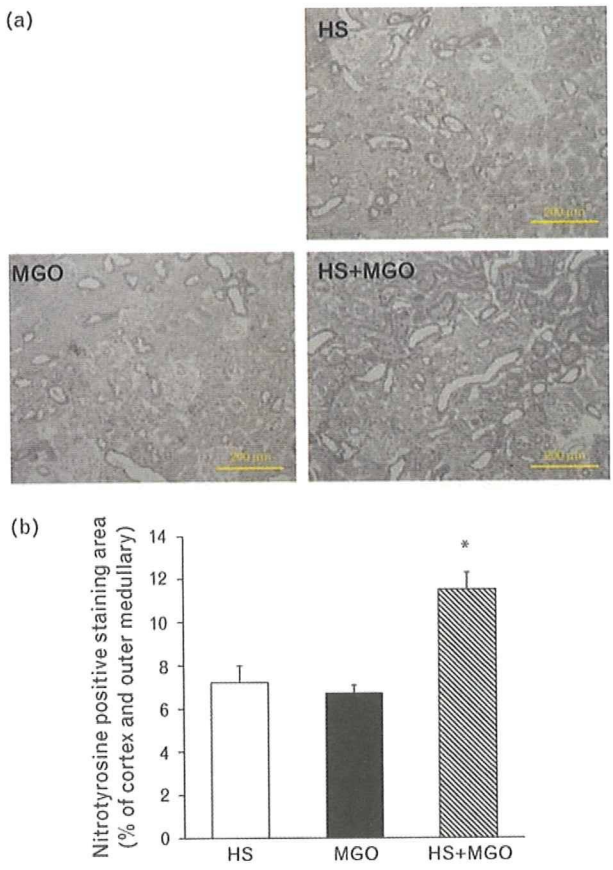
Fig. 6



Representation of nitrotyrosine positive staining area in rats. (a) Representative images of nitrotyrosine in the kidney from Sprague–Dawley rats. (b) Percentage of nitrotyrosine positive staining area of the cortex and outer medullary in HS, MGO and HS + MGO. HS, high-salt-fed rats; MGO, normal-fed rats treated with methylglyoxal; HS + MGO, high-salt-fed rats treated with methylglyoxal. Data are the means ± SE. $n = 6$ for each group. $^{*}P < 0.001$ vs. HS or MGO group.

metabolite may play a role in the development of insulin resistance. Several studies have shown that MGO inhibited the insulin signaling in cultured skeletal muscle cells [16], and in adipose tissue of fructose-induced hypertensive rats [17]. However, whether increased MGO would directly contribute to the development of insulin resistance in a normotensive state is still unsettled. To the best of our knowledge, the present study is the first to investigate the effects of MGO on insulin sensitivity by using a glucose clamp in normotensive Sprague–Dawley rats. We also successfully demonstrated that MGO-induced salt sensitivity. The results of the present study indicate that high plasma levels of MGO are implicated in the pathophysiology of chronic kidney disease.

The present study demonstrated that 4-week treatment with 1% MGO in drinking water significantly increased the M value (a marker of insulin resistance) without altering the blood pressure in Sprague–Dawley rats (Figs. 1 and 2). We also found that 4-week treatment with not only NAC, an aldehyde binding compound as a MGO scavenger, but also TM2002, a novel AGE inhibitor [12], completely improved MGO-induced insulin resistance to a level of control group. As enhanced AGEs have been demonstrated to increase insulin resistance [18], it may be that MGO-induced insulin resistance was at least partly mediated through AGEs in the present study.

Although we do not have further evidence for the exact mechanism of the MGO-induced insulin resistance in the present study, there are several possible mechanisms by which MGO affects insulin resistance. First, MGO and MGO-induced AGEs can induce nonenzymatic modifications of various amino acid residues (e.g., lysine and arginine) that are generally present in the active sites of various insulin-signaling proteins, and MGO-induced structural modifications of these proteins might cause impaired insulin-signaling transduction. Jia *et al.* [17] reported that elevation of the endogenous MGO level in fructose-fed rats reduced insulin receptor substrate (IRS-1)/phosphatidylinositol-3-kinase (PI3K) association and altered PI3K activity, which may lead to the decrease in insulin-stimulated glucose uptake in adipose tissue, thereby contributing to insulin resistance. Furthermore, MGO modifies insulin by attaching to an internal arginine residue in the β-chain of insulin [19]. The formation of this MGO-insulin adduct decreases insulin-mediated glucose uptake, impairs autocrine control of the insulin secretion, and decreases insulin clearance. These structural and functional abnormalities of insulin molecule may contribute to the pathogenesis of insulin resistance.

Role of methylglyoxal in the development of hypertension

There have been several reports implicating MGO in the development of hypertension, but the exact mechanism is not yet fully understood. Vasdev *et al.* [20] have

Table 1 Body weight, kidney weight, kidney weight/body weight, urinary albumin excretion (Ualb) and Ualb/creatinine ratio (Ualb/Cre) after the 4-week study

	BW (g)	KW (mg)	KW/BW (mg/g)	Ualb (μg/day)	Ualb/Cre (μg/mg)
HS	302 ± 4	1000 ± 17	3.3 ± 0.1	429 ± 96	40 ± 9
MGO	290 ± 12	994 ± 36	3.5 ± 0.2	210 ± 25	21 ± 1
HS + MGO	296 ± 10	1107 ± 41*,†	3.8 ± 0.1*	570 ± 278	50 ± 28

Data are the means ± SE. $n = 6$ for each group. BW, body weight; HS, high-salt-fed rats; MGO, normal-fed rats treated with methylglyoxal; HS + MGO, high-salt-fed rats treated with methylglyoxal; KW, kidney weight; Ualb, urinary albumin excretion; Ualb/Cre, Ualb/creatinine ratio. $^{*}P < 0.05$ vs. HS group. $^{\dagger}P < 0.05$ vs. MGO group.

proposed a role of MGO in the Ca^{2+} channels of the arteries. They have demonstrated that methylglyoxal binds to the sulfhydryl groups of vascular Ca^{2+} channels and increases intracellular cytosolic Ca^{2+} levels, which enhance vascular tension. Oxidative stress is another mechanism that could explain MGO-induced hypertension. Wang *et al.* [2,3] have demonstrated that the MGO level was elevated in parallel with increased oxidative stress and AGEs in SHR compared with normotensive WKY rats. These results indicated that elevated MGO levels can lead to increased production of ROS and AGEs, and thereby might contribute to the development of hypertension. Enhanced production of ROS leads to structural and functional alterations, such as endothelial dysfunction and, vascular smooth muscle hypertrophy and hyperplasia, all of which contribute to the development of hypertension [21]. A number of studies [22–24] have shown that there is a synergistic relationship between MGO, AGEs and oxidative stress. Accumulation of AGEs further magnifies ROS damage by inducing glycation of the enzymes involved in the antioxidant system and by providing precursors of oxidative stress.

Vasdev *et al.* [20] have demonstrated that treatment with MGO (0.2–0.8% in drinking water) significantly increased blood pressure in WKY rats. In the present study, although we expected that MGO would induce a significant increase in blood pressure, treatment with 1% MGO alone had no effect on blood pressure for up to 4 weeks in Sprague–Dawley rats. The reason for the different results of MGO on blood pressure between in Sprague–Dawley rats and in WKY rats is not clear, but except difference in the rat strains, higher plasma angiotensin II level [25] and the oxidative stress level of renal proximal tubules [26] in WKY than Sprague–Dawley rats may be involved. In addition, although it is reported that treatment of Sprague–Dawley rats with 4% high-salt diet for 4-week increases urinary excretion rate of H_2O_2 [11], 4% high-salt diet do not increase blood pressure. In the present study, the levels of urinary TBARS excretion and nitrotyrosine expression in the kidney (markers of oxidative stress) were found to be similar between the MGO and high-salt diet groups (Figs. 4 and 6). Taken together, these results indicate that neither the increase in oxidative stress by salt loading nor the formation of AGEs by MGO loading is sufficient in itself to induce the development of hypertension, but when the two are combined, hypertension develops.

Methylglyoxal induces salt sensitivity in Sprague–Dawley rats

Interestingly, coadministration of MGO and high-salt diet significantly increased SBP and the urinary Na^+ excretion rate after 4-week treatment compared with either MGO or high-salt diet alone in the present study (Figs. 2 and 3). These data suggested that MGO caused an increase in salt sensitivity in normotensive Sprague–Dawley rats. Although the exact mechanisms of the MGO-induced salt sensitivity in normotensive Sprague–Dawley rats remains to be investigated, increased oxidative stress could be a trigger for salt sensitivity [27].

