesting that diabetes shifts the distribution of GFR to the high and low ranges. We speculated that hyperfiltration plays a major role in this shift, and may contribute to the rapid decline in GFR in diabetic individuals. Hyperfiltration may aid the development of microalbuminuria in type 1 diabetic patients. Amin and colleagues reported a strong relationship between the risk for the development of microalbuminuria in individuals who had diabetes for five and ten years and the development of glomerular hyperfiltration in individuals who had diabetes for five years, independent of glycemic control [18].

The prevalence of CKD comorbid with other concurrent conditions in the Japanese population was similar to the corresponding prevalences in the US and Chinese populations. The prevalence of CKD was higher among hypertensive and diabetic individuals in the white US population, as previously reported [19]. The prevalence of CKD was reported to increase in hypertensive and diabetic populations in Chinese [20]. This study also supports the notion that prevalence of CKD comorbidity is higher in hypertensive and diabetic populations than in the normal population.

We previously reported that the rate of decline of GFR was more than twofold faster when the eGFR was less than 50 ml/min/1.73 m^2 in adults [17]. From the viewpoint of risk stratification for progression to ESKD, we estimated that 3.1% of the adult population (3.17 million) had GFR <50 ml/min/1.73 m^2 in 2005 (Table 3). Presence of proteinuria is a strong risk factor for ESKD and CVD. From Table 3, 2.74 million (2.7%) of the adult population have proteinuria and GFR >50 ml/min/1.73 m^2. Taken together, the CKD population with risk of progression to ESKD is predicted to be 5.91 million, 5.8% of the adult population in 2005.

The limitations of the present study are as follows. First, the study cohort was a proportion of the general population that participated in an annual health check program; it was not representative of the whole Japanese population. Second, the serum creatinine was not measured at a single laboratory, so the values of serum creatinine may have drifted. Third, we only measured proteinuria once. Therefore, the presence of proteinuria was confirmed, not persistent proteinuria.

In conclusion, about 13% of Japanese adult population, approximately 13.3 million people, were predicted to have CKD in 2005. From the viewpoint of risk stratification to progression to ESKD, about 5.8% of the adult population—approximately 6 million people—who have proteinuria or GFR <50 ml/min/1.73 m^2 are estimated to have CKD in Japan.

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Angiotensin II type 1 receptor blocker attenuates the activation of ERK and NADPH oxidase by mechanical strain in mesangial cells in the absence of angiotensin II

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[1]*Department of Internal Medicine 3, Fukushima Medical University School of Medicine, Fukushima, Japan;* [2]*Department of Pathology, University of Virginia Health System, Charlottesville, Virginia; and* [3]*Departments of Pediatrics, Physiology and Biophysics, Georgetown University Medical Center, Washington, District of Columbia*

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Yatabe J, Sanada H, Yatabe MS, Hashimoto S, Yoneda M, Felder RA, Jose PA, Watanabe T. Angiotensin II type 1 receptor blocker attenuates the activation of ERK and NADPH oxidase by mechanical strain in mesangial cells in the absence of angiotensin II. *Am J Physiol Renal Physiol* 296: F1052–F1060, 2009. First published March 4, 2009; doi:10.1152/ajprenal.00580.2007.—It has been reported that mechanical strain activates extracellular signal-regulated protein kinases (ERK) without the involvement of angiotensin II (Ang II) in cardiomyocytes. We examined the effects of mechanical strain on ERK phosphorylation levels in the absence of Ang II using rat mesangial cells. The ratio of phosphorylated ERK (p-ERK) to total ERK expression was increased by cyclic mechanical strain in a time- and elongation strength-dependent manner. With olmesartan [Ang II type 1 receptor (AT1R) antagonist] pretreatment, p-ERK plateau levels decreased in a dose-dependent manner ($EC_{50} = 1.3 \times 10^{-8}$ M, maximal inhibition 50.6 ± 11.0% at 10^{-5} M); a similar effect was observed with RNA interference against Ang II type 1A receptor ($AT_{1A}R$) and Tempol, a superoxide dismutase mimetic. In addition to the inhibition of p-ERK levels, olmesartan blocked the increase in cell surface and phosphorylated $p47^{phox}$ induced by mechanical strain and also lowered the mRNA expression levels of NADPH oxidase subunits. These results demonstrate that mechanical strain stimulates AT1R to phosphorylate ERK in mesangial cells in the absence of Ang II. This mechanotransduction mechanism is involved in the oxidative stress caused by NADPH oxidase and is blocked by olmesartan. The inverse agonistic activity of this AT1R blocker may be useful for the prevention of mesangial proliferation and renal damage caused by mechanical strain/oxidative stress regardless of circulating or tissue Ang II levels.

