ORIGINAL ARTICE

The Effects of Alkaline Reduced Water Administration to the Fasting Blood Glucose Levels in Patients with Type 2 Diabetes Mellitus

SATRIO ADI WICAKSONO[1], DINA HISAN NABYLA[1], SULISTIYATI BAYU UTAMI[2]
[1]*Department of Anesthesiology and Intensive Care, Faculty of Medicine, Diponegoro University, Semarang*
[2]*Department of Cardiology and Vascular Medicine, Faculty of Medicine, Diponegoro University, Semarang*
Correspondence to Satrio Adi Wicaksono, Email: drsaw11@yahoo.com, *Telephone/Fax . +62 (0) 24-76928010.*

ABSTRACT

Background: Alkaline reduced water (ARW) is an electrolyzed water with pH>7. It showed an antioxidant and an antidiabetic benefit, but the results still vary.
Aim: To investigate the effect of ARW to fasting blood glucose (FBG) and two-hours post prandial glucose (2hPPG) in Indonesian patients with type-2 diabetes mellitus (T2DM).
Methods: This was a randomized double blind controlled trial performed in September 2017- September 2018. Thirty patients that had been diagnosed with T2DM in one hospital in Semarang and met the inclussion criteria were determined by consecutive sampling. Subjects were randomly divided into two groups: ARW group (pH 9) (n=15) and control group (mineral water, OMW) (pH 7) (n=15). Both were administered orally 1 liter per day for 12 days; FBG and 2hPPG were measured before and after 12 days treatment.
Results: There were no differences in FBG and 2hPPG at pre-treatment, in 2hPPG at post-treatment, and in decrease beween pre-and post-treatment 2hPPG (∆2hPPG) between ARW group and OMW group. At post-treatment, there was a lower FBG in ARW group compared to OMW group, although it was not significant (214.8±12.66 mg/dL vs 225.1±15.44 mg/dL, *p*=0.056). There was a wider decrease of FBG (∆FBG) in ARW group compared to OMW group (19.4±1.68 mg/dL vs 14.3±3.64 mg/dL, *p*=0.000).
Conclusion: ARW decreased FBG in patients with T2DM yet 2hPPG. These findings might have important implications for the management of T2DM.
Keywords: alkaline reduced water, fasting blood glucose levels, type-2 diabetes mellitus, T2DM

INTRODUCTION

World Health Organization (WHO) Global Report on diabetes mellitus (DM) in 2016 estimated that there was 422 million adults in the world population were living with DM in 2014, compared to 108 million in 1980. The global prevalence of DM has doubled since 1980, rising from 4.7% to 8.5% in the adult population[1]. International Diabetes Federation (IDF) in 2015 estimated that the world numbers of patients with DM in 2015 were 415 million and were expected to increase until 642 million in year 2040.[2] This reflected an increase in associated risk factors including overweight or obese. Diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries in the past decade, including in Indonesia.[1] Indonesian Basic Health Research (Riset Kesehatan Dasar, RISKESDAS) in 2013 showed that the prevalence of DM in Indonesia was 1.5–2.1%[3].

Type 2 diabetes mellitus (T2DM) is a metabolic disorder with several etiologies represented with chronic hyperglycemia and disorders of carbohydrate, lipid, and protein metabolism that is related to insulin resistence in which its progressivity may also impact on insulin secretion defect or their combination. Hyperglycemia has been one of pivot diagnosis criteria of T2DM represented by fasting blood glucose and post prandial glucose[4]. In some patients, optimal glucose level is often difficult to be obtained although under optimal dose of oral anti-diabetic agents.

Type 2 DM (T2DM) may bring acute and chronic complications. Overall, DM have caused 1.5 million deaths in 2012, and an additional 2.2 million deaths have occured in a higher-than-optimal blood glucose, by increasing the risks of cardiovascular and other diseases[1]. Its chronic complications include microangiopathy such as diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, and macroangiopathy such as atherosclerotic cardiovascular disease (ASCVD), stroke or cerebrovascular disease (CVD), and peripheral arterial disease (PAD)[5].

Studies showed that the increased of extracellular and intracellular glucose concentration might lead to oxidative stress that played role in the pathogenesis of onset, progressivity and complication of T2DM[6-8]. Oxidative stress is related to the onset of T2DM through insulin resistance.[9] The source of oxidative stress came from reactive oxygen species (ROS) leaking from mitochondria[10,11]. Several antioxidants have been studied to handle or prevent oxidative stress in T2DM[12,13]. However some studies with antioxidant supplements were failed to prevent oxidative stress-related diseases[14].

