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The effect of carbon dioxide water immersing on inflammation biomarker in patients with chronic kidney disease undergoing hemodialysis

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Abstract

Background: Chronic kidney disease (CKD) patients undergoing hemodialysis (HD) often exhibit elevated levels of ICAM-1 and VCAM-1, key biomarkers of inflammation, and are prone to microcirculatory dysfunction. Recent evidence suggests that carbon dioxide (CO_2)-rich water immersion may enhance microcirculation, yet its impact on inflammatory biomarkers in CKD patients on HD remains unclear.
Purpose: To evaluate the effect of CO_2-rich water immersion on ICAM-1 and VCAM-1 levels in CKD patients receiving HD.
Method: This experimental study involved 22 CKD patients randomized into two groups: 14 received CO2-rich water immersion and 8 received freshwater immersion. CO2-rich water was generated using JESC CREABC-2000, with both immersions maintained at 37–38°C. Patients immersed both legs for 10 minutes, 2–3 times weekly for one month, prior to HD sessions. Blood samples were collected on day 1 and day 28. Data were analyzed using the Wilcoxon test, with statistical significance set at $P < 0.05$.
Results: After one month, ICAM-1 and VCAM-1 levels decreased in the CO2-rich water group (42.895±58.833 to 39.690±41.911 ng/mL, P=0.650; 140.520±177.43 to 96.170±30.17 ng/mL, P=0.917). Conversely, levels increased in the freshwater group (ICAM-1: 38.642±40.975 to 63.978±80.659 ng/mL, P=1.000; VCAM-1: 100.430±34.89 to 131.920±59.80 ng/mL, P=0.461).
Conclusion: CO2-rich water immersion tended to reduce ICAM-1 and VCAM-1 levels in CKD patients undergoing HD, although the changes were not statistically significant. These findings suggest potential anti-inflammatory effects warranting further investigation with larger cohorts.

Keywords: **Carbon Dioxide; Chronic Kidney Disease; Hydrotherapy; Inflammation; Intercellular Adhesion Molecule-1; Vascular Adhesion Molecule-1.**

INTRODUCTION

Chronic Kidney Disease (CKD) is a non-communicable chronic disease with a very high prevalence. Globally, the prevalence of CKD in 2017 reached 697.5 million patients, while in Indonesia, the prevalence of CKD in 2018 was reported to have reached 3.8% of the population (Wang, Naghavi, Allen, Barber, Bhutta, Carter, & Bell, 2016; Hill, Fatoba, Oke, Hirst, O'Callaghan, Lasserson, & Hobbs, 2016; Ministry of Health of the Republic of Indonesia, 2017). Chronic Kidney Disease is characterized by a progressive and irreversible decline in kidney function which results in metabolic

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and electrolyte imbalances in the body which in the final stages requires hemodialysis (HD) as the main therapy (Zatz, Seguro, & Malnic, 2011). According to the 8th Report of the Indonesian Renal Registry in 2015, there was an increase in HD patients from 11,689 patients in 2014 to 30,554 patients in 2015 (Indonesian Renal Registry, 2015). The percentage of CKD patients who recover from HD treatment is relatively low, ranging from 0.3-8%. The average survival time for HD patients in Indonesia is about 3 years, but the largest proportion of patients survive only 6-12 months (Ministry of Health of the Republic of Indonesia, 2017). Meanwhile, in developed countries, the value of patient survival is very high. In the USA was reported to be 38% (Saran, Robinson, Abbott, Bragg-Gresham, Chen, Gipson, Shahinian, 2020). Meanwhile, in Japan, the survival time for HD patients who reach 5-25 years is 95.52% (Nitta, Goto, Masakane, Hanafusa, Taniguchi, Hasegawa, & Nakamoto, 2020).

Patients undergoing HD are experiencing a risk of oxidative stress and inflammation that accelerates cardiovascular disease (Lakshmi, Devi, Suchitra, Rao, & Kumar, 2018). Inflammation that occurs causes blood vessels, especially the endothelium layer to release inflammatory modulators which include several adhesion molecules, specifically Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Adhesion Molecule-1 (VCAM-1) (Gimbrone & Garcia-Cardena, 2016; Čejková, Králová-Lesná, & Poledne, 2016). Adhesion molecule concentration will increase in endothelial dysfunction presence and it has been reported that this biomarker will be useful for predicting future inflammatory events (de Faria, Ritter, Sabbatini, Corrêa, Brunelli, Modolo, & Moreno, 2016). Increased levels of ICAM-1 and VCAM-1 result in a decrease in the process of vasodilation of blood vessels, which is a sign that Peripheral Arterial Disease (PAD) has occurred (Rahawarin, Bahar, Akbar, Masadah, Wuysang, Bintang, & Halim, 2025).

