Novel haemodialysis (HD) treatment employing molecular hydrogen (H₂)-enriched dialysis solution improves prognosis of chronic dialysis patients: A prospective observational study

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Recent studies have revealed unique biological characteristics of molecular hydrogen (H₂) as an anti-inflammatory agent. We developed a novel haemodialysis (E-HD) system delivering an H₂ (30–80 ppb)-enriched dialysis solution by water electrolysis, and conducted a non-randomized, non-blinded, prospective observational study exploring its clinical impact. Prevalent chronic HD patients were allocated to either the E-HD (n = 161) group or the conventional HD (C-HD: n = 148) group, and received the respective HD treatments during the study. The primary endpoint was a composite of all-cause mortality and development of non-lethal cardio-cerebrovascular events (cardiac disease, apoplexy, and leg amputation due to peripheral artery disease). During the 3.28-year mean observation period, there were no differences in dialysis parameters between the two groups; however, post-dialysis hypertension was ameliorated with significant reductions in antihypertensive agents in the E-HD patients. There were 91 events (50 in the C-HD group and 41 in the E-HD group). Multivariate analysis of the Cox proportional hazards model revealed E-HD as an independent significant factor for the primary endpoint (hazard ratio 0.59; [95% confidence interval: 0.38–0.92]) after adjusting for confounding factors (age, cardiovascular disease history, serum albumin, and C-reactive protein). HD applying an H₂-dissolved HD solution could improve the prognosis of chronic HD patients.

The combination of enhanced oxidative stress and inflammation in patients on chronic haemodialysis (HD) treatment plays a crucial role in the occurrence of excessive cardiovascular events and death¹,². The bio-incompatibility of the HD procedure is supposed to be involved with this pathology. HD may exaggerate leukocyte activation and injury¹³–⁵, which enhance oxidative stress and inflammation. Therefore, we hypothesized that ameliorating the stress to leukocytes during HD may have a beneficial effect on patient outcomes.

Molecular hydrogen (H₂) is an inert gas with no known side effects. Recent studies have shown that H₂ acts as an antioxidant and an anti-inflammatory agent, and ameliorates cellular and organ damage⁶,⁷. We therefore

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developed a novel HD system using highly dissolved H₂ water rendered by the water electrolysis technique⁸–¹⁰. Previous pilot studies, including ours, have reported that suppression of interleukin-6, high-sensitivity C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1)/chemokine (C-C motif) ligand 2 (CCL2), and myeloperoxidase (MPO), decrease oxidative injury of lymphocytes, improve the redox status of serum albumin, and ameliorate hypertension⁸–¹⁴. In reference to these findings, we conducted a non-randomized, non-blinded, prospective observational study to compare the outcomes between patients receiving haemodialysis using an H₂-enriched dialysis solution (E-HD group) and patients receiving conventional haemodialysis (C-HD group).

Results

Patient registration and characteristics. Patients were recruited during April 2011 and October 2012. Of the 327 prevalent chronic HD patients who were pre-registered, 18 were excluded because of the lack of data and withdrawal. Ultimately, 148 patients were allocated to the C-HD group and 161 patients were allocated to the E-HD group (Fig. 1). The patients’ characteristics in the two groups at baseline are shown in Table 1. All subjects were treated by the standard HD schedule (three sessions/week, 4–5 h/session), using high-performance biocompatible dialyzers with fixed blood flow rate (QB) (200 mL/min) and dialysate flow rate (QD) (500 mL/min). Patients who had been treated by a vitamin-E coated dialyzer were excluded from this study. At baseline, there was no statistical difference between the groups in the blood urea nitrogen (BUN) reduction rate (69.7 ± 6.9% in the C-HD group and 70.3 ± 8.4% in the E-HD group; p = 0.485).