Alkaline reduced water, an electrolysis-treated water, that can increase its reduction potential, is a promising solution in providing a safe source of free electrons to block the oxidation of normal tissue by free oxygen radicals. Some studies revealed that hydrogen (H_2) molecule showed some therapeutic effects by acting as new antioxidant.[15,16] Reactive hydrogen was also thought as ideal scavanger of ROS in T2DM[17]. However previous studies revealed vary interpretations in the effects of alkaline reduced water to the blood glucose levels[18-20].

In Indonesia, electrochemically alkaline reduced water have been freely marketed and consumed, however there was still lack of study to prove its clinical effects in patients with T2DM in Indonesia. The effects of alkaline reduced water in Indonesian patients with T2DM might differ from other countries since they could be influenced by race, genetic, or socioeconomic factors.

MATERIAL AND METHODS

Study Design and Population: This study was a randomized double blind controlled trial conducted to investigate the effects of alkaline reduced water administration on fasting and two hours post prandial blood glucose levels in patients with T2DM. The study participants comprised 30 patients that had been diagnosed with T2DM. Subjects were determined by consecutive sampling that met the inclusion criteria, namely Indonesian men and women, aged from 20 to 80 years old, undergoing outpatient care at one teaching hospital in Semarang, Indonesia, between September 2017 to September 2018 and had been diagnosed with T2DM. The exclusion criteria were insulin therapy, cardiovascular diseases, diabetic ulcer, chronic kidney disease, anemia, cancer or hematologic malignancies, lung tuberculosis, previously known autoimmune diseases, sepsis, increased AST/ALT and/or creatinine of three times above normal value and major physical or mental disabilities.

Participants were randomly divided into two groups: treatment group who received alkaline reduced water with pH 9 (n=15) and control group who received placebo (ordinary mineral water) with neutral pH of 7 (n=15). Both alkaline reduced water or placebo was administered orally ad libitum with maximun of 1 liter per day for 12 days. Placebo was made to have the same appearance with alkaline reduced water. Electrochemically alkaline reduced water showed an alkaline pH 9 and was produced with electrolysis machine (Leveluk® SD 501, Japan). It was examined for its alkaline pH of 9 before distributed to the participant of study.

Clinical and Laboratory Measurements: Study data included demography and medical history, physical examination, information provided by questionnaire, anthropometric measurements and laboratory measurements. The medical and drug prescription history was assessed by the examining physicians.

Diabetes mellitus was defined as either participant had history of fasting serum glucose level ≥126 mg/dl, or serum hemoglobin A1c (HbA1c) level ≥6.5%, or the participant ever having been diagnosed with diabetes, or the current use of blood glucose-lowering agents. Hypertension was defined as either the participant ever having been diagnosed with hypertension or as having a measured blood pressure (BP) ≥140/90 mmHg at initial examination. Trained nurses measured participants' seated BP 3 times using automated equipment (53000-E2, Welch Allyn, NY, USA) after a 5 minutes rest. Final BP was calculated as the average of BP measurements. The body mass index (BMI) was calculated by dividing weight (kilograms) by the square of height (m^2).

Participants who have been fulfilled the inclusion criteria underwent a blood sampling from an antecubital vein at baseline (before treatment) and after 12 days treatment. Serum creatinine were determined using the Jaffe reaction method (Advia 1650 kit, Bayer Corp, PA, USA). Levels of AST and ALT were examined with International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) method without Pyridoxal Phosphate in 37ºC. The serum fasting glucose and 2 hours post prandial glucose were measured by biochemical analyzer with hexokinase assay (Roche Cobas C311, Roche, Germany). All lipid profiles were collected in the fasting state and included total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglyceride levels.

Statistical Analysis: Data of patients with T2DM were collected, sorted, tabulated, coded and processed using descriptive statistical methods. Data were presented as mean±standard deviation for continuous variables and as proportions (n, %) for categorical variables. Categorical variables were presented as frequency distribution tables. The chi-square test was used to determine the differences in proportions for categorical variables. The continuous independent variables were compared using independent *t*-test, if normally distributed, or non-parametric Mann-Whitney test, if not normally distributed. Statistically significance was considered as $p<0.05$. All statistical analyses were performed using statistical computing program.

Ethical Clearance: Ethics approval for the study protocol and analysis of data was obtained from the Ethics Committee in Health and Medical Research (KEPK) Faculty of Medicine, Diponegoro University Semarang, Indonesia (No. 426/EC/FK-RSDK/VII/2017). All participants had been given explanation of the purpose, benefits, research protocols, possible side effects, questionnaire, and a written informed consent.