hypertension

HYPERTENSION IS A DOMINANT pathogenetic factor in target organ damage such as ischemic heart disease, stroke, and renal dysfunction. Emerging evidence indicates that renal sclerosis is a major cause of renal dysfunction in hypertension, and mesangial proliferation is known to play a pivotal role in the pathophysiology of renal sclerosis. Since mesangial cells are located between the vascular and urinary space in glomeruli, they can be influenced by many hormonal/humoral substances, including angiotensin II (Ang II). Ang II stimulates cell differentiation and proliferation of glomerular mesangial cells, leading to glomerulosclerosis (1). Extracellular signal-regulated kinases (ERK), among the mitogen-activated protein kinases (MAPK), are serine/threonine kinases acting as transducers of signals from the cell membrane to the nucleus in response to various cellular stresses, such as cytokines or signals from G protein-coupled receptors, including the Ang II type 1 receptor (AT1R) (27). The activation of ERK, via Ang II through AT1R, modulates cell growth and differentiation in mesangial cells, as well as in cardiac myocytes. Presently, AT1R blockers (ARBs) are widely used clinically for the treatment of hypertension and the prevention of heart disease and renal injury. However, Ang II-independent effects of ARBs on AT1R are not well defined.

Recently it was reported that mechanical strain activates the MAPK pathway via the AT1R without the involvement of Ang II in cardiomyocytes (29). We have also demonstrated that selective knockdown of renal AT1R expression with the use of antisense oligodeoxynucleotides markedly reduces urinary protein excretion and glomerular sclerosis in spontaneously hypertensive rats, independent of circulating Ang II levels (28). However, in the kidney, the influence of mechanical strain and the renin-angiotensin system on mesangial cells remains unclear. It is possible that mechanical strain caused by elevated blood pressure that is not primarily attributable to increased activity of the renin-angiotensin system may be aggravated by Ang II-independent activation of AT1R.

It is well recognized that ERKs are activated by mechanical strain through modulation of intracellular calcium ion concentration (10) or through integrins that connect the cytoskeleton to the extracellular matrix (10, 15). It is also known that NADPH oxidase is activated by Ang II via AT1R, which leads to phosphorylation of ERKs (3, 4, 7). We tested the hypothesis that NADPH oxidase activity can be modulated by mechanical strain through AT1R and activation of ERK in the absence of Ang II. In the present study, we examined ERK phosphorylation caused by mechanical strain in the absence of Ang II using primary cultures of rat mesangial cells (RMCs) and also examined the role of NADPH oxidase in this phenomenon.

MATERIALS AND METHODS

Isolation and cultivation of RMCs. RMCs were isolated from 5–7-wk-old male Sprague-Dawley rats with the use of a conventional sieving method as reported previously (22). Isolated RMCs (*passages 4–6*) were cultured in DMEM (Gibco-Invitrogen, Carlsbad, CA) containing 20% fetal bovine serum and penicillin-streptomycin at 37°C in a humidified 5% CO_2 water-jacketed incubator. All procedures were approved by the Fukushima Medical University School of Medicine Animal Committee.