Changes in laboratory and subjective/objective parameters during the study. HD-related laboratory parameters at the time of the first HD session of the respective weeks are shown in Table 2. No differences were noted between the two groups during the study period. Regarding subjective symptoms, there was a significant difference in the grade of pruritus between the two groups at baseline (with more severe symptoms in the E-HD group); however, no differences were found during the course of the study. Small but significant differences were noted between the two groups in the fatigue grade (fewer symptoms in the E-HD group) at 48 weeks. No differences were observed in the time-course pre-dialysis blood pressures (BPs); however, post-dialysis BPs differed between the two groups. In sub-analysis of the post-dialysis systolic BP (SBP) levels at baseline, there were significant differences in post-dialysis SBP (6 months) and Defined Daily Dose¹⁵ of antihypertensive medication (6, 12, 18 months) in patients with post-dialysis SBP ≥ 140 mmHg at baseline, while no statistical differences were found in those parameters in patients with post-dialysis SBP < 140 mmHg (Fig. 2).

Composite events summary and multivariate analysis of risk factors for events. During the mean observation period of 3.28 years, there were 91 events: 50 in the C-HD group and 41 in the E-HD group.
E-HD and those on C-HD, reflects the influence of H2 during HD.

Thus, the different clinical profiles between the two groups, patients on mortality and development of non-lethal cardio-cerebrovascular events. In the study, all HD systems employed results revealed E-HD as an independent significant factor for reducing the risk of the primary events of all-cause mortality. We speculate that the primary mechanism of BP reduction could not be attributed to changes in fluid volume, since there were no significant differences in body weight after HD. Rather, the primary mechanism of BP reduction might be related to vasodilation or to a reduction in vascular resistance. Recent studies in deoxycorticosterone acetate (DOCA)-salt hypertension have revealed a crucial role of superoxide anion release from macrophages in mesenteric peri-arteries, due in part to impaired function of the Alpha 2-adrenergic autoreceptors, which provide negative feedback on the release of norepinephrine from the sympathetic nerves associated with the blood during treatment, as reported elsewhere. During the mean observation period of 3.28 years, the study provided negative feedback on the release of norepinephrine from the sympathetic nerves associated with the blood during treatment, as reported elsewhere.

### Table 1. Patient characteristics. C-HD, conventional haemodialysis; E-HD, electrolyzed water haemodialysis.

| Characteristic                  | C-HD          | E-HD          | P Value |
|--------------------------------|---------------|---------------|---------|
| N                              | 148           | 161           | —       |
| Age (y)                        | 67.4 ± 11.8   | 64.0 ± 11.9   | <0.05   |
| Gender, male (%)               | 92 (62.2)     | 85 (52.8)     | NS      |
| Dialysis vintage (months)      | 60 (3, 263)   | 80 (2, 478)   | <0.01   |
| Cause of renal failure (DM, (%)) | 62 (41.9)    | 55 (34.2)     | NS      |
| Patients with CVD history (%)  | 36 (24.3)     | 53 (32.9)     | NS      |
| with multiple CVDs (%)         | 5 (3.4)       | 10 (6.2)      | NS      |
| with cardiac disease (%)       | 25 (16.9)     | 31 (19.3)     | NS      |
| with apoplexy (%)              | 11 (7.4)      | 29 (18.0)     | <0.01   |
| with PAD (%)                   | 5 (3.4%)      | 3 (1.9%)      | NS      |
| Body weight (pre HD, kg)       | 59.3 ± 12.0   | 58.9 ± 11.2   | NS      |
| Body weight (post HD, kg)      | 57.0 ± 11.7   | 56.3 ± 10.9   | NS      |
| CTR (%)                        | 48.7 ± 6.0    | 48.7 ± 5.5    | NS      |
| Pre-dialysis SBP (mmHg)        | 154 ± 27      | 154 ± 25      | NS      |
| Pre-dialysis DBP (mmHg)        | 79 ± 15       | 80 ± 16       | NS      |
| Post-dialysis SBP (mmHg)       | 142 ± 24      | 135 ± 24      | <0.05   |
| Post-dialysis DBP (mmHg)       | 75 ± 14       | 73 ± 14       | NS      |
| Patients on Anti-hypertensive agents (%) | 108 (73.0) | 107 (66.5) | NS      |
| Patients with ESA (%)          | 124 (83.8)    | 140 (87.0)    | NS      |
| Fatigue Grade                  | 2.9 ± 1.0     | 2.9 ± 1.1     | NS      |
| Pruritis Intensity Grade       | 3.4 ± 0.9     | 3.2 ± 0.9     | <0.05   |
| Pruritis Frequency Grade       | 5.2 ± 1.0     | 3.0 ± 1.1     | NS      |