RESULTS

Characteristics of Participant: Clinical and demographic characteristics of participants are presented in Table 1. As many as 30 participants were recruited in this study consisted of 16(53.3%) male and 14(46.7%) female. There was no difference in gender distribution between alkaline reduced water group with pH 9 and mineral water group with pH 7 ($p=0.464$). The mean age was 52.9±1.06 years old. There was no difference in the mean age between alkaline reduced water group and mineral water group (53.0±1.19 vs 52.8±0.94 years old, $p=0.547$). The mean BMI of study population was 24.0±0.77 kg/m^2, and there was no difference in mean BMI between alkaline reduced water group and mineral water group (23.9±0.68 vs 24.0±0.86 kg/m^2, $p=0.637$). There were no differences in distribution of hypertension ($p=0.309$) and dyslipidemia ($p=0.409$) between both groups (Table 1).

There was no difference in medication history between both groups, including sulfonylureas ($p=0.690$), biguanides ($p=0.409$), angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) ($p=0.715$), ß-blockers ($p=0.068$), calcium channel blockers (CCBs) ($p=0.464$), diuretics ($p=0.666$), and statins ($p=0.409$) (Table 2).

Glucose and Lipid Profiles between Alkaline Reduced Water and Mineral Water: Glucose profile in pre- and post-treatment between both groups are presented in Table 3. At baseline (pre-treatment), there were no differences between alkaline reduced water group and mineral water group in fasting blood glucose (234.2±12.67 mg/dL vs 239.4±16.05 mg/dL, respectively, $p=0.333$) and two hours post prandial glucose (2h PPG) (290.8±12.18 mg/dL vs 289.8±17.84 mg/dL, respectively, $p=0.589$) (Table 3).

At post-treatment, there was a lower fasting blood glucose in the alkaline reduced water group in comparison with in the mineral water group, although it was not significant (214.8±12.66 mg/dL vs 225.1±15.44 mg/dL, respectively, *p*=0.056). There was a wider decrease beween pre- and post-treatment fasting blood glucose (∆FBG) in alkaline reduced water group compared to mineral water group (19.4±1.68 mg/dL vs 14.3±3.64 mg/dL, respectively, *p*=0.000) (Table 3, Figure 1).

At post-treatment, there was no difference in 2h PPG between the alkaline reduced water group and the mineral water group (254.4±15.54mg/dL vs 250.0±17.58mg/dL, *p*=0.474). There was no significant difference of pre- and post-treatment 2h PPG (∆2h PPG) in alkaline reduced water group compared to mineral water group (36.4±14.63mg/dL vs 39.8±13.11mg/dL, *p*=0.534) (Table 3).

There were no differences between alkaline reduced water group and mineral water group in all lipid profiles at before and after treatment, including total cholesterol (pre-treatment: 205.8±17.76 mg/dL vs 203.8±16.20 mg/dL, *p*=0.750, and post-treament: 204.4±15.76 vs 201.1±13.17 mg/dL, *p*=0.535), LDL-C (pre-treatment: 123.8±6.14mg/dL vs 124.0±8.27 mg/dL, *p*=0.921 and post-treatment: 122.8±3.77mg/dL vs 123.4±5.13mg/dL, *p*=0.718), HDL-C (pre-treatment: 41.1±4.92mg/dL vs 38.5±4.61 mg/dL, *p*=0.147 and post-treatment: 41.9±3.75 mg/dL vs 41.2±3.70 mg/dL, *p*=0.416), and triglyceride (pre-treatment: 179.6±3.52mg/dL vs 180.5±3.90mg/dL, *p*=0.498 and post-treatment: 180.0±1.85 mg/dL vs 179.7±3.10 mg/dL, *p*=0.950), respectively.