In the present study, renal nitrotyrosine expression was observed strongly in the tubules of the outer medulla. It has been shown that enhanced medullary oxidative stress plays an important role in the pathophysiology of salt-sensitive hypertension. The balance between nitric oxide and superoxide in this region, which participates in the regulation of renal medullary blood flow, has been shown to determine the level of blood pressure and salt sensitivity [28,29]. Taylor *et al.* [30] have demonstrated that renal medullary H_2O_2 concentration is higher in Dahl salt-sensitive rats than in salt-resistant ($SS13^{BN}$) control rats, determined the role of oxidative stress to the salt sensitivity. In their study, the reduction of renal medullary oxidative stress induced by the antioxidant apocynin reduced the salt sensitivity in Dahl salt-sensitive rats [30]. On the contrary, renal medullary interstitial infusion of H_2O_2 in $SS13^{BN}$ rats induced a salt-sensitive form of hypertension [31]. Increased salt sensitivity was shown to be associated with reduced medullary blood flow [31]. Therefore, the renal medullary oxidative stress observed in the present study may have reduced medullary blood flow and induced salt sensitivity.

Oral methylglyoxal intake induces renal oxidative stress and advanced glycation endproducts

The present study demonstrated that orally administered MGO could contribute to salt sensitivity and insulin resistance. Although we have not determined whether oral administration of MGO could directly increase renal MGO, the expression of CEL and nitrotyrosine in the renal tubules indicates that oral MGO has at least some effect on AGEs and oxidative stress. This is consistent with the previous report by Vasdev *et al.* [20] that oral administration of MGO increased renal aldehyde in WKY rats. In addition, it is well recognized that the rats orally given fructose developed the pathogenesis of metabolic syndrome (including hypertension and insulin resistance) and increased accumulation of endogenous MGO (plasma and tissues MGO levels) [17,32]. Therefore, oral administration of MGO comparable to that of endogenously produced MGO could be involved in the pathogenesis of renal injury, insulin resistance and hypertension. MGO and associated AGEs are also formed by nonenzymatic reactions and could be formed in heat-treated foods [33–35]. A high AGE diet has been shown to increase plasma CEL and MGO derivatives in healthy human individuals [34]. Moreover, macrovascular and microvascular function is attenuated in diabetes patients with high AGE intake, indicating that oral MGO and/or AGEs play a role in the pathogenesis of diabetic vasculopathy [33]. Therefore, we suspect that MGO also plays a role in the insulin resistance and salt sensitivity in

diabetes, and this hypothesis should be tested in a future clinical study.

Study limitation

There was a notable limitation in the present study. Although the limited sensitivity in the detection of insulin resistance, we tried to look at the maximum response and used the highest concentration of MGO as possible. In the present study, 4-week treatment with 1% MGO in drinking water increased plasma MGO level by about 7.5-fold compared with the control rats in Sprague–Dawley rats (data not shown). The increased magnification of plasma MGO level in the present study is as high as that in patients with chronic kidney disease (CKD) stages 5 compared with the healthy controls (about 5–10 fold) [36]. With the technical limitation of measurements and the control of plasma and/or tissue concentration of MGO, we were not able to determine whether MGO on insulin resistance or salt sensitivity were dose dependent. Further investigation is required in the future study by amelioration of these techniques.

In summary, we have demonstrated that MGO induces insulin resistance as well as salt sensitivity of blood pressure in normotensive Sprague–Dawley rats. Our results suggest that these effects of MGO may be mediated at least in part by increased oxidative stress or AGEs formation, or both. Our present study provides further evidence that MGO is one of the causative factors in the pathogenesis of insulin resistance and salt-sensitive hypertension. Antioxidants and ACE inhibitors may be useful for the treatment of chronic kidney disease individuals with insulin resistance and salt sensitivity.

Acknowledgement

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Biological Effects of Electrolyzed Water in Hemodialysis

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Key Words
Hemodialysis · Electrolyzed water · Oxidative stress · Methylglyoxal · Neutrophils

Abstract
Background/Aims: The application of electrolyzed water (EW) at the cathode side to manufacture reverse osmosis (RO) water and hemodialysis (HD) solution can actually lead to less oxidative capacity in chemical terms. The present study examined the biological actions of this water on human polymorphonuclear leukocytes (PMNs), and the clinical feasibility of applying this technology to HD treatment. ***Methods:*** RO water using EW (e-RO) exhibited less chemiluminescence in luminol-hydrogen peroxide and higher dissolved hydrogen levels (~99.0 ppb) compared with control RO water. The effects of e-RO on PMN viability were tested. HD using e-RO was performed for 12 consecutive sessions in 8 patients for the feasibility test. ***Results:*** Basal cellular viability and function to generate superoxide radicals of PMNs were better preserved by e-RO application. In the clinical trial, reductions of blood pressure were noted, but no adverse events were observed. There were no changes in the blood dialysis parameters, although methylguanidine levels were significantly decreased at the end of study. ***Conclusion:*** The present study demonstrated the capacity of e-RO to preserve the viability of PMNs, and the clinical feasibility of applying this water for HD treatment. The clinical application of this technology may improve the bio-compatibility of HD treatment.

Introduction

Evidence suggests that enhanced oxidative stress plays a crucial role in poor outcomes of patients on maintenance hemodialysis (HD) [1, 2]. In addition to a uremic milieu [1], several factors in the HD system have been found to be involved in the pathological mechanism of these poor outcomes, including bio-incompatibility of the dialysis membrane, contamination of the HD solution and loss of antioxidants during HD [3–7].

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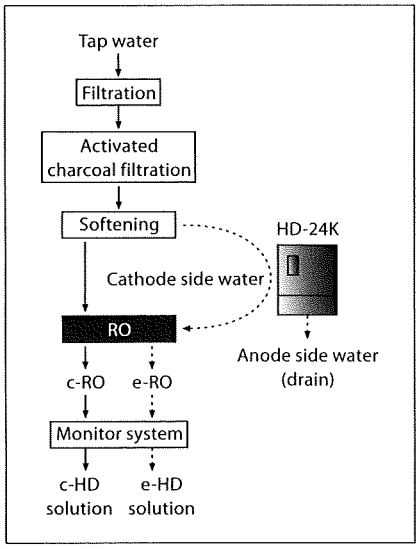

Fig. 1. Manufacturing process for HD solution using reverse osmosis water from the cathode-side of electrolyzed water. c-RO = control reverse osmosis water; e-RO = reverse osmosis water using electrolyzed water at the cathode side.

Water electrolysis renders 2 types of water: acidic water at the anode side and alkali water at the cathode side. It has been shown that alkali water (called electrolyzed water, EW) at the cathode side exhibits unique chemical properties, such as highly dissolved hydrogen with antioxidative capacity [8, 9]. An in vitro study showed that chemical reactions to generate superoxide anions and hydrogen peroxide (H_2O_2) were suppressed by EW treatment [8]. Based on these findings, therapeutic application of EW or water with highly dissolved hydrogen has been studied in animal models of diabetes [10, 11] and ischemic brain injury [12].

Furthermore, EW has been used to manufacture HD solution to reduce oxidative stress in patients [13–15]. To date, the limited clinical experience using this technology shows that EW suppresses oxidative/inflammatory markers in HD patients [13, 14]; however, most of the biological and clinical effects of EW remain unclear. The present study aimed to: (1) examine the biological action of EW in terms of whether it could ameliorate injury to human polymorphonuclear leukocytes, which may play a central role in excess inflammation or oxidative stress in HD patients [16], and (2) to test the clinical feasibility of applying this technology to HD treatment.

Materials and Methods

Manufacture of Test Solutions

Details of the manufacturing process were reported previously [15]. Briefly, test solutions were manufactured as follows (fig. 1): prefiltered water was processed by activated charcoal filtration and water softening to supply the water electrolysis system HD-24K (Nihon Trim, Osaka, Japan), where water was electrolyzed by direct current supply to the anode and cathode electrode plates. Water at the anode side was drained out, and EW was collected to supply the reverse osmosis equipment (MH500CX, Japan Water System Corp., Tokyo, Japan) at 500 ml/min. The intensity of the electrolysis was adjusted to maintain a pH ≤10.0. The reverse osmosis water made by EW (e-RO) was supplied to a personal HD monitoring system (DBB-22B, Nikkiso, Tokyo, Japan) to make the HD solution by mixing with a liquid dialysis solution concentrate.

The pH of the e-RO ranged from 9.0 to 10.0, with mean dissolved hydrogen levels of 99.0 ppb. The dissolved hydrogen level was detected by a gas analyzer (DH-35A hydrogen gas analyzer, Mitsuwa Rikagaku, Osaka, Japan). The hemodialysis solution made by e-RO (e-HD) did not differ from the control HD (c-HD) solution in respect of the electrolyte composition or pH; however, the former solution had a higher level of dissolved hydrogen (80 vs. 0 ppb).