### Discussion

This prospective observational study primarily aimed to examine the clinical effects of the addition of H2 to HD dialyse (an average of 30–80 ppb of H2), which was delivered continuously through the dialyzer membrane to the blood during treatment, as reported elsewhere. During the mean observation period of 3.28 years, the study results revealed E-HD as an independent significant factor for reducing the risk of the primary events of all-cause mortality and development of non-lethal cardio-cerebrovascular events. In the study, all HD systems employed an endotoxin-eliminating filter system. Thus, the different clinical profiles between the two groups, patients on E-HD and those on C-HD, reflects the influence of H2 during HD.

The mechanisms by which E-HD delivers clinical benefits remain to be elucidated, since there were no differences in dialysis-related clinically relevant parameters between the two groups during the study. However, we could speculate several possibilities. The observation that the amelioration of post-dialysis hypertension (SBP ≥ 140 mmHg) in E-HD patients may suggest an idea to elucidate the benefits of E-HD, because intra-dialysis systolic hypertension, as well as high SBP, are well-known risk factors for all-cause mortality in HD patients. On the other hand, low SBP (<110 mmHg) has also been reported as a risk for excessive mortality. Interestingly enough, there were no differences during the course of the study in post-dialysis SBP levels among the patients with SBP < 140 mmHg at baseline (Fig. 2). Furthermore, there were no differences between the two groups in the proportion of patients with SBP < 110 mmHg (Supplementary Fig. S1). Thus, taken together the observations, the improved post-dialysis BP may have played a role, at least partially, for the better outcomes in patients with post-dialysis hypertension.

Other possible mechanisms could be suggested in the previous studies, i.e., increased reduced albumin redox status by acute as well as long-term E-HD, improved patients' anti-oxidative capacity, amelioration of micro inflammation with reduction of pro-inflammatory cytokines, and suppression of T-cell damage. These possible mechanisms need to be clarified in the context of patients' clinical outcomes in the future.