In several studies related to CO2 water immersion in PAD patients, it was reported that there was an improvement in microcirculation through a vasodilation effect which significantly increased microvascular vasodilator function (Dogliotti, Galliera, Valserra, Solimene, & Corsi, 2011; Izumi, Yamaguchi, Yamazaki, Yamashita, Nakamura, Shiota, & Iwao, 2015; Finzgar, Melik, & Cankar, 2015; Xu, Elimban, & Dhalla, 2017; Ogoh, Washio, Suzuki, Ikeda, Hori, Olesen, & Muraoka, 2018). Although the results of previous studies were reported to be quite good, this type of research has never been found in Indonesia. In addition, the pathomechanism of the success of this therapy in improving inflammation in CKD patients has not been discovered. Therefore, we are interested in research to examine the benefits of carbon dioxide water immersion in patients with CKD undergoing HD in Indonesia, especially in the city of Makassar by measuring ICAM-1 and VCAM-1 levels.

RESEARCH METHOD

The design of this study was the randomized Pretest-posttest Control Group. The population of this study were patients with Chronic Kidney Disease (CKD) undergoing hemodialysis who were registered as outpatients at the Hemodialysis Clinic at Hasanuddin University Hospital in 2023. The population in this study was 28 patients. The entire population was included as a sample in this study which was then divided into 2 groups randomly. There were 14 samples each from the CO2 and freshwater immersion groups. However, at the end of the study, 6 samples from the freshwater group failed (1 person was declared cured, 1 person died, and 4 other people were lost to follow-up).

In the CO2 immersion group, immersing water was produced by combining warm water and CO2 using JESC CREA, BC-2000. The pH of the water produced for this group is guaranteed to be around 4.5-5.6. Meanwhile, in the freshwater group, the immersing water is warm water without CO2. Both immersions were guaranteed to be around 37-38°C. Both legs of each patient were immersed for 10 minutes, 2-3 times a week for 1 month, before each hemodialysis.

This research has received approval from the Hasanuddin University Faculty of Medicine Ethics Committee number 334/UN4.6.4.5.3.1/PP36/2023. Secondary data in the form of disease history, age, gender, body weight, blood pressure, GFR value, and frequency of hemodialysis, as well as urea and creatinine levels, were obtained through patient medical records.

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Foot immersion protocol

The CO2 immersion water solution in this study was produced using a high concentration hydrogen carbonate ion spring (JESC, CREA BC2000, Nagoya, Japan) connected to an electric water heater (Ariston, TI-SHAPE 15 OR 500 ID, Marche, Italy) and a gas cylinder contains CO2 with a pressure of 1300 ppm. The output is considered to be the CO2-immersing solution at pH 4.5-5.6. A total of 24 liters of the resulting solution was then placed into a container (Figure 1). Meanwhile, in the freshwater immersion group, immersion water is obtained through tap water connected to a water heater which is also stored in in container of as much as 24 liters. Both CO2 and freshwater immersions are guaranteed to have temperatures ranging from 37-38°C. Both feet of each research subject in each group were soaked for 10 minutes immediately before receiving hemodialysis therapy (Figure 2).

Blood samples were taken through a hemodialysis tube before hemodialysis on day 1 and day 28. The blood samples were put into the EDTA tube as much as 3cc. The blood in the EDTA tube was centrifuged and transferred to Eppendorf tubes and stored in a refrigerator at -80°C.



Figure 1: JESC CREA,BC200



Figure2: Immersion of both feet

ICAM-1 dan VCAM-1 Measurement

Blood plasma ICAM-1 and VCAM-1 concentrations on day 1 and day 28 of immersion were measured using ELISA. The respective reagents used are Human Soluble Intercellular Adhesion Molecule 1 (sICAM) with catalogue number MBS3800942 and Human Soluble Vascular Cell Adhesion Molecule 1 (sVCAM) with catalogue number MBS3800948 from MyBiosource.

The basic principle of the ELISA method is the use of enzymes to detect the bond between antigen and antibody, namely changing the colour from blue to yellow and measuring the color intensity at 450 nm using a spectrophotometer for 15 minutes to measure the concentration of ICAM-1 and VCAM-1. The spectrophotometer is connected to a computer so that the concentrations of ICAM-1 and VCAM-1 can be read in the form of Excel data.

Data Analysis

The normality test in this study was tested using the Shapiro-Wilk test. Changes before and after intervention in each group were analyzed using the Wilcoxon test. The results were considered statistically significant at $P < 0.05$. All data was processed using SPSS 25.