Biological Effects of EW on Polymorphonuclear Leukocytes

Measurement of Cellular Viability

Human polymorphonuclear leukocytes (PMNs) were obtained from healthy volunteers or patients on chronic HD as indicated. Briefly, whole blood was withdrawn from healthy volunteers, and heparinized samples were placed onto the Mono-poly Resolving Medium (Dainippon Pharmaceutical, Osaka, Japan) to collect PMNs. After centrifugation at 1,800 rpm for 30 min at 18°C, the intermediate PMN-enriched layer was recovered, washed, and resuspended in RPMI 1640 culture medium supplemented with 10% fetal calf serum or phosphate buffered saline (PBS). Collected PMNs were adjusted to a concentration of 5×10^5 cells/ml. 50 μl of cell suspension was placed in a 96-well microplate and 50 μl of the test solution containing methylglyoxal (MGO), a toxic dicarbonyl compound elevated in uremic patients, and/or 4-β phorbol 12-β-myristate 13-α-acetate (PMA; a stimulator of the respiratory burst of PMNs) in PBS, was added. The viability of PMNs was examined using a commercially available kit (CellTiter-Glo® luminescent cell viability assay, Promega Corp., Wisc., USA) as previously reported [17]. After each treatment, 100 μl of the sample was placed in a microplate and 100 μl CellTiter-Glo® reagent (Promega), which contains beetle luciferin and luciferase was added immediately. This reacts with adenosine triphosphate (ATP), which was released from lysed cells, to produce oxyluciferin; it also generates chemiluminescence as a function of the increased ATP levels. After incubation, luminescence was measured using a chemiluminescence analyzer (Glo-Max™ 20/20n luminometer, Promega). Each measurement was made 5 times, and the mean values for each sample were calculated for analysis after correction for cell-free levels.

Measurement of Radical Generation from PMN

The rate of superoxide release from human PMNs was determined by measuring the reduction of ferricytochrome C, as reported elsewhere [18]. PMNs obtained by the same procedure described above were adjusted to a concentration of 1×10^6 cells/ml

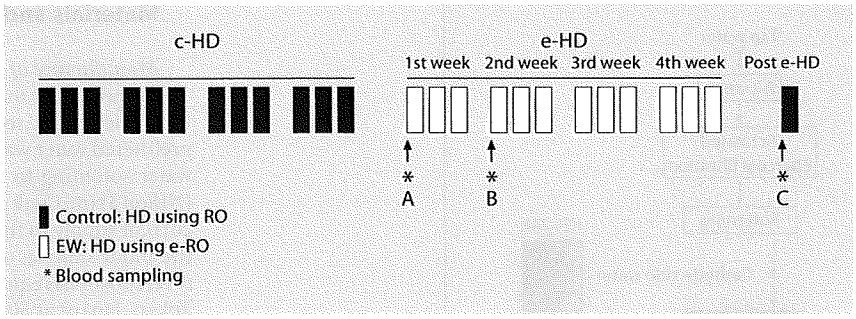

Fig. 2. Feasibility study protocol of HD applying e-RO based HD solution (e-HD). e-RO = reverse osmosis water using electrolyzed water at the cathode side; A = the start of the first e-HD session (first week); B = the start of the fourth e-HD session (second week); C = the start of c-HD after 12 e-HD sessions (post-e-HD).

Table 1. Patient demographics

Case	Sex	Age years	HD vintage months	Underlying renal disease	Dialyser	Prescription	
						AHD	statin
1	female	65	58	NS	BG-1.6	ARB	–
2	male	35	111	CGN	APS-2.1	–	–
3	female	64	216	CGN	BG-1.8	–	–
4	male	70	70	DN	BG-1.8u	ARB	–
5	male	75	77	CGN	AMBC-1.5	ARB	+
6	male	67	335	CGN	APS-185	–	–
7	male	72	116	DN	BG-2.1	CCB+ARB	–
8	male	70	6	NS	PS-1.6	ARB	–

AHD = Anti-hypertensive drug; ARB = angiotensin receptor blockade; CCB = calcium channel blocker; CGN = chronic glomerulonephritis; DN = diabetic nephropathy; NS = nephrosclerosis.

in a solution of 80 μM cytochrome C diluted in PBS. Immediately after adding 10 μl of control or PMA (100 μg) to 700 μl of the sample, 250 μl of the sample was placed on the microplate. Absorbance was measured for 15 min at 550 nm, and was expressed as nanomoles of superoxide production per 0.25 × 10^6 cells/10 min.

In these 2 experiments, PBS was prepared either with control reverse osmosis water (c-RO) or e-RO. Manufactured e-RO was stocked in the closed flask immediately after being made and then used in the study.

Clinical Feasibility of a HD System Using EW

Patients and Study Design

Eight patients on regular HD treatment at Kashima Hospital Dialysis Center (Iwaki, Japan) were enrolled in the trial (table 1). They consisted of 6 men and 2 women, with a mean age of 67 years (range 35–75 years) and a mean dialysis duration of 85 months (range 6–33.5 months). Their underlying renal diseases were chronic glomerulonephritis in 4 patients, nephrosclerosis in 2 and diabetic nephropathy in 2. All patients had been on regular HD treatment 3 times a week for 4 h (n = 6) or 5 h (n = 2) each session. All patients had been using high-flux membrane dialyzers. Among them, 4 were receiving an angiotensin receptor blocker, 1 was receiving an angiotensin receptor blocker and a calcium channel blocker, and 1 was receiving a statin. Patients who were taking ascorbic acid or tocopherol were excluded.

Patients were treated by regular HD regimen using c-HD solution for 1 month, followed by the use of e-HD solution for another month. Both 1-month regimens comprised 3 sessions per week, for a total of 12 sessions (fig. 2). During the study, no changes in HD modes or concomitant medications were made.

Blood sampling was obtained just before each HD session. Blood was immediately centrifuged and serum was stored at –80°C until measurements were made by commercially available kits or high performance liquid chromatography (HPLC). Interleukin-6 was assessed by the CLEIA method (Human IL-6 CLEIA, Fujirebio C, Tokyo, Japan), highly sensitive C reactive protein by nephrometry (N High Sensitive CRP, Dade Behring, Marburg, Germany), creatol and methylguanidine by HPLC, 8-OHdG by an enzyme-linked immunosorbant assay (ELISA) kit (high sensitive 8-OHdG check, Nikken C, Shizuoka, Japan) and pentosidine by an ELISA kit (FSK pentosidine, Fushimi C, Marugame, Japan).

All patients were monitored regarding subjective symptoms during the study periods. Blood pressure was measured using a sphygmomanometer at the upper arm with the patient in the supine position just before and after each HD session. The mean of the 12 measurements obtained was determined.

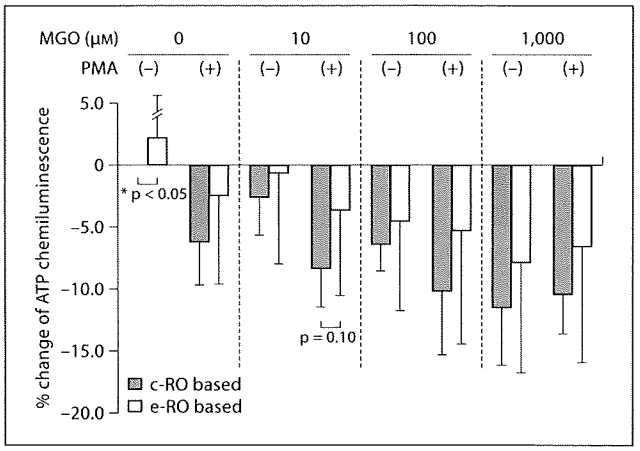

Fig. 3. Effect of e-RO: human neutrophil viability against MGO and/or PMA. Neutrophils from 7 healthy volunteers were suspended in PBS (n = 7). c-RO = treatment by control RO water; e-RO = treatment by RO water by electrolysis. Data are expressed as means ± SD. * $p < 0.05$.

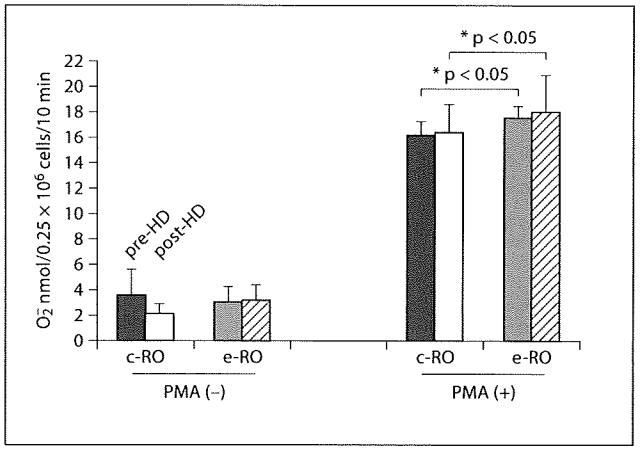

Fig. 4. Effect of e-RO: superoxide anion generation from human neutrophils by PMA. Neutrophils from patients on hemodialysis were suspended in PBS (n = 6). c-RO = treatment by control RO water; e-RO = treatment by RO water by electrolysis. Data are expressed as means ± SD. * $p < 0.05$.

The present study was approved by the ethical committees of Kashima Hospital and Tohoku University (No. 2006-101, No. 2007-207), and informed consent was obtained from all patients. All values are expressed as mean ± SD. Paired t test and 1-way repeated measure analysis of variance were used for statistical analysis. $p < 0.05$ was considered statistically significant.