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Test compounds. Ang II was supplied from Peptide Institute (Osaka, Japan). Olmesartan was kindly supplied by Daiichi-Sankyo Pharmaceutical (Tokyo, Japan), and losartan potassium was purchased from Wako Pure Chemical Industries (Osaka, Japan). Tempol, BAPTA-TM, and cytochalasin D were purchased from Sigma-Aldrich Japan (Tokyo, Japan).

Mechanical strain application. Mesangial cells were seeded into six-well silicon elastomer-base culture plates with collagen type I coating (Flex I plates; Flexcell International, Hillsborough, NC) or standard six-well cell-culture plates at a density of 12,000 cells/cm^2. The cells achieved confluence after 5–7 days. After serum starvation for 12 h, the cells were preincubated with the test compounds or their corresponding vehicle as control for designated periods. Thereafter, cells were subjected to stretch/relaxation cycles using the Flexercell Tension Plus system, FX-4000T (Flexcell International). Vacuum was cyclically applied (60 cycles/min) to the rubber-based plates via the base plate, which was placed in a water-jacketed incubator with 5% CO_2 at 37°C (9). Cells were exposed to elongation stretch strengths of 5, 10, 15, or 20% for periods ranging from 2.5 min to 12 h.

Silencing AT1R by miR RNAi. BLOCK-iT Pol II miR RNAi expression vector (Invitrogen) with the target sequence of TGTCATCCACCGAAATGTATA was used for silencing of Ang II type 1A receptor ($AT_{1A}R$) in *Rattus norvegicus*. Purified oligodeoxynucleotides were designed and synthesized by Invitrogen primer team (Tokyo, Japan). $AT_{1A}R$ micro RNA (miRNA) insert caused a 85.48 ± 4.71% decrease in $AT_{1A}R$ transcript, as verified by real-time RT-PCR. Transfection was performed using Lipofectamine LTX and Plus Reagent (Invitrogen). Briefly, trypsinized and suspended cells in normal growth medium were seeded at a concentration of 1×10^5 cells/well in six-well plates containing miR vector (500 ng) in transfection reagent mixture. After incubation at 37°C for 12 h, the culture medium containing transfection reagent was replaced with fresh medium with Blastcidin for selection. Transfection efficiency was determined by the fluorescent intensity of the Emerald-green fluorescent protein tag.

Membrane protein preparation and immunoprecipitation. Protein sample preparation and immunoblotting analysis were performed as previously described (28). Whole cell protein samples were extracted with single detergent lysis buffer containing 50 mM Tris·HCl, 150 mM NaCl, 0.02% sodium azide, 1% NP-40, and Complete (a protease inhibitor cocktail; Roche Diagnostics, Mannheim, Germany). Phosphatase inhibitor cocktails (Sigma-Aldrich, St. Louis, MO) were also added to the lysis buffer to prevent dephosphorylation. Membrane protein samples were separated using a cell-surface biotinylation method. In brief, cells were incubated in ice-cold PBS supplemented with cell-impermeant and noncleavable sulfo-NHS-SS-biotin (500 μg/ml) with gentle agitation on a rocking platform. After quenching excess biotin with HEPES-buffered saline, cells were lysed with single detergent lysis buffer and cell debris removed with low-speed centrifugation. Biotinylated membranes in lysis buffer were captured by Immobilized NeutrAvidin gel and then eluted in LDS sample buffer with heating at 75°C. Immunoreactive $p47^{phox}$ protein sample was separated and purified by immunoprecipitation. The whole cell protein sample (500 μg protein/ml) was incubated with anti-$p47^{phox}$ antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 2 μl/ml for 2 h, followed by incubation with protein-G agarose at 4°C overnight. The immunoprecipitates were pelleted and washed four times with lysis buffer containing the protease inhibitor cocktail. The pellets were suspended in sample buffer (Invitrogen), heated at 75°C for 10 min, and subjected to immunoblotting with the anti-$p47^{phox}$ and anti-phosphoserine antibodies.