The mitigating effect on elevated SBP, as observed in the present study and previous studies, is very unique. We speculate that the primary mechanism of BP reduction could not be attributed to changes in fluid volume, since there were no significant differences in body weight after HD. Rather, the primary mechanism of BP reduction might be related to vasodilation or to a reduction in vascular resistance. Recent studies in deoxycorticosterone acetate (DOCA)-salt hypertension have revealed a crucial role of superoxide anion release from macrophages in mesenteric peri-arteries, due in part to impaired function of the Alpha 2-adrenergic autoreceptors, which provide negative feedback on the release of norepinephrine from the sympathetic nerves associated with the blood during treatment, as reported elsewhere.
| Months     | 0m       | 6m       | 12m      | 18m      | 24m      | 30m      | 36m      | 42m      | 48m      |
|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| WBC count (µL) |
| (n)  | 148     | 160      | 152      | 145      | 131      | 123      | 121      | 112      | 105      |
| Hemoglobin (g/dL) |
| (n)  | 161     | 159      | 152      | 145      | 131      | 123      | 121      | 112      | 105      |
| BUN (mg/dL) |
| (n)  | 148     | 136      | 128      | 126      | 117      | 109      | 104      | 84       | 80       |
| creatinine (mg/dL) |
| (n)  | 161     | 160      | 152      | 145      | 131      | 123      | 121      | 112      | 105      |
| Ca (mg/dL) |
| (n)  | 148     | 136      | 128      | 126      | 117      | 109      | 104      | 84       | 80       |
| Pi (mg/dL) |
| (n)  | 161     | 159      | 152      | 145      | 131      | 123      | 121      | 112      | 105      |
| B2-microglobulin (mg/L) |
| (n)  | 148     | 135      | 128      | 126      | 117      | 109      | 104      | 84       | 80       |
| CRP (mg/dL) |
| (n)  | 161     | 160      | 152      | 145      | 131      | 123      | 121      | 112      | 105      |
| albumin (g/dL) |
| (n)  | 161     | 159      | 152      | 145      | 131      | 123      | 121      | 112      | 107      |
| Dry weight (kg) |
| (n)  | 148     | 133      | 128      | 126      | 116      | 108      | 102      | 80       | 78       |
| CTR (%) |
| (n)  | 161     | 155      | 148      | 133      | 129      | 123      | 119      | 108      | 101      |
| pre-dialysis MBP (mmHg) |
| (n)  | 148     | 137      | 121      | 112      | 101      | 88       | 78       | 66       | 62       |
| post-dialysis MBP (mmHg) |
| (n)  | 161     | 163      | 152      | 146      | 131      | 125      | 120      | 115      | 105      |

Continued
mesenteric arteries. The mesenteric arteries constitute a major resistance arterial bed for BP regulation. In addition, one fourth of the systemic blood volume is present in the splanchnic circulation. Therefore, an increase in arteriolar resistance will elevate the arterial BP, and an increase in the mesenteric venmotor tone will lead to an increase in the cardiac venous return and the cardiac load due to a decrease in the venous capacity.

| Months | 0m   | 6m   | 12m  | 18m  | 24m  | 30m  | 36m  | 42m  | 48m  |
|--------|------|------|------|------|------|------|------|------|------|
| C-HD   | 1.04 | 1.03 | 1.00 | 1.00 | 1.22 | 1.36 | 1.34 | 1.12 | 1.00 |
| E-HD   | 0.57 | 0.57 | 0.57*| 0.57*| 0.57*| 0.57*| 0.57*| 0.57*| 0.57*|
| (n)    | 147  | 137  | 130  | 127  | 118  | 112  | 105  | 105  | 105  |
| Table 2. Dialysis-related and subjective/objective parameters in the two groups. vs. C-HD; *p < 0.05, **p < 0.01 MBP, mean blood pressure; CTR, cardiothoracic ratio; DDD, defined daily dose of anti-hypertensive agents. C-HD, conventional haemodialysis; E-HD, electrolyzed water haemodialysis; WBC, white blood cell; BUN, blood urea nitrogen; Ca, serum Calcium; Pi, serum phosphate; CRP, C-reactive protein.

Figure 2. Changes in post-dialysis systolic blood pressure, and prescription of antihypertensive agents during the study. Patients with post-dialysis SBP ≥ 140 mmHg (n = 139) at baseline (0 month): changes in post-dialysis SBP (a), and changes in DDD (b); Patients with post-dialysis SBP < 140 mmHg (n = 168) at baseline: changes in post-dialysis SBP (c), and changes in DDD (d). Abbreviations: C-HD, conventional haemodialysis; E-HD, electrolyzed water haemodialysis; SBP, systolic blood pressure; DDD, daily defined dose of antihypertensive agents. (a,c) There were significant differences in post-dialysis SBP (6 months; p < 0.05), and DDD (6, 12, 18 months; p < 0.05, respectively) between the two groups. (b,d) No differences were observed in post-dialysis SBP or DDD between the two groups.
combination of these two pathological processes results in a severe cardiac load. Interestingly, a recent study showed that the chemokine (C-C motif) receptor type 2 blockade suppresses vascular macrophage infiltration and reduces blood pressure. Upon the observation that MCP-1 decreased in E-HD patients in the previous study, it is possible to speculate the possible action of E-HD on macrophage of patients. The question of whether the HD procedure activates the residential macrophages, or activates extrinsic macrophages to infiltrate the mesenteric vascular area, needs to be addressed.