Results

Changes in ATP-chemiluminescence of PMNs against MGO in the presence or absence of PMA are shown in figure 3. At basal levels (MGO 0 μM) in the absence of PMA, ATP-chemiluminescence was significantly higher with e-RO than c-RO ($p < 0.05$). Significant decreases in ATP-chemiluminescence were associated with MGO levels in both the e-RO and c-RO groups ($p < 0.001$ for both groups). However, no differences were found between the e-RO and c-RO groups in MGO levels. For PMA load, further significant decreases in ATP-chemiluminescence were associated with MGO levels in the e-RO and c-RO groups (at MGO levels 0–100 μM, $p < 0.01$ in both groups); however, no differences were found between c-RO and e-RO.

Figure 4 shows generated superoxide anions in both groups. Under basal levels, no differences were found between groups, whereas in stimulated conditions, levels of generated radicals were significantly higher for e-RO than for c-RO ($p < 0.05$ in pre-HD and post-HD).

Table 2. Change in laboratory parameters

	Pre	Post	p value
WBC, n/μl	6,795 ± 863	6,644 ± 945	0.35
Hb, g/dl	11.3 ± 1.2	11.2 ± 1.2	0.19
Creatinine, mg/dl	11.0 ± 3.2	10.6 ± 3.0	0.07
BUN, mg/dl	64.6 ± 6.2	60.8 ± 9.0	0.1

Data are means ± SD. Blood samples were obtained from the arteriovenous fistula before starting dialysis. Pre = e-HD day 1 (fig. 1); Post = c-HD day 13 (fig. 1); WBC = white blood cells; Hb = hemoglobin; BUN = blood urea nitrogen.

Changes in laboratory parameters during the clinical trial of e-HD are shown in table 2. No changes were observed in white blood cell count, hemoglobin, blood urea nitrogen and creatinine levels. Figure 5 shows changes in inflammatory or oxidative stress markers in the blood. There were significant decreases in plasma methylguanidine levels ($p < 0.05$; fig. 5c). There were also significant decreases in mean blood pressure during the study in the c-HD and e-HD groups between the pre- and post-HD sessions ($p < 0.01$ and $p < 0.001$, respectively; table 3). No adverse symptoms were observed in any patient.

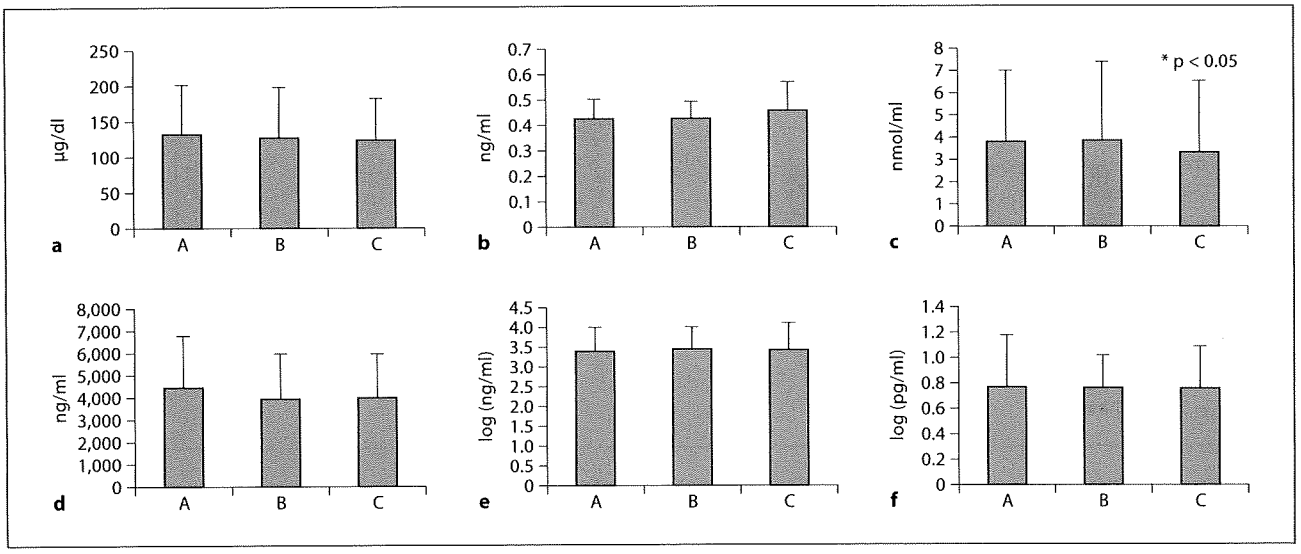

Fig. 5. Changes in laboratory variables after HD using the e-RO based HD solution. **a** Creatol. **b** 8-OHdG. **c** Methylguanidine. **d** Pentosidine. **e** Highly sensitive C reactive protein. **f** Interleukin-6. Data are expressed as means ± SD. A = the start of the first e-HD session (first week); B = the start of the fourth e-HD session (second week); C = the start of c-HD after 12 e-HD sessions (post-e-HD). * $p < 0.05$.

Discussion

It has been demonstrated that e-RO exhibits antioxidative capacity [13–15]. The present study aimed to investigate the biological effect of e-RO on the viability of PMNs, and the clinical feasibility of applying this technology to HD treatment.

Plasma MGO levels are reportedly elevated in patients on dialysis [19, 20]. MGO is highly reactive and biologically toxic due to the formation of advanced glycation end products, protein and DNA modifications [21–23]. Recently, we demonstrated that MGO plays a role in the enhancement of injury to PMNs in combination with H_2O_2 [24], which may be involved with the pathological mechanism of microinflammation in dialysis patients.

In the c-RO group in the present study (fig. 3), decreased viabilities of PMNs were found to be associated with the MGO level, and the toxicity of MGO was further increased in the presence of PMA. Basal cellular viability was better preserved in the e-RO group than in c-RO group. Furthermore, MGO-related PMN injury in the e-RO group was relatively ameliorated compared with the c-RO group, although differences did not reach statistical significance. In addition, in PMNs of uremic patients (fig. 4), the cellular function to generate superoxide anions by a physiological respiratory burst was significantly higher with e-RO than with c-RO.

Table 3. Blood pressure and heart rate pre- and post-dialysis

	c-HD (n = 12)	e-HD (n = 12)	p value
Pre-dialysis			
SBP, mm Hg	165 ± 25	157 ± 21	<0.01
DBP, mm Hg	85 ± 12	81 ± 10	<0.05
MBP, mm Hg	112 ± 15	106 ± 12	<0.01
HR, beats/min	76 ± 12	78 ± 14	0.71
BW gain, %	3.8 ± 0.85	4.0 ± 0.93	0.9
Post-dialysis			
SBP, mm Hg	124 ± 19	117 ± 15	<0.01
DBP, mm Hg	69 ± 11	64 ± 9	<0.001
MBP, mm Hg	87 ± 13	81 ± 11	<0.001
HR, beats/min	80 ± 12	81 ± 14	0.63
Volume removed, kg	2.2 ± 0.8	2.4 ± 0.8	0.97

Data are the mean ± SD levels of 12 HD sessions in each patient. BW gain = Inter-dialytic body weight gain over dry weight; c-HD = hemodialysis using control reverse osmosis water; DBP = diastolic blood pressure; e-HD = hemodialysis using reverse osmosis water by electrolyzed water; HR = heart rate; MBP = mean blood pressure; pre-dialysis = just before the hemodialysis session; post-dialysis = just after the hemodialysis session; SBP = systolic blood pressure; volume removed = intra-dyalitic volume removal per HD session.

These findings indicate that the application of e-RO does not stimulate PMNs, but rather benefits PMNs in preserving cellular viability as compared with c-RO. However, suppression of MGO toxicity by e-RO application was not clearly demonstrated in this study (fig. 3). In terms of the lack of e-RO effect on MGO cytotoxicity in this study (fig. 3), we think that the change in chemical properties of e-RO, such as levels of dissolved hydrogen, may contribute to our findings, as indicated by recent studies that suggested a significant role for dissolved hydrogen in ameliorating oxidative stress [25, 26]. Further studies are needed in this area to address the context of the preservation of e-RO chemical properties.

Next, we examined the feasibility of the application of this technology to regular HD treatment. No symptomatic adverse events were observed during the study period, although there were nonsymptomatic reductions in mean blood pressure before and after the HD session. No changes were found in laboratory parameters or in interdialytic changes of body weight, which may indicate the feasibility of an e-HD system.

In previous reports, Huang et al. [13, 14] showed decreases in surrogate markers of inflammation and oxidative stress following the introduction of an e-HD system. In the present short trial, plasma methylguanidine was significantly decreased, but no other parameters, including C-reactive protein, interleukin-6, creatol and 8-OHdG, were changed (fig. 5). We propose the following reasons for these contradictory results. First, methylguanidine is the nonspecific oxidative end product that could reflect the overall oxidative milieu, and thus could be changed significantly. Second, we only enrolled clinically stable patients, and thus those who presented with inflammation or malnutrition were not included in the study. This clinical background might influence the results of our study.