Table 1: Baseline Characteristics of Study Subjects

Parameter	Group		Total (n=50)	*P*
	Alkaline Reduced Water (n=15)	Mineral Water (n=15)		
Gender Male Female	 7 (23.3%) 8 (26.7%)	 9 (30.0% 6 (20.0%)	 16 (53.3%) 14 (46.7%)	0.464*
Age (months)	53.0±1.19; 53(51– 5.0)	52.8 ± 0.94; 53(51– 55)	52.9 ± 1.06 53.0 (51.0–55.0)	0.547***
Body Weight (kg)	66.8 ± 3.21; 66(60–71.0)	68.7±3.20; 70.0 (62– 73)	67.7 ± 3.29; 68.5 (60.0 – 73.0)	0.122**
Height (cm)	167 ± 2.85;166(163–172)	169±3.08; 169 (164–173)	168.0 ± 3.10;168.5 (163.0 – 173.0)	0.067**
Body Mass Index (BMI) (kg/m^2)	23.9±0.68; 23.9 (22.3–24.9)	24.0 ± 0.86; 24.1(22.7– 25.7)	24.0 ± 0.77; 24.0 (22.3 – 25.7)	0.637**
Hypertension (n, %)	15 (50.0%)	14 (46.7%)	29 (96.7%)	0.309*
Dyslipidemia (n, %)	10 (33.3%)	12 (40.0%)	22 (73.3%)	0.409*
Current smoker	7 (23.3%)	5 (16.7%)	12 (40.0%)	0.456*
Exercise >5 hours/week	3 (10.0%)	4 (13.3%)	7 (23.3%)	0.666*
Systolic Blood Pressure (mmHg)	112.6 ± 5.94; 110.0 (100.0 – 120.0)	112.6 ± 7.98; 10 (100–120)	112.7 ± 6.91; 110.0 (100.0–120.0)	0.856***
Diastolic Blood Pressure (mmHg)	74.7 ± 5.16; 70.0 (70.0 – 80.0)	75.0 ± 5.00; 75.0 (70– 80)	74.8 ± 4.99; 72.5 (70.0 – 80.0)	0.850***
Mean Arterial Pressure (mmHg)	87.3 ± 4.94; 87.0 (80.0 – 93.0)	87.3 ± 4.77; 86 (80– 93)	87.3 ± 4.77; 86.5 (80.0 – 93.0)	0.949***
Heart Rate (beats/min)	74.0 ± 3.77; 74.0 (70.0 – 81.0)	74.7 ± 5.65; 75(64–85)	74.4 ± 4.73; 74.5 (64.0 – 85.0)	0.722***
Hemoglobin (g/dL)	13.1 ± 1.07; 13.3 (11.0 – 14.6)	13.4 ± 0.78; 13.1 (12.1 – 14.9)	13.2 ± 0.93; 13.2 (11.0 – 14.9)	0.370**
Leukocyte (10^3/µL)	9.1 ± 0.70; 9.3 (8.0 – 9.9)	8.6 ± 0.46; 8.5 (8.1 – 9.8)	8.8 ± 0.63; 8.8 (8.0 – 9.9)	0.071***
Platelet (10^3/µL)	267.2 ± 28.72; 275.0 (213.0 – 320.0)	279.0 ± 53.97; 284.0 (173.0 – 415.0)	273.1 ± 42.89; 277.5 (173.0 – 415.0)	0.464**
Ureum (mg/dL)	25.2 ± 4.96; 25.0 (19.0 – 39.0)	25.2 ± 4.64; 26.0 (19.0 – 35.0)	25.2 ± 4.72; 25.0 (19.0 – 39.0)	0.967***
Creatinine (mg/dL)	0.5 ± 0.12; 0.5 (0.4 – 0.8)	0.5 ± 0.07; 0.5 (0.4 – 0.6)	0.5 ± 0.09; 0.5 (0.4 – 0.8)	0.948***

significant if *p*< 0.05. Presented as mean ± SD; median (min – max).
*Chi-square test; **Independent *t*-test; ***Non-parametric Mann-Whitney test.

Table 2. Medication History

Characteristics	Alkaline Reduced Water (n=15)	Mineral Water (n=15)	Total	*P*
Sulfonylureas				
Yes	10 (33.3%)	11 (36.7%)	21 (70.0%)	0.690
No	5 (16.7%)	4 (13.3%)	9 (30.0%)	
Biguanides				
Yes	10 (33.3%)	12 (40.0%)	22 (73.3%)	0.409
No	5 (16.7%)	3 (10.0%)	8 (26.7%)	
ACEi/ARBs				
Yes	8 (26.7%)	7 (23.3%)	15 (50.0%)	0.715
No	7 (23.3%)	8 (26.7%)	15 (50.0%)	

ß-Blockers				
Yes	10 (33.3%)	5 (16.7%)	15 (50.0%)	0.068
No	5 (16.7%)	10 (33.3%)	15 (50.0%)	
CCBs				
Yes	6 (20.0%)	8 (26.7%)	14 (46.7%)	0.464
No	9 (30.0%)	7 (23.3%)	16 (53.3%)	
Diuretics				
Yes	3 (10.0%)	4 (13.3%)	7 (23.3%)	0.666
No	12 (40.0%)	11 (36.7%)	23 (76.7%)	
Statins				
Yes	10 (33.3%)	12 (40.0%)	22 (73.3%)	0.409
No	5 (16.7%)	3 (10.0%)	8 (26.7%)	

significant if $p < 0.05$ using Chi-square test.
ACEi: angiotensin converting enzyme inhibitors; ARBs: angiotensin receptor blockers; CCBs: calcium channel blockers.