There are several issues and limitations in this study. First, the observed results in the E-HD group were slightly complicated, i.e., the rate of the primary composite endpoint was lower in the E-HD group than in the C-HD group, although the rate of death was not different between the groups. In univariate analysis of the Cox proportional hazards model, E-HD was not a strong factor for the primary endpoint, although multivariate analysis showed E-HD to be a strong factor after adjusting for confounding factors. Regarding the reasons for this, we speculate that a potential bias existed in the patients who were allocated to the E-HD group in that these patients had a relatively higher incidence of CVD history. This may have influenced the results of the univariate analysis, since the presence of a CVD history was the most influential risk factor for the occurrence of the primary endpoint. To clarify this point, we performed a sub-analysis on this profile according to the presence or absence of CVD history. And it was revealed that E-HD was a significant factor for reducing the risk of primary

| C-HD     | E-HD     |
|----------|----------|
| Observation vintage (patient-year) | 467 | 544 |
| Number of Primary events | 50 | 41 |
| (all causes of deaths and non-lethal CVD events) | 29 | 20 |
| Cardiac events including death | 11 | 8 |
| Congestive heart failure | 13 | 9 |
| Ischemic heart disease | 1 | 1 |
| Sudden cardiac arrest | 4 | 2 |
| Apoplexy including death (bleeding/infarction) | 6 (1/5) | 10 (2/8) |
| PAD including death | 8 | 2 |
| Primary events rate (1000 patients-year: 95%CI) | 107.1 (81.2–141.1) | 73.4 (55.6–102.2) |
| Number of deaths | 17 | 20 |
| Deaths rate (1000 patients-year: 95%CI) | 36.4 (22.7–58.3) | 36.8 (23.8–56.8) |

Table 3. Summary of events in the two groups. C-HD, conventional haemodialysis; E-HD, electrolyzed water haemodialysis. PAD, peripheral artery disease (with surgical procedure).

Figure 3. Cox proportional hazards model demonstrating events-free differences between patients on C-HD and those on E-HD. Treatment with E-HD was an independent predicting factor for events (hazard ratio: 0.593; p < 0.05). Abbreviations: C-HD, conventional haemodialysis; E-HD, electrolyzed water haemodialysis.
that there are generation of H\textsubscript{2} in average of 24 ml/min in healthy human (approximately over 15 mmol daily) in

water haemodialysis; HD, haemodialysis; CVD, cardio-cerebral vascular disease; DM, diabetes mellitus; BMI, body mass index; Pre SBP, pre-dialysis systolic blood pressure; CRP, C-reactive protein; Hg, hemoglobin.

unknown whether the applied H\textsubscript{2} levels were best in regards to provide clinical effects, and higher levels of H\textsubscript{2} may

The study used a non-randomized, non-blinded, prospective observational study was conducted to evaluate the clinical impact of the E-HD system (UMIN-ICDR Clinical Trial: Study Title: “Prospective observational study of the clinical effect of haemodialysis using electrolyzed water”; Unique ID issued by UMIN: UMIN000004857, Date of disclosure of the study information: 2011/01/11, Link to view the page (ICDR): https://upload.umin.ac.jp/cgi-bin/icdr_e/ctr_view.cgi?recptno=R000005491).

The primary composite endpoints included all-cause mortality, and concomitant disease such as cardiac disease (heart failure or myocardial infarction requiring hospitalization, coronary artery disease requiring invasive therapy), stroke (symptomatic cerebral hemorrhage or cerebral infarction confirmed by diagnostic imaging), and obstructive arteriosclerosis requiring leg amputation.