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RESEARCH RESULTS

Table 1. Characteristics of the Participants (N=22)

Variable	VCAM-1 and ICAM-1 Levels		p-value
	CO2 Immersion (n=14)	Freshwater Immersion (n= 8)	
[a]Age (Mean±SD)(Year)	44.93 ±14.69	48.25±14.09	0.657
[b]Gender			
Male	4/28.6	6/75.0	-
Female	10/71.4	2/25.0	-
[b]HD Frequency			
2x/week	9/64.3	4/50.0	
3x/week	5/35.7	4/50.0	-
[a] Weight (Kg)	52.93±8.11	66.75±11.17	-
[a] Systolic (mmHg)	147.14±20.164	122.38±24.66	0.031
[a] Diastolic (mmHg)	82.86±6.112	72.75±10.58	0.016
[a] GFR (mL/Min/1.73 m2)	7.12±3.51	8.50±4.12	0.040
[a] Urea (mg/dL)	72.71±32.552	96.00±35.246	0.065
[a] Creatinine (mg/dl)	9.029±6.48	8.05±3.086	0.811

p= Mann-Withney Test
[a]=Parameters for numeric data (Mean±SD)
[b]=Parameters for categorical data (n/%)

Table 1 shows that the mean baseline blood pressure and GFR values in the CO2 immersion group appeared to be worse than those in the freshwater immersion group, while other characteristic data were similar between the two groups. Although the patient groups were divided randomly, our data relies on patients undergoing hemodialysis at Hasanuddin University Hospital.

Table 2. Differences in ICAM-1 Levels Between Groups

ICAM-1 Levels	n	Day 1	Day 28	*p-value*
CO2 immersion (ng/ml)	14	42.895± 58.833	39.690±41.911	0.650
Freshwater immersion (ng/ml)	8	38.642±40.975	63.978±80.659	1.000

Table 3. Differences in VCAM-1 Levels Between Groups

VCAM-1 Levels	n	Day 1	Day 28	*p-value*
CO2 immersion (ng/ml)	14	140.520±177.43	96.170±30.17	0,917
Freshwater immersion (ng/ml)	8	100.430±34.89	131.920±59.80	0,461

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Tables 2 and 3 show that the levels of ICAM-1 and VCAM-1 after immersion for one month tend to decrease in CO2 immersion, while the levels of ICAM-1 and VCAM-1 tend to increase in freshwater immersion, although it is not statistically significant (P>0 .05)

DISCUSSION

Research regarding the beneficial effectiveness of CO2 balneotherapy has continued to be developed since 1834 until now. Piderit and Beneke were the first to describe the primary direct effects of CO2 balneotherapy on the submerged parts of the body and the resulting sensation of warmth and redness on the skin (Pagourelias, Zorou, Tsaligopoulos, Athyros, Karagiannis, & Efthimiadis, 2011). Furthermore, a research was conducted at Sapporo Medical University, Hokkaido, Japan in 2016 showed that this therapy can improve endothelial function by providing a vasodilating effect on blood vessels with less heat stress. In contrast, immersion of the lower limbs in warm water without CO2 does not increase Flow Mediated Dilatation (FMD), requiring an increase in temperature of up to 43°C to produce increased skin blood flow (Ogoh, Nagaoka, Mizuno, Kimura, Shidahara, Ishii, Kudoh, & Iwamoto, 2016). From these two studies it can be concluded that there is a beneficial effect in the form of increasing peripheral blood flow in areas exposed to CO2. This also proves that CO2 is indeed a potential vasodilator.

Changes in blood vessel diameter due to decreased vascular stiffness are closely related to endothelium-dependent vascular changes (Makino, Maeda, & Abe, 2015). When endothelial dysfunction or inflammation occurs, the endothelium layer releases inflammatory modulators that include several adhesion molecules, namely Intercellular Adhesion Molecule-1 (ICAM-1) and Vascular Adhesion Molecule-1 (VCAM-1) (Rahawarin et al., 2025). The mechanism of ICAM-1 and VCAM-1 when inflammation occurs at the site of trauma, activated macrophages will produce pro-inflammatory cytokine mediators, namely IL-1 and TNF-α. Tumour Necrosis Factor-α induces vascular endothelial cells to express adhesion molecules. These adhesion molecules bind to their receptors on leukocytes. Leukocytes in the bloodstream express adhesion molecules, namely resting integrins LFA-1 and GPCR (G-Protein Coupled Receptor) Adhesion molecules. This causes the leukocytes to move to the walls of the blood vessels and adhere to the endothelium. The attachment becomes more assertive so that the force of the blood flow cannot break the bond. The force of the urge to blood flow causes the leukocytes to roll along the blood vessels' endothelium. The adhesion between the leucocytes and the endothelium becomes more substantial due to the activation of the adhesion molecule factor. The leukocytes that roll on the endothelium surface eventually stop because the leukocyte bonds with the adhesion molecules expressed by the activated endothelium, namely Intercellular Adhesion Molecule-1 and Vascular Cell Adhesion Molecule-1). These adhesion molecules bind to the integrins on the surface of the leukocyte cells resulting in strong adhesion. After there is a stronger attachment between the leukocytes and the endothelium, the leukocytes that have stopped rolling, penetrate the endothelial wall with a diapedesis process through the gaps between endothelial cells (Muller, 2014; Gimbrone & Garcia-Cardena, 2016; Steyers & Miller, 2014).This uncontrolled situation initiates plaque formation which causes a decrease in blood flow (Kunutsor, Bakker, & Dullaart, 2017).