Interestingly, MBP was decreased by the introduction of e-HD. Taken together, the findings of decreased MBP and no changes in heart rate or inter-dialytic body weight gain lead us to speculate that e-HD may lead to vasodilation. Vasoconstrictive action by radical oxygen species such as peroxynitrite could play a role in increased blood pressure in HD patients, and dissolved hydrogen in EW may reduce superoxide anions, as previously reported [8], during the course of e-HD to suppress its pathological process. Further studies are needed in this area.

There are some unresolved issues in the present HD system. Data on the microbiological quality of the dialysis solution were not available, and water volume consumed per session was not clearly measured. These issues need to be clarified in follow-up studies.

In conclusion, the present study demonstrated the capacity of e-RO to preserve the viability of PMNs, and the clinical feasibility of using this water for HD treatment. The clinical application of this technology may improve bio-compatibility of HD treatment.

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ORIGINAL ARTICLE

Geographic difference in the prevalence of chronic kidney disease among Japanese screened subjects: Ibaraki versus Okinawa

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Abstract
Background In Japan, there is a geographic difference in the prevalence of end-stage renal disease (ESRD). Few epidemiologic studies, however, have compared the prevalence of chronic kidney disease (CKD) among different geographic areas. Other than genetic factors, socioeconomic conditions and lifestyle are targets for modification.
Methods We examined the prevalence of CKD among two large community-based screened populations, 40 years of age and older, in Japan: Ibaraki ($N = 187{,}863$) and Okinawa ($N = 83{,}150$). Prevalence of CKD was defined as an estimated glomerular filtration rate (eGFR) of less than 60 ml/min/1.73 m^2 using the coefficient modified abbreviated Modification of Diet in Renal Disease (aMDRD) study equation using a standardized serum creatinine value. CKD prevalence was compared among screenees with (+) or without (−) hypertension (systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg) and hyperglycemia (plasma glucose ≥ 126 mg/dl).
Results Both male and female participants in Okinawa had a significantly lower prevalence of hypertension (−)/hyperglycemia (−) than did patients in Ibaraki. The prevalence of CKD in Okinawa was higher than that in Ibaraki among screenees with hypertension (−)/hyperglycemia (−), and highest among screenees with hypertension (+)/hyperglycemia (−).
Conclusion The regional difference in CKD prevalence may underlie the variation in ESRD prevalence observed in Japan.

Keywords Chronic kidney disease · Glomerular filtration rate · Prevalence · Screening

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Introduction

The prevalence of end-stage renal disease (ESRD) is linearly increasing and is as high as 2,000 per million people in Japan [1]. The geographic difference in the prevalence of ESRD in Japan is well known; Okinawa has the highest ESRD population, whereas the ESRD population in Ibaraki is smaller than the National average [1]. This trend might be explained by either a high prevalence of chronic kidney disease (CKD), a faster progression of CKD, or both. The north-south gradient in the incidence and prevalence of certain diseases, such as stroke and hypertension are also well known in Japan [2]. Populations in northern Japan have a higher salt intake and other dietary habits also vary [3]. People in Okinawa tend to be more obese and have a

higher prevalence of metabolic syndrome, which causes CKD [4, 5]. The prevalence of CKD may reflect the health and functional status of the community, such as the proportion of the population with diabetes and hypertension, as well as differences in muscle mass, diet, and lifestyle.

We compared the prevalence of CKD between two large community-based screening registries available in two target prefectures (Ibaraki and Okinawa). To define CKD, we applied the newly developed and modified abbreviated Modification of Diet in Renal Disease (MDRD) study equation as it provides the most accurate formula for this purpose [6]. Determining the factors related to the regional difference in CKD prevalence might be useful for preventing ESRD. The present study is the first to demonstrate a regional difference in CKD prevalence in Japan.

Methods

The Japanese Society of Nephrology has organized an epidemiology work group and has collected data to estimate CKD population in Japan [7, 8]. The authors are participating with the epidemiology work group. Among the community-based screening programs, we selected two cohorts because the details of these subjects were previously reported and the method of serum creatinine measurement was verified. Okinawa, 128°E 27°N, is in the southern-most part of Japan, and Ibaraki, 140°E 36°N, is in northern Japan. Screening was performed during April 2005 to March 2006. Hypertension was defined as 140/90 mmHg and over and hyperglycemia was defined as fasting plasma glucose 126 mg/dl and over.

Community-based screening registry

(Okinawa) Details of the screening in Okinawa were published previously [9, 10]. For this study, we used the 2005 Okinawa General Health Maintenance Association (OGHMA) registry, and analyzed data for those aged 40 years and over at the time of screening. There were 83,150 screenees, 13.0% of the target population of 0.64 million in 2005 (Total 1.36 million).

(Ibaraki) Details of the screening in Ibaraki were published previously [11–13]. For this study, we used the 2005 registry, and analyzed data for those aged 40 years and over at the time of screening. There were 187,863 screenees, 11.6% of the target population of 1.62 million in 2005 (Total 2.98 million). The central laboratory measured creatinine using an autoanalyzer (Hitachi 7170). Data were provided after written agreement by the research committee for each registry.

GFR estimation

GFR was estimated using the coefficient modified MDRD study equation after obtaining the standardized serum creatinine (SCr) from the Cleveland Clinic. Serum creatinine (C-SCr) was calibrated using the following formula: for Okinawa, C-SCr = 1.03557343 × SCr + 0.00736639; for Ibaraki, C-SCr = 1.01758277 × SCr − 0.0643799. Both laboratories measure SCr using an enzymatic method. We confirmed the accuracy of creatinine measurement using a calibration panel composed of 42 serum samples, whose values were determined by the Cleveland Clinic (kindly provided by Dr. Van Lente at the Cleveland Clinic). eGFR (ml/min/1.73 m^2) = 175 × $Age^{-0.203}$ × $S\text{-}Cr^{-1.154}$ × (if female × 0.742) × (if Japanese × 0.741). Performance of the IDMS aMDRD equation for evaluating Japanese CKD patients was recently published [6].

Statistical analyses

Data are expressed as means ± standard deviation (SD). The st CKD was defined as eGFR <60 ml/min/1.73 m^2 [6]. A statistical significance of differences in the characteristics among participants was examined using non-paired *t* test, the Wald chi-square test, and Wilcoxson test (categorical variables). Multivariate logistic analyses were performed using SAS (Version 8.2, SAS Institute Inc., Cary, NC). A *P* value of less than 0.05 was considered statistically significant.

Results

The demographics of the screened cohorts were different between the two community-based registries: 35.6% of the participants in Ibaraki and 42.6% of those in Okinawa were men. Therefore, the mean (SD) glomerular filtration rate (GFR) levels are summarized for each age-class for both men and women among the total number of screenees (Table 1). The mean GFR levels were significantly higher in Okinawa than in Ibaraki, except in those age 80 and over among both sexes. Prevalence of CKD in Ibaraki (Okinawa) was 18.1% (15.3%) in men and 16.0% (13.9%) in women, respectively. However, the fraction of screenees were different between the two cohorts. In Ibaraki (Okinawa), it was 8.9% (23.3%) in age 40–49, 18.7% (24.9%) in age 50–59, 35.1% (23.9%) in age 60–69, 30.6% (21.9%) in age 70–79, and 6.7% (6.0%) in age 80 and over in men. In women, that was 14.4% (21.2%) in age 40–49, 27.1% (25.1%) in age 50–59, 31.7% (23.9%) in age 60–69, 22.3% (22.1%) in age 70–79, and 4.5% (7.8%) in age 80 and over.

The proportion of screenees without either hypertension or high plasma glucose was significantly smaller in

Table 1 Comparison of GFR among screened subjects in Okinawa and Ibaraki: total screened

	Ibaraki	Okinawa	*P* value
Men			
40–49	76.8 (13.3), *N* = 5,961	78.4 (14.7), *N* = 8,238	<0.0001
50–59	74.8 (14.4), *N* = 12,485	75.6 (15.4), *N* = 8,810	<0.001
60–69	69.6 (14.3), *N* = 23,515	70.4 (15.1), *N* = 8,476	<0.0001
70–79	65.8 (14.8), *N* = 20,513	66.5 (15.5), *N* = 7,757	<0.001
80 and over	61.6 (15.6), *N* = 4,463	60.6 (15.9), *N* = 2,112	<0.05
Women			
40–49	80.7 (15.6), *N* = 17,388	86.1 (16.5), *N* = 10,120	<0.0001
50–59	77.1 (15.5), *N* = 32,798	80.8 (16.5), *N* = 11,991	<0.0001
60–69	72.8 (15.4), *N* = 38,309	74.7 (15.7), *N* = 11,401	<0.0001
70–79	67.8 (15.3), *N* = 27,008	68.7 (16.2), *N* = 10,541	<0.0001
80 and over	62.1 (15.7), *N* = 5,423	62.1 (19.3), *N* = 3,704	NS