Table 3. Blood Glucose Profile between groups

Parameter	Group		*P*
	Alkaline Reduced Water (n=15)	Mineral Water (n=15)	
FBG Pre-treatment (mg/dL)	234.2 ± 12.67; 234.0 (213.0 – 254.0)	239.4 ± 16.05; 234.0 (213.0 – 267.0)	0.333*
FBG Post-treatment (mg/dL)	214.8 ± 12.66; 212.0 (194.0 – 233.0)	225.1 ± 15.44; 221.0 (202.0 – 249.0)	0.056*
∆ FBG	19.4 ± 1.68; 9.0 (16.0 – 22.0)	14.3 ± 3.64; 13.0 (10.0 – 20.0)	0.000[a]*
2h PPG, Pre-treatment (mg/dL)	290.8 ± 12.18; 295.0 (275.0 – 312.0)	289.8 ± 17.84; 286.0 (267.0 – 347.0)	0.589**
2h PPG, Post-treatment (mg/dL)	254.4 ± 15.54; 256.0 (220.0 – 276.0)	250.0 ± 17.58; 249.0 (223.0 – 289.0)	0.474*
∆ 2h PPG	36.4 ± 14.63; 34.0 (18.0 – 57.0)	39.8 ± 13.11; 35.0 (26.0 – 75.0)	0.534**

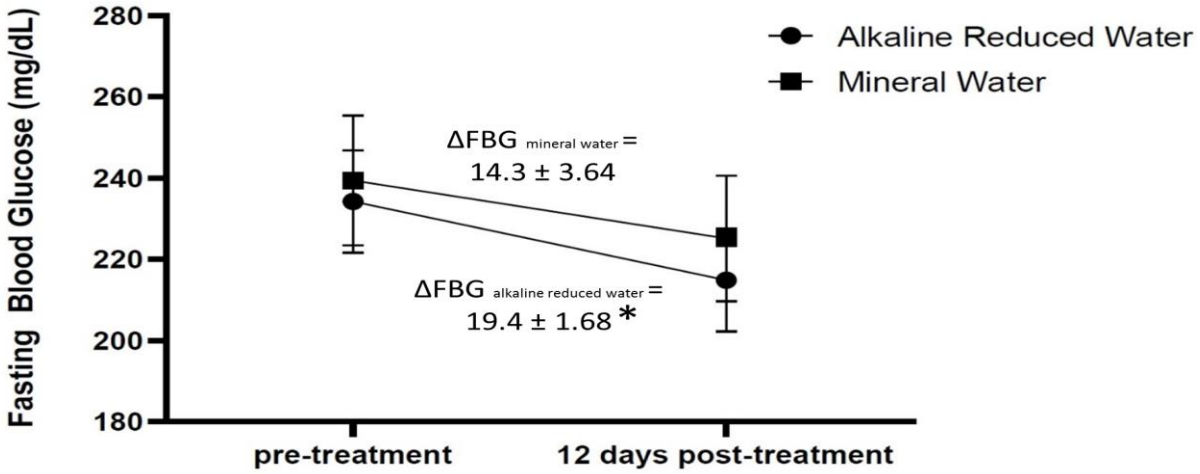
[a]significant if $p < 0.05$. Presented as mean ± SD; median (min – max).
*Independent *t*-test; **Non-parametric Mann-Whitney test.
FBG: fasting blood glucose; 2h PPG: two hours post-prandial glucose.

Table 4. Lipid Profile between groups

Parameter	Group		*P*
	Alkaline Reduced Water (n=15)	Mineral Water (n=15)	
Total cholesterol Pre-treatment (mg/dL)	205.8 ± 17.76; 206.0 (178.0 – 234.0)	203.8 ± 16.20; 201(183.0 – 237.0)	0.750*
Total cholesterol Post-treatment (mg/dL)	204.4 ± 15.76; 210.0 (182.0 – 229.0)	201.1 ± 13.17; 206(175.0 – 225.0)	0.535*
LDL-C Pre-treatment (mg/dL)	123.8 ± 6.14; 125.0 (116.0 – 139.0)	124.0 ± 8.27; 124.0 (110.0 – 141.0)	0.921*
LDL-C Post-treatment (mg/dL)	122.8 ± 3.77; 124.0 (116.0 – 132.0)	123.4 ± 5.13; 123.0 (116.0 – 136.0)	0.718*
HDL-C Pre-treatment (mg/dL)	41.1±4.92; 41.0 (31.0 – 48.0)	38.5 ± 4.61; 39.0 (31.0 – 46.0)	0.147*
HDL-C Post-treatment (mg/dL)	41.9±3.75; 43.0 (32.0 – 46.0)	41.2 ± 3.70; 41.0 (36.0 – 48.0)	0.416**
Triglyceride Pre-treatment (mg/dL)	179.6±3.52; 179.0 (172.0 – 185.0)	180.5 ± 3.90; 182.0 (174.0 – 187.0)	0.498*
Triglyceride Post-treatment (mg/dL)	180.0±1.85; 180.0 (178.0 – 184.0)	179.7 ± 3.10; 180.0 (175.0 – 185.0)	0.950**

significant if $p < 0.05$. Presented as mean ± SD; median (min – max).
*Independent *t*-test; **Non-parametric Mann-Whitney test;
LDL-C : low density lipoprotein cholesterol; HDL-C : high density lipoprotein cholesterol.