To see the effect of CO2 immersion in healing endothelial dysfunction and inflammation along with the underlying mechanisms, in this study ICAM-1 and VCAM-1 levels were measured on day 1 of immersion and day 28 of immersion. In this study it was found that the basal VCAM-1 value was higher than the basal ICAM-1 value. Higher VCAM-1 values were reported in patients with increased arterial stiffness (Kilic, Findikoqlu, Alihanoqlu, Yildiz, Uslu, Rota, & Evrengul, 2015). Perhaps, the patients in this study did experience blood vessel stiffness.

This study reports a tendency to decrease ICAM-1 and VCAM-1 levels in the CO2 immersion group, whereas in fresh water immersion there tends to be an increase in ICAM-1 and VCAM-1 levels. Based on previous studies that focused on changes in ICAM-1 and V-CAM-1 in patients treated with hemodialysis, it showed that there was a significant increase in circulating ICAM-1 and VCAM-1 levels in HD patients,

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with large amounts of ICAM-1 and VCAM -1 which is soluble (molecular weight range 90-110 kDa), these molecules cannot be completely cleared effectively through the membrane used so that HD does not affect the levels of ICAM-1 and VCAM-1 (Papayianni, Alexopoulos, Giamalis, Gionanlis, Belechri, Koukoudis, & Memmos, 2002; Mrowka & Sieberth, 1995). Indeed, increased levels of ICAM-1 and VCAM-1 in HD patients are often found because during HD treatment, patients are at high risk of experiencing increased oxidative stress and inflammation which accelerate atherosclerosis and the onset of cardiovascular disease (Gambo, Pretorius, Todd-Tzanetos, Luther, Yu, Alp Ikizler, & Brown, 2012). Therefore, through the results of our study, it can be said that administering CO2 immersion tends to improve blood microcirculation by reducing inflammation of peripheral blood vessels in kidney failure patients, although statistically this result was not significant in both treatments.

The decrease in ICAM-1 and VCAM-1 during CO2 immersion may be caused by endothelial activity, in which case there is an increase in endothelial function (Ogoh et al., 2016). It is known that ICAM-1 and VCAM-1 are expressed on endothelial cells and leukocytes, although ICAM-1 is also expressed in hematopoietic cells. VCAM-1 is known to be a marker of plaque activity, while ICAM-1 has been suggested to be used as a marker of low-grade inflammation (Kilic et al., 2015). When ICAM-1 and VCAM-1 levels increase, this can accelerate atherosclerosis-related inflammation by activating the adhesion of circulating leukocytes to endothelial cells, ICAM-1 and VCAM-1 mediate stronger adhesion of leukocytes to the endothelium (Ogoh et al., 2016; Dörr, Liebetrau, Möllmann, Gaede, Troidl, Rixe, Hamm, & Nef, 2014). By reducing ICAM-1 and VCAM-1, inflammation is reduced so plaque activity decreases. This research proves that with CO2 immersion the levels of ICAM-1 and VCAM-1 decrease, so it can be concluded that there is an improvement in microcirculation after CO2 immersion.

This study has certain limitations, it is short term, consists of a small number of subjects in both groups, and the subjects of this study are not homogeneous. Therefore, further research is needed with a larger sample and a long-term period.

CONCLUSION

Application of CO2 immersion for four weeks can reduce ICAM-1 and VCAM-1 in improving microcirculation in kidney failure patients. Further research is needed with a larger sample size and a longer time period.

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AUTHORS' CONTRIBUTIONS

All authors contributed equally to conceptualizing this research, conducting sample collection and data analysis, preparing manuscripts, and designing figures and tables.

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