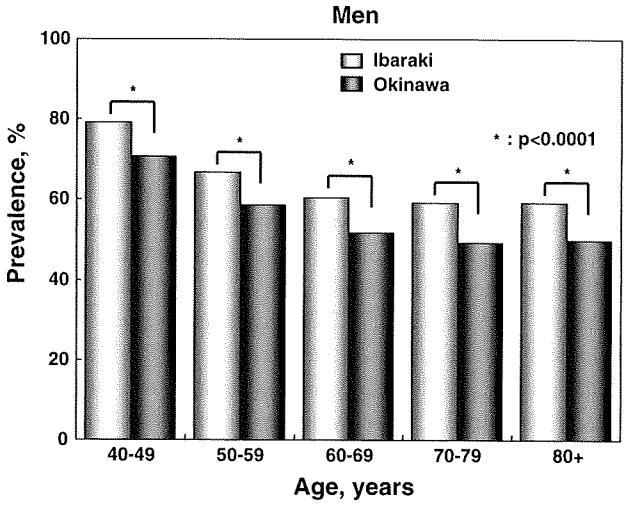

Fig. 1 Prevalence of screenees without hypertension or hyperglycemia in Okinawa and Ibaraki (men)

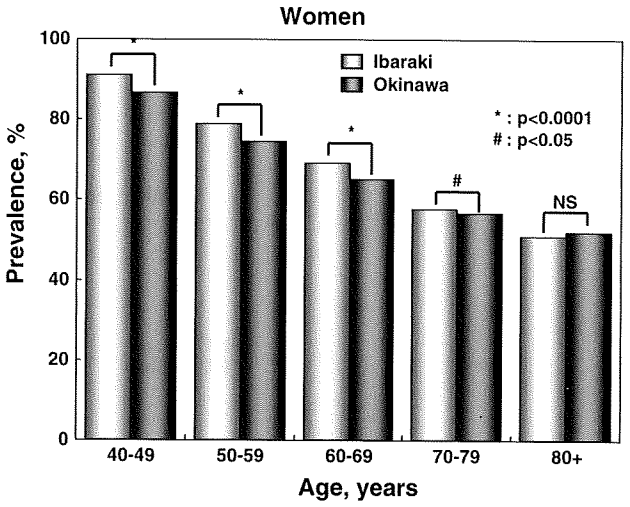

Fig. 2 Prevalence of screenees without hypertension or hyperglycemia in Okinawa and Ibaraki (women). *NS* not significant

Okinawa than in Ibaraki among men (Fig. 1) and women (Fig. 2) of all age-groups. Overall prevalence of hypertension and hyperglycemia in Okinawa was 29.9% and 10.4%: 35.5% and 14.2% in men, 26.2% and 7.6% in women, and that of Ibaraki was 27.9% and 5.1%: 31.9% and 8.4% in men, 25.9% and 3.4% in women. Among those 40–79 years of age, the prevalence of CKD of eGFR <45 ml/min/1.73 m^2, was higher in Okinawa than in Ibaraki in those with normal blood pressure and normal glucose levels, high plasma glucose, hypertension, and the total screened populations in men (Fig. 3). In each sex, the prevalence of CKD of eGFR <45 ml/min/1.73 m^2, was compared with Okinawa and Ibaraki (Fig. 4). The prevalence of CKD of eGFR <45 ml/min/1.73 m^2 among those with age 80 years and over in Okinawa (Ibaraki) was 12.6% (10.1%) in men ($P < 0.05$) and 13.0% (11.4%) in women ($P < 0.001$), respectively.

Similarly, mean GFR levels were high in Okinawa among those without either hypertension or high plasma glucose (Table 2). Compared to Ibaraki, the prevalence of low GFR (<45 ml/min/1.73 m^2) was significantly higher in Okinawa, particularly in those under 60 years of age (Table 3). Similar trends were observed among screenees without either hypertension or high plasma glucose (Table 4).

Discussion

We compared the CKD prevalence between two community-based screened cohorts using the standardized serum creatinine measurements and adopted a new, accurate GFR estimation formula for the screened Japanese populations. The strengths of the study include the large study population containing a comparable number of men and women

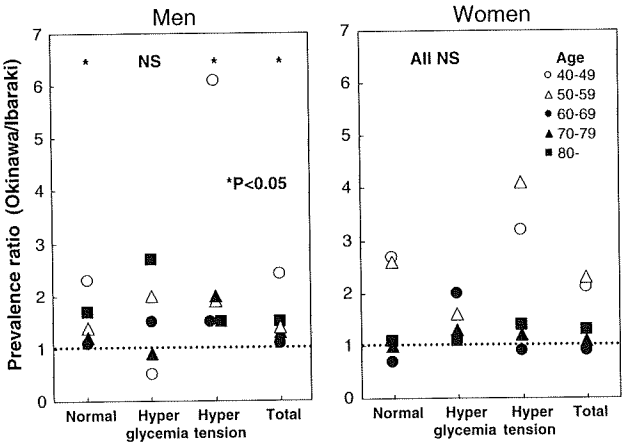

Fig. 3 Prevalence ratio of CKD, GFR <45 ml/min/1.73 m^2, in Okinawa and Ibaraki among screenees aged 40–79 years and those with age 80 years. Age-groups are 40–49 (*open circle*), 50–59 (*open triangle*), 60–69 (*filled circle*), 70–79 (*filled triangle*), and 80 and over (*open square*). In women, there was none with GFR <45 ml/min/1.73 m^2 among those with hyperglycemia age 40–49 years. $P < 0.05$ by the Wilcoxson test

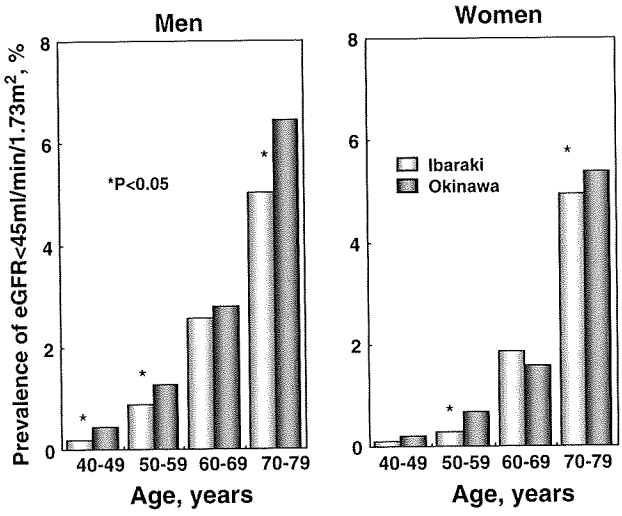

Fig. 4 Prevalence ratio of CKD, GFR <45 ml/min/1.73 m^2, by age in Okinawa and Ibaraki among screenees aged 40–79 years

and comparable age-groups, and the creatinine assays in each population were calibrated to standardized values. The key finding of the present study was that CKD prevalence was higher in Okinawa than in Ibaraki, even among groups of similar age and sex. As shown in Fig. 3, prevalence rate of GFR<45 ml/min/1.73 m^2 was higher in Okinawa, in particular age-class less than 60 years in both sexes. This may reflect the increase in obesity and metabolic syndrome in Okinawa. As a whole, mean levels of eGFR was higher in Okinawa (Table 1). This could be explained the two peaks of eGFR levels or wider distribution due to hyperfiltration related to obesity or hyperglycemia.

The findings of the present study may explain the high prevalence of ESRD in Okinawa [14]. According to the registry data of the Japanese Society for Dialysis Therapy, the prevalence of ESRD was 2,055 (Ibaraki), 2,704 (Okinawa), and 2,070 (Total) per million population in Japan in 2006 [1]. This number increased from the 2001 values of 1584 (Ibaraki), 2330 (Okinawa), and 1722 (Japan) per million population, respectively. The trend might also be explained by a rapid progression of CKD, insufficient therapy for CKD, or both.

Usami et al. [15] reported that the intake of angiotensin-converting enzyme inhibitors in Okinawa was lower than that in other parts of Japan, suggesting the insufficiency of CKD therapy in Okinawa. Because the income levels in Okinawa are the lowest in Japan, cheaper drugs are preferred. Other socioeconomically related conditions, such as a high smoking rate, a high motorization rate, and use of erythropoietin [16] may also be involved in the high prevalence of CKD.

The prevalence of CKD stages 3–5 differs among various ethnic groups. The CKD prevalence in Japan is one of the highest in the world [17–19]. The CKD prevalence might be explained by the age of the population in Japan, as more than 20% are 60 years and older. The prevalence of CKD is higher in those with hypertension and diabetes mellitus in the United States [20, 21]. In Okinawa, however, the prevalence of CKD was higher even in those without hypertension or hyperglycemia. GFR varies based on the presence of hyperglycemia, high protein intake, and obesity. Generally, Okinawan people are short in stature and have a higher prevalence of low birth weight than the national average [22]. A lower birth weight is associated with a lower nephron number and a significant risk of developing ESRD [23]. A low nephron number may result in the future development of hypertension and diabetes mellitus-related nephropathy [24]. Lifestyles have changed rapidly after the return of Okinawa to Japan in 1972, including a rapid increase in obesity.