Figure 1. The Decrease of Fasting Blood Glucose between Alkaline Reduced Water Group and Control Group



*significant if $p < 0.05$ with Independent *t*-test.
ΔFBG, decrease of fasting blood glucose between pre- and post-treatment.

DISCUSSION

Type 2 diabetes mellitus (T2DM) is a very complex and multifactorial metabolic disease characterized by insulin resistance and β-cell failure leading to elevated blood glucose levels. Hyperglycemia is thought to be correlated to oxidative stress and is the main cause of diabetic complications, which not only decrease life quality and expectancy, but are also becoming a problem regarding the financial burden for health care systems[5,21].

Oxidative stress has an important role in early events relevant for the development of T2DM and in hyperglycemia-induced tissue injury. The formation of advanced glycation end products (AGEs), a group of modified proteins and/or lipids with damaging potential, is one contributing factor in the development and progression of T2DM and their role in diabetic complications. It has been reported that AGEs that are formed endogenously and exogenously, increase ROS or free radicals formation and impair antioxidant systems. Reactive oxygen species are considered to cause extensive oxidative damage to biological macromolecules. Meanwhile, the formation of some AGEs is also induced by oxidative conditions. Thus, AGEs contribute to chronic stress conditions in diabetes[9].

Several studies on electrochemically alkaline reduced water have been developed in Japan. Reduced waters were expected to have role in counter oxidative stress-related diseases such as diabetes. Reduced water was reported to be able to scavenge ROS in cultured cells. It has been suggested that the active agents in reduced water are hydrogen (atoms and molecules), mineral nanoparticles, and mineral nanoparticle hydrides[15].

Our study showed that there was a lower fasting blood glucose in the post-treatment alkaline reduced water group in comparison with in the mineral water group, although it was not significant. Morever, we found a wider decrease of fasting blood glucose (∆FBG) in alkaline reduced water group as many as 19.4±1.68 mg/dL. However, we could not find any difference in 2h PPG and all lipid profiles after alkaline reduced water treatment for 12 days.

Similar to our studies, some studies revealed that hydrogen (H_2) molecule in alkaline reduced water could give therapetic benefits as a new antioxidant. One of the benefits were as anti-diabetic with reduced glucose levels as its indicators[15,16,22]. Other studies also showed similar results of alkaline reduced water to the blood glucose levels[15,19,20,23].

Electrolysed reduced water, Hita Tenryosui water and Nordenau water have been shown to scavange intracellular ROS in a hamster pancreatic β cell line HIT-T15 cells, and accelerate the secretion of insulin.[19,24] The oxidative damage induced by alloxan, a type 1 diabetes inducer, was suppressed by electrolysed reduced water and natural reduced water in cells and in alloxan-induced type 1 diabetes model mice[20].

Electrolysed reduced water, Hita Tenryosui water and Nordenau water scavanged ROS in rat L6 myotube cells and enhanced sugar uptake. Hita Tenryosui water and Nordenau water promoted the phosphorylation of the insulin receptors via suppression of the activity of tyrosine protein phosphatase, which was a redox-sensitive protein, and activate phophatidylinositol-3-kinase (PI-3-kinase) and Akt, as well as promote the translocation of the sugar transport carrier GLUT4 to the cell membrane to promote sugar uptake[15,34]. These waters also alleviate sugar tolerance damage in type 2 diabetes model mice[19,24,25]. Electrolysed reduced water derived from tap water improves the symptoms of diabetes model mice[17].

Insulin that is consist of amino acids series, are secreted by pancreatic beta cells and has role in glucose uptake from blood to the cells. In insulin, there are amino acids that are responsible in transporting glucose that is known as glucose transporter (GLUT). The majority of GLUT that is responsible in glucose absorption in cells is GLUT-4. Gadek *et al*[24] also showed that alkaline reduced water could increase the phosphorilation of insulin receptors via inhibition of the activity of tyrosine phosphatase protein that was a redox-sensitive protein, and activation of PI3K and Akt, as well as help the translocation of GLUT-4 to cell membran so that increased intracellular glucose uptake.

Shirahata *et al.* showed that the ideal scavenger for active oxygen should be active hydrogen. They mentioned that active hydrogen could be produced in reduced water near the cathode during electrolysis of water. Shirahata *et al*. mentioned that, in the electrolysed water, the cations (positive ions) were gathered at the negative cathode of the electrolysis unit to form cathodic water or electrochemically alkaline reduced water[15,23].