The study used a non-randomized design, and the candidate patients were selected by decision of the patient's physician. In two centers (KH and NMH), candidates for the E-HD group were selected by chief physicians; subsequently, matched control patients in the C-HD group were selected from the rest of the patients in the respective centers in terms of demographic background such as age and sex. In two of the study centers (HMC and HHC), all patients were selected for the E-HD group since the centers were to employ a central E-HD system to completely replace the conventional HD system. In three study centers in which the E-HD group was not available (NH, TJJC, GJC), more than one patient was selected as part of the matched control group to the E-HD group of the above four centers in terms of age and sex as much as possible. Patients who were receiving on-line hemodiafiltration or combination therapy with peritoneal dialysis, and potential subjects with serious disease at the time of enrollment, i.e., severe heart failure (New York Heart Association III/IV), severe liver disease, psychological problems, dementia, malignant disease within the previous 3 months, or an evidently poor systemic condition with an evidently poor short-term prognosis, were excluded from this study. History of CVD included cardiac disease, stroke (these definitions were comparable to those of the primary composite endpoints mentioned above), and symptomatic peripheral arterial disease requiring medical intervention.

| Univariate HR | 95%CI | P value | Multivariate HR | 95%CI | P value |
|---------------|-------|---------|----------------|-------|---------|
| E-HD          | 0.687 | 0.454-1.039 | 0.077 | 0.593 | 0.384-0.916 | 0.019 |
| HD vintage    | 1.000 | 0.997-1.002 | 0.824 |       |         |       |
| Age           | 1.036 | 1.017-1.055 | 0.000 | 1.014 | 0.993-1.036 | 0.183 |
| Gender (female) | 0.698 | 0.454-1.074 | 0.102 |       |         |       |
| History of CVD | 3.085 | 2.040-4.665 | 0.000 | 3.037 | 1.977-4.665 | 0.000 |
| non DM        | 0.865 | 0.569-1.314 | 0.497 |       |         |       |
| BMI           | 0.987 | 0.933-1.044 | 0.644 |       |         |       |
| Pre SBP       | 0.999 | 0.990-1.007 | 0.783 |       |         |       |
| Albumin       | 0.195 | 0.101-0.377 | 0.000 | 0.328 | 0.160-0.674 | 0.002 |
| CRP           | 1.266 | 1.017-1.576 | 0.035 | 1.323 | 1.005-1.740 | 0.046 |
| Hg            | 0.893 | 0.741-1.075 | 0.230 |       |         |       |

Table 4. Cox proportional hazards model analysis for the composite primary endpoints. E-HD, electrolyzed water haemodialysis; HD, haemodialysis; CVD, cardio-cerebral vascular disease; DM, diabetes mellitus; BMI, body mass index; Pre SBP, pre-dialysis systolic blood pressure; CRP, C-reactive protein; Hg, hemoglobin.
The study was approved by the Ethics Committee of Fukushima Medical University (No. 1155: Supplementary file of study protocol), and the clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all patients registered.