In the present study, we applied the Japanese coefficient to improve the accuracy of the abbreviated MDRD equation to identify patients with stage 3 and 4 CKD. We used a coefficient of 0.741 obtained from the data of patients with a Cin <90 ml/min/1.73 m^2 as the Japanese coefficient with the IDMS traceable abbreviated MDRD (aMDRD) study equation. The equation provided a reasonably accurate GFR estimation in the range of less than 90 ml/min/1.73 m^2 [25]. This equation can be easily used by Japanese clinicians because the equation does not require that serum creatinine values be calibrated to the 1990 Cleveland Clinic values, where creatinine was measured using the non-compensated Jaffe method [26]. An accurate measurement of serum creatinine, however, is critical for use of IDMS aMDRD equation. In Japan, almost all clinical laboratories use the

Table 2 Comparison of GFR among screened subjects in Okinawa and Ibaraki: normal blood pressure and normal fasting plasma glucose

	Ibaraki	Okinawa	*P* value
Men			
40–49	76.5 (12.9), *N* = 4,416	77.9 (13.8), *N* = 5,812	<0.0001
50–59	74.4 (13.5), *N* = 7,356	74.9 (14.3), *N* = 5,155	NS
60–69	69.3 (13.6), *N* = 12,093	70.1 (14.2), *N* = 4,364	<0.01
70–79	65.7 (14.4), *N* = 10,095	66.7 (15.0), *N* = 3,807	<0.001
80 and over	61.4 (15.2), *N* = 2,174	61.2 (16.2), *N* = 1,037	NS
Women			
40–49	80.5 (15.3), *N* = 15,428	85.9 (16.1), *N* = 8,765	<0.0001
50–59	76.6 (15.0), *N* = 24,392	80.5 (16.1), *N* = 8,921	<0.0001
60–69	72.5 (15.0), *N* = 24,103	74.7 (15.1), *N* = 7,419	<0.0001
70–79	67.4 (14.9), *N* = 13,801	68.6 (15.5), *N* = 5,946	<0.0001
80 and over	61.9 (15.6), *N* = 2,403	62.1 (19.2), *N* = 1,847	NS

Table 3 Comparison of the prevalence of low GFR, <45 ml/min/1.73 m^2 and <60 ml/min/1.73 m^2 among screened subjects in Okinawa to those in Ibaraki (reference): total screened

	GFR <45	*P* value	GFR <60	*P* value
Men				
40–49	2.37	<0.01	0.93	NS
50–59	1.44	<0.01	1.42	<0.0001
60–69	1.10	NS	0.84	<0.0001
70–79	1.29	<0.0001	0.85	<0.0001
80 and over	1.50	<0.0001	1.06	<0.05
Total	1.04	NS	0.76	<0.0001
Women				
40–49	2.1	<0.05	0.65	<0.0001
50–59	2.34	<0.0001	1.40	<0.0001
60–69	0.86	NS	0.56	<0.0001
70–79	1.11	<0.05	0.76	<0.0001
80 and over	1.26	<0.0001	0.95	<0.05
Total	1.27	<0.0001	0.75	<0.0001

Table 4 Comparison of the prevalence of low GFR, <45 ml/min/1.73 m^2 and <60 ml/min/1.73 m^2 among screened subjects in Okinawa to those in Ibaraki (reference): normal blood pressure and normal fasting plasma glucose

	GFR < 45	*P* value	GFR < 60	*P* value
Men				
40–49	2.28	NS	0.86	<0.05
50–59	1.43	NS	1.47	<0.0001
60–69	1.08	NS	0.84	<0.0001
70–79	1.19	<0.05	0.84	<0.0001
80 and over	1.65	<0.0001	1.00	NS
Total	0.97	NS	0.73	<0.0001
Women				
40–49	2.72	<0.01	0.65	<0.0001
50–59	2.60	<0.0001	1.37	<0.0001
60–69	0.71	<0.01	0.53	<0.0001
70–79	1.01	NS	0.73	<0.0001
80 and over	1.14	NS	0.92	<0.05
Total	1.18	<0.001	0.72	<0.0001

enzymatic method to measure serum creatinine. The enzymatic method is more precise and accurate than the Jaffe method, which usually overestimates serum creatinine due to interference from the non-creatinine chromogen. Nevertheless, we further confirmed that the difference is still evident when using the original Japanese Society of Nephrology GFR estimation equation (S. Matsuo et al., personal observation).

The strengths of the present study were as follows: (1) eGFR was calculated using the serum creatinine value after calibration and standardization, (2) both cohorts were large enough to compare by age and sex, (3) CKD prevalence was also evaluated using the two equations currently available in Japan.

There were some limitations of the present study: (1) Serum creatinine was not measured at a single laboratory, although assay methods of the participating laboratories were evaluated by standard samples from the Cleveland Clinic and the inter-laboratory coefficient of variation was very small (0.88%), (2) The formula for estimating GFR was developed using CKD patients; therefore, it is not applicable to a healthy population. In particular, underestimation is possible in those with an eGFR of more than 60 ml/min/1.73 m^2 [6]. Serum creatinine concentration is affected not only by GFR, but by various other factors as well, such as muscle mass, sex, race, diet, drugs, and tubular function. Ideally, the clearance of exogenous GFR markers, such as inulin, should be measured for GFR estimation, but the method is time-consuming and difficult and is not feasible for community-based screening. The Kidney Disease Improving Global Outcomes (KDIGO) group has initiated an action to improve clinical practice by

introducing GFR estimating equations that were developed for a large cohort of a variety of racial and other groups for international comparisons [27–29]. Asian populations, including the Japanese, generally have low muscle mass and low protein intake, which could impair the performance of the MDRD study equation, (3) Clinical information, such as inflammation, nutritional status, or drug treatment, was not included in the registry data.

In conclusion, the findings of the present study revealed that there are significant regional differences in CKD prevalence among screened subjects in Japan. Although, our results may need to be confirmed in other parts of Japan. Reasons for the difference in CKD prevalence remain speculative. Generally, people in Okinawa are short in stature and have a larger body mass index. Lifestyle habits, such as smoking, drinking, and exercise among people in Okinawa also differ from those in Ibaraki. The observed differences in ESRD prevalence might be at least partly due to the difference in the CKD prevalence. Further studies on CKD progression and background demographics in the two cohorts are warranted.

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Conflict of interest statement We have no conflict of interest.

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ORIGINAL ARTICLE

Prevalence of anemia according to stage of chronic kidney disease in a large screening cohort of Japanese

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Abstract
Background The prevalence of chronic kidney disease (CKD) is high in developed countries, including Japan. However, little is known about the prevalence of anemia according to the estimated glomerular filtration rate (eGFR) among Japanese.
Methods We studied screenees on the Okinawa General Health Maintenance Association (OGHMA) registry in 1993 ($N = 94,602$; 54,848 women and 39,754 men) who had both serum creatinine and hematocrit data. Anemia was defined as follows: hematocrit level <40% in men, <32% in women aged <50 years, and <35% in women aged ≥50 years. GFR was estimated using a new Japanese equation: eGFR (ml/min per 1.73 m^2) = 194 × serum creatinine$^{1.094}$ × age$^{0.287}$ × 0.739 (if female).
Results The prevalence of anemia clearly increased as CKD progressed below an eGFR of 60 ml/min per 1.73 m^2 in both genders. Logistic analysis adjusted with body mass index and older age (≥70 years) revealed that the odds ratio for complications of anemia was significantly increased below an eGFR of 45 ml/min per 1.73 m^2 in women and 90 ml/min per 1.73 m^2 in men. The association of lower kidney function with anemia was found to be more prevalent: adjusted odds ratio ≥2.0, from approximately 50 ml/min per 1.73 m^2.
Conclusion The present study suggested that there might be as many as 1,000,000 people with CKD stage 3–5 complicated with anemia in Japan.

Keywords Chronic kidney disease · Anemia

Introduction

Accumulating evidence has shown that even early-stage chronic kidney disease (CKD) is a risk factor for developing cardiovascular disease (CVD) [1–3]. In addition to traditional risk factors such as hypertension, anemia may be associated with CVD among general subjects [4]. Similarly, it has been reported that low hemoglobin, especially together with CKD, increases the risk of coronary heart disease (CHD), CHD-related death, and stroke [5–8]. Since anemia accelerates the progression of CKD and advanced CKD is likely to be complicated with anemia, the combination of anemia and CKD, which promote each other in a vicious circle, could result in an increased risk of CVD and vice versa, that is, cardio-renal anemia syndrome [9]. Therefore, it is critical to identify CKD patients complicated with anemia.

Recent studies have estimated that the incidence of mild kidney dysfunction is substantially high in the general population worldwide, though it varies across countries [10–13]. In the advanced stages of kidney failure, anemia is a common complication due to an inappropriately reduced endogenous erythropoietin production [14]. However, previous studies performed in the USA have found that even mild kidney dysfunction, with an estimated glomerular filtration rate (eGFR) of 60 ml/min per 1.73 m^2, had a

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significant impact on the occurrence of anemia [15, 16]. The study by Astor et al. [16] also demonstrated that there was a significant racial difference in the relationship between kidney function and anemia, with Japanese reported to have a much higher prevalence of CKD than US subjects [12, 17]. However, it is not yet known whether Japanese have a much higher prevalence of CKD complicated with anemia.