Immunoblotting. Each sample was electrophoretically size separated under denaturing conditions in 10% bis-Tris-polyacrylamide gels, followed by the transfer of proteins onto polyvinylidene difluoride membranes. The blots were soaked overnight at 4°C in commercially available blocking agent (BlockAce; Dainihon Pharmacy, Osaka, Japan). The membranes were then probed for 1 h with polyclonal rabbit anti p42/p44 MAP kinase (ERK), anti-phospho-p42/p44 MAP kinase (phospho-ERK; Cell Signaling, Beverly, MA), anti-$p47^{phox}$, anti-AT1R, anti-phosphoserine, or anti-β-actin antibodies (Santa Cruz Biotechnology) in Tris-buffered saline containing 0.1% Tween 20. The membranes were subsequently washed and incubated with peroxidase-conjugated goat anti-rabbit or goat anti-mouse secondary antibody. When necessary, probed membranes were stripped in Tris buffer solution containing 2% SDS and 100 mM β-mercaptoethanol at 65°C. Quantitative assessment of band densities was performed by scanning densitometry.

Quantitative real-time RT-PCR. Total RNA was prepared from RMCs growing on elastic-bottomed plates using TRIzol (Invitrogen) and column-purified after DNase treatment with RNase-free DNase set (Qiagen, Valencia, CA). Subsequently, 1 μg of total RNA was reverse transcribed into cDNA using oligo (dT) method (iScript cDNA synthesis kit; Bio-Rad, Hercules, CA) in 20 μl reaction volume. cDNA in 1 μl of reaction mix was used for real-time quantitative PCR using Light Cycler and FastStart DNA Master SYBR Green I (Roche Diagnostics). Quantification of mRNA was based on C_t value, normalized to β-actin, and expressed as the magnitude of change under mechanical strain application relative to the appropriate control. Primers for rat angiotensinogen, $p22^{phox}$, $p47^{phox}$, $p67^{phox}$, $gp91^{phox}$, Nox-1, Nox-4, and β-actin were used to replicate cDNAs reverse transcribed from the experimental, positive, and negative control RNA samples, as described previously (12).

Determination of Ang II concentration in culture medium. Supernatant was collected from the culture medium after the cells were subjected to cyclic mechanical strain. The concentration of Ang II was measured by an enzyme-linked immunosorbent assay using an Ang II enzyme immunoassay (EIA) Kit (Cayman Chemical, Ann Arbor, MI).

Statistical analysis. Differences among groups were analyzed by one-way ANOVA with Bonferroni correction. Statistical significance was assumed at $P < 0.05$. Data are given as means ± SE.

RESULTS

Mechanical strain-induced ERK phosphorylation is decreased by olmesartan in the absence of Ang II. In primary cultures of rat renal mesangial cells, cyclic mechanical strain alone significantly increased the ratio of phospho-ERK (p-ERK) to total ERK expression in a time-dependent manner. ERK phosphorylation peaked after 5 min of strain (Fig. 1*A*). With continued strain, ERK phosphorylation decreased and reached a nadir at 60 min and plateaued at a higher level than baseline thereafter. The increase in p-ERK at the peak phase was also elongation strength dependent, and maximal response was achieved with 20% elongation (Fig. 1*B*). Ang II-induced activation of ERK was confirmed; Ang II administration induced the phosphorylation of ERK in a time- and concentration-dependent manner (Fig. 1, *C* and *D*, respectively).

In cardiomyocytes, it has been recently reported that AT1R can be activated by mechanical strain in the absence of its specific agonist, Ang II (29). Since AT1R stimulation leads to ERK phosphorylation, we hypothesized that the stretch-induced ERK phosphorylation is mediated by AT1R activation in mesangial cells. To determine whether mechanical strain can activate ERK through AT1R in mesangial cells, the effect of olmesartan, an AT1R blocker, on mechanical strain-induced ERK activation in the absence of Ang II was examined. Pretreatment with olmesartan for 60 min before the application of mechanical strain significantly inhibited ERK phosphorylation at the plateau phase, which occurred at 60 min after the initiation of stretch stress. As shown in Fig. 2*A*, p-ERK levels at the plateau phase were reduced in a concentration-dependent