Shirahata *et al.* showed that electrolyzed reduced water exhibited high pH (alkaline), low dissolved oxygen, and extremely high dissolved molecular hydrogen. It contained a large amount of active hydrogen (or electrons, e^-) that was produced through the electrolytic promotion liquid. This reduced water had a negative redox potential (ORP) value of approximately -250 mV to -350 mV. It had a large mass of electron ready to donate to electron-thieving active oxygen. Electrolyzed reduced water could scavenge $O^{\cdot -2}$ produced by the hypoxanthine-xanthine oxidase (HX-XOD) system in sodium phosphate buffer (pH 7.0). Moreover, electrolysis in reduced water also made the cluster of H_2O reduced in size from about 10 – 13 molecules per cluster to 5 – 6 molecules per cluster.[15,23] While ordinary water with pH 7 was approximately neutral on the pH scale. The redox potential (ORP) value of the ordinary water was approximately +400 to +500 mV. Because ordinary water had a positive redox potential, it was apt to acquire electrons and oxidize other molecules[15,16,23].

The effects of alkaline reduced water to glucose levels might be correlated to its anti-oxidative role. The SOD-like activity of reduced water was due to the dissolved atomic hydrogen (active hydrogen), but not due to the dissolved molecular hydrogen. The superoxide dismutase (SOD)-like activity of reduced water was stable at 4ºC for over a month and was not lost even after neutralization, repeated freezing and melting, deflation with sonication, vigorous mixing, boiling, repeated filtration, or closed autoclaving, but was lost by opened autoclaving or by closed autoclaving in the presence of tungsten trioxide which efficiently adsorbs active atomic hydrogen[15].

Reduce water suppressed single-strand breakage of DNA caused by ROS produced by the Cu(II)-catalyzed oxidation of ascorbic acid in a dose-dependent manner. Lee *et al.* showed that pre-treatment, co-treatment, and post-treatment with electrolyzed-reduced water enhanced human lymphocyte resistance to the DNA strand breaks induced by H_2O_2 in vitro.[26] It seemed that reduced water could scavenge not only $O2^{\cdot-}$ and H_2O_2, but also O_2 and OH^-. Reduced water decreased the amount of H_2O_2 produced by XOD in an SOD accumulated H_2O_2 in the HX-XOD system[23].

The formation of ROS was thought to cause extensive oxidative damage to biomolecules such as DNA, RNA, and protein. Lee *et al.* studied the preventive, suppressive, and protective effects of in vitro supplementation with electrolyzed-reduced water on H_2O_2-induced DNA damage in human lymphocytes that were examined using a comet assay. Pre-treatment, co-treatment, and post-treatment with electrolyzed-reduced water improved human lymphocyte resistance to the DNA strand breaks induced by H_2O_2 in vitro. Electrolyzed reduced water was better than diethylpyrocarbonate-treated water in preventing total RNA degradation at 4º and 25ºC. It also prevented the oxidative cleavage of horseradish peroxidase, as determined using sodium dodecyl sulfate-polyacrylamide gels. Enhancement of the antioxidant activity of ascorbic acid dissolved in electrolyzed-reduced water was about threefold that of ascorbic acid dissolved in non-electrolyzed deionized water, as measured by a xanthine-xanthine oxidase superoxide scavenging assay system, suggesting an inhibitory effect of electrolyzed reduced water on the oxidation of ascorbic acid[26].

Nonetheless, several limitations should be considered. First, we only studied patients with T2DM who were stable non-complicated condition, we were not able to generalize this results to patients in more severe clinical condition. Second, the real mechanism of how reduced ionized water could reduce FBG could not be fully explained yet from our study. Third, we could not avoid the influence of patients' complience to anti-diabetic or other drugs, diet, and physical activities. In conclusion, our study was the first study that provided evidence for a positive effect of reduced ionized water with pH 9 in reducing fasting blood glucose in Indonesian patients with T2DM.

CONCLUSIONS

The findings of this study concluded that alkaline reduced water highly decreased fasting blood glucose compared to mineral water (19.4±1.68 mg/dL vs 14.3±3.64 mg/dL, p=0.000). This study recommends bigger sample size including all diabetes categories for the next studies.