In this study, we investigated the prevalence of anemia according to CKD stage in a large community-based screening of Japanese subjects.

Methods

About OGHMA

Screening program: The Okinawa General Health Maintenance Association (OGHMA), a nonprofit organization founded in 1972 and currently under the direction of Drs. Ikemiya and Kinjo, conducts a large community-based annual health examination. Once each year, the staff, doctors, and nurses visit residences and workplaces throughout the prefecture to carry out health examinations. All subjects participate voluntarily in the screening. The OGHMA personnel provide mass screening, inform the participants of their results, and when necessary, recommend further evaluation or treatment. This process includes an interview concerning health status, a physical examination, and urine and blood tests. A nurse or doctor measures blood pressure using a standard mercury sphygmomanometer with the subject in sitting position. Dipstick testing for proteinuria, hematuria, and glucosuria (Ames Dipstick, Tokyo, Japan) is performed in spontaneously voided fresh urine. Proteinuria is defined as a dipstick urinalysis score of 1+ or more. Body mass index (BMI) is calculated as weight (kg) divided by the square of height (m). Computer-based data were available from April 1, 1993 through March 31, 1994 (n = 143,948) for the 1993 screening.

Participants

For the purposes of the present study, we examined OGHMA 1993 screenees who had both serum creatinine (SCr) and hematocrit data (N = 94,602; 54,848 women and 39,754 men). SCr was measured using a modified Jaffe's reaction in an autoanalyzer at the OGHMA laboratory.

Assessment of kidney function

Kidney function was evaluated by eGFR, which was calculated using the new Japanese equation: eGFR (ml/min per 1.73 m^2) = 194 × serum creatinine$^{1.094}$ × age$^{0.287}$ × 0.739 (if female) [18]. For calculating eGFR, we applied the value of SCr in enzymatic methods, which was calculated by the following equation: SCr (enzyme) = (SCr (Jaffe) − 0.194)/1.079 [19].

Definition of anemia, clinical data, and analysis

Anemia was defined according to the Japanese Society for Dialysis Therapy (JSDT) guidelines and the kidney disease outcomes quality initiative (K/DOQI) guidelines, which take both age and sex into account: men, <40%; women aged <50 years, <32%; and women aged ≥50 years, <35% [20, 21]. Diabetes mellitus (DM) was diagnosed when fasting plasma glucose levels were >126 mg/dl. Subjects who were already on chronic dialysis were excluded from the screening registry. To analyze the effect of kidney function on the prevalence and risk of anemia, subjects were divided into following six groups: less than 15 ml/min per 1.73 m^2, from 15 to 29 ml/min per 1.73 m^2, from 30 to 44 ml/min per 1.73 m^2, from 45 to 59 ml/min per 1.73 m^2, from 60 to 90 ml/min per 1.73 m^2, and more than 90 ml/min per 1.73 m^2.

According to the recently published JSDT Guideline for Renal Anemia in Chronic Kidney Disease, anemia was defined as <35% in women [22]. We also analyzed using this definition in women.

Statistics

Statistical significance of differences in characteristics across participants was examined using the t test (continuous variables), and the Wald chi-square test (categorical variables) was carried out. We compared values of hematocrit and prevalence of anemia between the different levels of clinical variables such as BMI, age, and eGFR by Scheffé's multiple comparison methods after analysis of variance (ANOVA). Multiple logistic analysis was done to examine the correlates of anemia by variables such as eGFR category, sex, older age (>70 years), and BMI category. Data are expressed as mean (standard deviation, SD). A P value of less than 0.05 was considered statistically significant.

Results

OGHMA population

Of total of 143,948 OGHMA subjects, 94,602 (65.7%: 54,848 women and 39,754 men) had measurements of both SCr and hematocrit levels. The clinical characteristics of the screened subjects according to gender are summarized in

Table 1 Characteristics of screened subjects in 1993 in Okinawa, Japan

Variable	All (N = 94,602)	Men (N = 39,754)	Women (N = 54,848)	P value
Age (years)	54.7 ± 15.3	53.5 ± 15.7	55.6 ± 14.9	<0.0001
BMI (kg/m^2)	24.0 ± 3.4	24.1 ± 3.2	23.9 ± 3.5	<0.0001
SBP (mmHg)	127.4 ± 17.7	129.4 ± 16.8	126.0 ± 18.1	<0.0001
DBP (mmHg)	76.6 ± 10.5	78.6 ± 10.4	75.1 ± 10.3	<0.0001
Urine protein (%)	3504 (3.8)	1774 (4.5)	1730 (3.3)	<0.0001
Hematocrit (%)	41.4 ± 4.1	44.5 ± 3.3	39.2 ± 3.0	<0.0001
Estimated GFR (ml/min per 1.73 m^2)	79.3 ± 20.1	79.8 ± 18.6	78.9 ± 21.1	<0.0001
Anemia (%)	5450 (5.8)	3056 (7.7)	2399 (4.4)	<0.0001
Serum creatinine (mg/dl)	0.98 ± 0.21	1.10 ± 0.20	0.89 ± 0.17	<0.0001
Diabetes (FPG ≥ 126 mg/dl)	3103 (4.8)	1711 (6)	1392 (3.8)	<0.0001
Hypertension	28312 (30.0)	13309 (33.6)	15003 (27.4)	<0.0001
Age (years)				
20–29	5423 (5.7)	2773 (7.0)	2650 (4.8)	
30–39	11802 (12.5)	5746 (14.5)	6056 (11.0)	
40–49	17612 (18.6)	7723 (19.4)	9889 (18.0)	
50–59	19996 (21.1)	7684 (19.3)	12312 (22.4)	
60–69	22446 (23.7)	9035 (22.7)	13411 (24.5)	
≥70	17323 (18.3)	6793 (18.3)	10530 (19.2)	
Estimated GFR (ml/min per 1.73 m^2)				
≥90	25258 (26.7)	10709 (26.9)	14549 (26.5)	
60–89	54042 (57.1)	24100 (60.6)	29942 (54.1)	
45–59	13287 (14.0)	4360 (11.0)	8927 (16.3)	
30–44	1829 (1.9)	524 (1.3)	1305 (2.4)	
15–29	151 (0.2)	47 (0.1)	104 (0.2)	
<15	35 (0.04)	14 (0.04)	21 (0.04)	

SBP systolic blood pressure, *DBP* diastolic blood pressure, *FPG* fasting plasma glucose

Table 1. The prevalence of subjects aged 60 years or older was approximately 40%, which included about 20% of subjects 70 years or older (both genders). Male subjects were younger overall, but had a higher prevalence of diabetes, hypertension, and proteinuria than did female subjects. The prevalence of eGFR less than 60 ml/min per 1.73 m^2 was about 16%. The distribution of eGFR according to gender is shown in Fig. 1. As expected, the prevalence of anemia in women increased from 4.4% to 7.3% when the JSDT anemia criteria were applied; consequently the overall prevalence was 7.4% in overall subjects.

Relationship between kidney function and hematocrit

Table 2 shows the mean hematocrit levels and prevalence of anemia according to BMI category, age category, and eGFR category for men and women. The lower the BMI category or the higher the age category, the lower the mean hematocrit level and the greater the prevalence of anemia. At age 70 years, the prevalence of anemia was clearly high. The mean hematocrit levels decreased and the prevalence of anemia increased as kidney function decreased below an

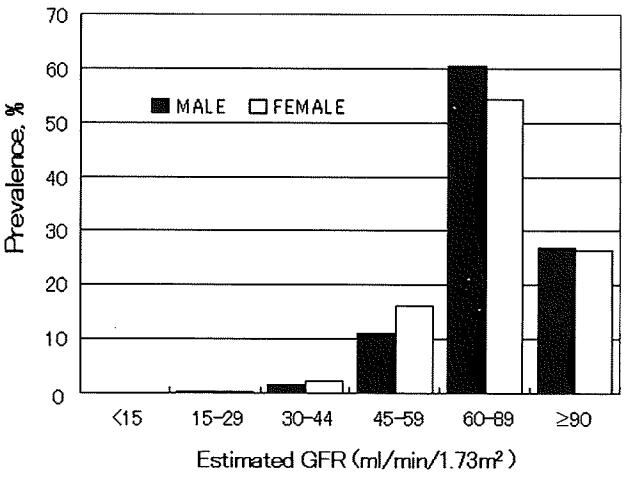

Fig. 1 Distribution of the estimated glomerular filtration rate in the cohort

eGFR of 60 ml/min per 1.73 m^2 among both men and women. In women, prevalence of anemia was 4.7% (age 20–29 years), 12.4% (age 30–39 years), 14% (age 40–49 years), 10.5% (eGFR ≥90 ml/min per 1.73 m^2), 5.7%