**Data collection.** All patients were monitored for subjective symptoms and objective signs during the study period. Blood pressure was measured using a sphygmomanometer on the upper arm with the patient in a supine position just before starting each HD session, and data were recorded into the clinical record. Iron, erythropoiesis-stimulating agents (ESA) to correct anemia, and agents to control calcium and phosphate were administered according to the guidelines of the Japanese Society of Dialysis Treatment. Antihypertensive agents and adjustment of body weight after HD (dry weight) were administered as needed by the attending physician. Quantities of antihypertensive agents were standardized using DDD. Regular monitoring of blood was performed at the first HD session of the week (Monday or Tuesday) at least once a month to monitor dialysis status. Patients were requested to fill out a self-assessment questionnaire every 6 months, which asked about the subjective symptoms of fatigue on the HD day and pruritus according to the following criteria: Fatigue (subjective level and daily activities)—Grade 1: Intense fatigue/Disturbed activity and required rest; Grade 2: Moderate fatigue/Reduced activity; Grade 3: Mild fatigue/Normal activity; Grade 4: Tireless/Normal activity; Grade 5: Inexhaustible/Active; Pruritus (subjective intensity and frequency)—Grade 1: Intense/Always; Grade 2: Moderate/Sometimes; Grade 3: Mild/Rarely; Grade 4: None/None. Levels of H₂ were determined using the gas chromatograph with a semiconductor detector (TRAlgizer mBA-3000, Taiyo Instruments Co., Osaka, Japan) according to the manufacturer’s instruction, as reported elsewhere.

All data generated or analysed during this study are included in this published article.

**Statistical Methods.** The target sample size of the original study (n = 70 < each) was based on an estimated event-free rate of 10% differences at 3 years between groups with 1:1 ratio between them, and calculated from the rationale that a statistical power of 90% and the alpha level 0.05, using a two-sided log-rank test.

All values are expressed as the mean ± standard deviation (SD) or median (interquartile range) as appropriate. For comparisons between the two groups, Student’s t-test or the Mann-Whitney U test was used for continuous variables and chi-square test or Fisher’s exact test was used for nominal variable, as appropriate. Values of p < 0.05 were considered statistically significant. Data were statistically analyzed using IBM SPSS Statistics version 22.0 for Windows (Chicago, IL, USA).

**H₂ delivery HD system.** Figure 4 details of the system have been reported previously. Briefly, test solutions were prepared as follows: pre-filtered water was processed using activated charcoal filtration and water softening to supply the HD-24K water electrolysis system (Nihon Trim, Osaka, Japan), where water was electrolyzed by direct current supply to the anode and cathode electrode plates. Water on the anode side was drained, and water from the cathode side (electrolyzed water) was collected to supply the reverse osmosis equipment (MH500CX; Japan Water System, Tokyo, Japan) at 500 mL/min. The intensity of electrolysis was adjusted to maintain a pH of 10.0. The reverse osmosis water produced by the water electrolysis system was supplied to prepare...
the HD solution. The composition of the inflow H₂-HD solution was the same as the standard HD solution with the exception of the presence of dissolved H₂ in the H₂-HD, and there were no differences in terms of electrolytes levels and pH, as compared to the standard HD solution, as reported elsewhere.11. Whereas regarding H₂ levels of control group, dialyze and blood H₂ levels were less than 1 ppb.

The present E-HD system could deliver H₂ (30–80 ppb)-enriched dialysis solution. H₂ levels of inflow blood and HD solution reached an equivalent state in the dialyzer, and the H₂ level of outflow blood from dialyzer showed approximately the same as that of inflow H₂-HD solution under QB 200 ml/min and QD 500 ml/min. Therefore, H₂ load to patient is determined by time of HD treatment and H₂ levels of HD solution if QB and QD are fixed, i.e., it is estimated that about 1.2 mmol of H₂ is loaded in case of 4-hour treatment, and HD solution with 50 ppb H₂. Regarding the H₂ dynamics in the body, previous studies revealed that no changes were found in H₂ levels of inflow blood after 4-hour treatment, and there were increases of constant H₂ levels in expired air of patients by treatment, and they soon returned to the basal levels by stop of treatment. Therefore, it is supposed that delivered H₂ into blood during the HD treatment is mostly excreted from lung during the time on HD.

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Author Contributions
M.N. wrote the main manuscript text and prepared all figures. M.N., I.S., K.R., M.M. and S.I. organized the study group. N.I., H.S., H.H., R.Y., K.T., N.O. and H.N. collected data. M.N. and Y.M. analyzed the data. M.N. and S.I. supervise the progress of research of all aspects.
**Additional Information**

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