REFERENCES

1. Global Report on Diabetes. Geneva: WHO; 2016.
2. International Diabetes Federation Diabetes Atlas. 7 ed. Karakas: International Diabetes Federation; 2015.
3. Riset Kesehatan Dasar (RISKESDAS) 2013. Jakarta: Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI; 2013.
4. Grant RW, Donner TW, Fradkin JE, Hayes C, Herman WH et al. Classification and diagnosis of diabetes: standards of medical care in diabetes 2015. Diabetes Care. 2015;38(Suppl. 1):S8–S16.
5. Chiang JL, Berg EG, McElvaine AT. American Diabetes Association: Standards of Medical Care in Diabetes 2016. Diabetes Care. 2016;39(Supplement 1):S1-S112.
6. Paneni F, Beckman JA, Creager MA, Cosentino F. Diabetes and vascular disease: pathophysiology, clinical consequences, and medical therapy: part I. Eur Heart J. 2013;34(31):2436–43.
7. Singhania N, Puri D, Madhu SV, Sharma SB. Assessment of oxidative stress and endothelial dysfunction in Asian Indians with type 2 diabetes mellitus with and without macroangiopathy. Q J Med. 2008;101(6):449-55.
8. Rosen P, Nawroth PP, King G, Moller W, Tritschler HJ, Packer L. The Role of Oxidative Stress in the onset and progression of diabetes and Its complications: a summary of a Congress Series sponsored by UNESCO-MCBN, the American Diabetes Association and the German Diabetes Society. Diabetes Metab Res Rev. 2001;17(3):189-212.
9. Nowotny K, Jung T, Hohn A, Weber D, Grune T. Advanced glycation end products and oxidative stress in type 2 diabetes mellitus. Biomolecules. 2015;5(1):194-222.
10. Niedowicz DM, Daleke DL. The Role of Oxidative Stress in Diabetic Complications. Cell Biochem Biophys. 2005;43(2):289.
11. West IC. Radicals and oxidative stress in diabetes. Diabet Med. 2000;17(3):171-80.
12. Bajaj S, Khan A. Antioxidants and diabetes. Indian J Endocrinol Metab. 2012;16(Suppl 2):S267-71.
13. Bloomgarden ZT. Antioxidants and Diabetes. Diabetes Care. 1997;20(4):670-3.
14. Steinhubl SR. Why Have Antioxidants Failed in Clinical Trials? Am J Cardiol. 2008;101(Supplement 10):14D–9D.
15. Shirahata S, Hamasaki T, Teruya K. Advanced research on the health benefit of reduced water. Trends Food Sci Technol. 2012;23(2):124-31.
16. Ohta S, Nakao A, Ohno K. The 2011 Medical Molecular Hydrogen Symposium: An inaugural symposium of the journal Medical Gas Research. Med Gas Res. 2011;1(1):10.
17. Kim M-J, Kim HK. Anti-diabetic effects of electrolyzed reduced water in streptozotocin-induced and genetic diabetic mice. Life Sci. 2006;79(24):2288-92.
18. Kajiyama S, Hasegawa G, Asano M, Hosoda H, Fukui M, Nakamura N, et al. Supplementation of hydrogen-rich water improves lipid and glucose metabolism in patients with type 2 diabetes or impaired glucose tolerance. Nutrition Research. 2008;28(3):137-43.
19. Osada K, Li Y, Hamasaki T, Abe M, Nakamichi N, Teruya K, et al. Anti-Diabetes Effects of Hita Tenryou-Sui Water®, a Natural Reduced Water. In: Kamihira M, Katakura Y, Ito A, editors. Animal Cell Technology: Basic & Applied Aspects. 16. Dordrecht: Springer, Dordrecht; 2010. p. 307-13.
20. Li Y, Hamasaki T, Teruya K, Nakamichi N, Gadek Z, Kashiwagi T, et al. Suppressive effects of natural reduced waters on alloxan-induced apoptosis and type 1 diabetes mellitus. Cytotechnology. 2012; 64(3):281-97.
21. American Diabetes A. Economic costs of diabetes in the U.S. In 2007. Diabetes Care. 2008;31(3):596-615.
22. Hayashi H, Kawamura M. Clinical Applications of Electrolyzed-Reduced Water. In: Shirahata S, Teruya K, Katakura Y, editors. Animal Cell Technology: Basic & Applied Aspects. 12. Dordrecht: Springer, Dordrecht; 2002. p. 31-6.
23. Shirahata S, Kabayama S, Nakanoa M, Miura T, Kusumoto K, Gotoh M, et al. Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage. Biochem Biophys Res Commun. 1997;234(1):269-74.
24. Gadek Z, Hamasaki T, Shirahata S. "Nordenau Phenomenon" - Application of Natural Reduced Water to Therapy: Follow-Up Study upon 411 Diabetes Patients. In: Shirahata S, Ikura K, Nagao M, Ichikawa A editors. Animal Cell Technology: Basic & Applied Aspects. 15. Dordrecht: Springer, Dordrecht; 2008: 265.
25. Gadek Z, Shirahata S. Changes in the Relevant Test Parameters of 101 Diabetes Patients under the Influence of the So-Called "Nordenau-Phenomenon". In: Shirahata S, Teruya K, Katakura Y, editors. Animal Cell Technology: Basic & Applied Aspects. 12. Dordrecht: Springer, Dordrecht; 2002.
26. Lee MY, Kim YK, Ryoo KK, Lee YB, Park EJ. Electrolyzed-reduced water protects against oxidative damage to DNA, RNA, and protein. Appl Biochem Biotechnol. 2006;135(2):133-4