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Oxidative Stress and Hemodialysis: The Role of Aerobic Exercise Training on the Various Laboratory Parameters

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Abstract

Background: Oxidative stress (OS) and inflammation are common factors in patients with chronic kidney disease and increase the risk of cardiovascular disease (CVD) and death in these patients, particularly in hemodialysis (HD) cases. Exercise training is a therapeutic approach to reduce morbidity and mortality in these patients. The purpose of this study was to determine the effect of aerobic exercise training on OS factors in HD patients.

Methods: This case-control study was performed over one year (From March 2019 to February 2020) at Kerman (south of Iran). Nineteen outpatient HD patients participated in an aerobic exercise training program during HD. Cycling was performed by mini-cycle in the first hour of HD, pedaling was started with the least tolerable time by the patients, and increased up to three-quarters of an hour based on the patient's tolerance, three times a week for eight weeks. Also control group, 18 outpatient HD patients were selected and did not enter the exercise training program. Blood samples were collected before and after the intervention in both groups. Inflammatory factors such as albumin (Alb), C-reactive protein (CRP), ferritin, white blood cell (WBC) as well as, oxidative and nitrogenous factors such as malondialdehyde (MDA), carbonyl groups, uric acid, and ionic regenerating capacity were measured. Chi-square and independent *t* test were used to analyze the data and compare the two groups.

Results: There were no significant differences in CRP, Alb, and uric acid between the two groups. In the test group, variables such as total antioxidant capacity (TAC), glutathione (GSH), protein carbonyl (PC), lipid peroxidation (LPO) products, ferric reducing ability of plasma (FRAP), and thiobarbituric acid reactive substances (TBARST) showed a significant difference after the intervention. The values of TAC, GSH, and FRAP in the case group were significantly higher than the same values in the control group ($P \leq 0.0001$ for all), but PC ($P \leq 0.006$) and TBARS ($P \leq 0.026$) values were higher in the control group after the intervention. The intervention had no effect on Alb and ferritin. But, uric acid levels decreased significantly in both groups after the intervention. In the case group, TAC, GSH, and FRAP increased ($P = 0.0001$ for all), but PC, LPO, and TBARST decreased ($P = 0.0001$, $P \leq 0.03$, and $P \leq 0.02$ respectively).

Conclusion: The results of the present study showed that aerobic exercise training had no effect on inflammatory factors such as CRP, ferritin, and Alb.

Keywords: Hemodialysis, Aerobic exercise training, Inflammation factors, Oxidative stress

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Introduction

Chronic renal failure (CRF) patients have a 3-5 folds higher risk for cardiovascular events than the general population and this morbidity is higher than 3.5 to 50 times in hemodialysis (HD) patients (1). In these patients, the emphasis on traditional risk factors such as hypertension, anemia, diabetes, and the related atherosclerotic cardiovascular and inflammatory phenomena associated with a rise in oxidative stress (OS) has been highlighted (2,3). Extreme oxidant products and/or low antioxidant levels have appeared as vital pathogeneses in the development of long-term complications such as cardiovascular disease (CVD), malnutrition, amyloidosis, anemia, and infections occurring in long-term HD cases (4,5). Some markers of OS include lipid, protein, polysaccharide, and nucleotide acid metabolites (6). Increase of lipid-based peroxidation products such as malondialdehyde (MDA), 4-hydroxynonenal, and F2-isoprostanes, produced by enzymatic activity from the free radical-catalyzed peroxidation of N-arachidonoyl glycine have been constantly reported in HD patients (7). MDA is an end-stage product of polyunsaturated fatty acid peroxidation in cells. Various studies show an inverse relationship between different markers of OS and

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glomerular filtration rate (8,9). Decreased antioxidant factors such as vitamin C, selenium deficiency, and low activity of the glutathione system lead to the high production of OS in CRF patients (10). So, old age, diabetes, chronic inflammation, uremic syndrome, dialysis solution, and biocompatibility of membranes used in the treatment of CRF accelerate the formation of OS. The presence of glucose in HD fluid, which activates the pentose phosphate pathway, can increase OS (11).

There is evidence to suggest that aerobic exercise training during dialysis increases dialysis efficiency and hemoglobin in HD patients (12). Exercise also improves laboratory parameters. Regular physical exercise training has many benefits, including reducing the risk of mortality in HD patients, CVD, cancers, and diabetes (13). Previous studies showed that aerobic exercise training has a significant effect on various aspects of quality of life, relieving fatigue, improving muscle strength and blood pressure in HD (14). There are many studies on the relationship between exercise and OS in the general population and many disorders, but studies in HD patients are limited and conflicting results are available on the effect of exercise on antioxidants and OS (15). However, the purpose of this study was to investigate the impact of aerobic exercise training on the various parameters such as albumin (Alb), C-reactive protein (CRP), white blood cell (WBC), ferritin, in addition to oxidative and nitrosative factors such as MDA, carbonyl groups, uric acid, ferric reducing ability of plasma (FRAP), glutathione (GSH), Thiobarbituric acid reactive substances (TBARS) and total antioxidant capacity (TAC) in HD patients at Shafa hospital in Kerman, Iran.

Materials and Methods

Patients

In this case-control study, 37 chronic HD patients enrolled in the study after obtaining their informed consent. The case group (19 patients) who were undergoing dialysis on an outpatient basis in the HD center participated in an aerobic exercise training program during HD for eight weeks and three times a week. The control group included 18 randomly selected patients undergoing HD who did not enter the exercise program. Since the appropriate exercise training recommendations for HD patients are mild to moderate aerobic exercise training, pedaling during HD sessions with mini-cycle advised, and depending on the patient's tolerance and lack of discomfort such as breathlessness and palpitation by the patients, the time was determined. Because the complications of dialysis are more common in the first session and the first hour of exercise training, intervention started from the second hour of the dialysis session. The duration of the exercise was determined by the time and tolerance level of the patient. Most patients continued the exercise for 20 -30 minutes, and in those who were more active, it lasts up to 45 minutes. The type of exercise was pedaling with a bicycle that could be placed on the patient's bed (mini-cycle). Smokers, patients with chronic hepatitis, hematological and inflammatory disorders, infectious disease, cardiac and/or cerebral ischemia, cancer, chemotherapy, radiotherapy, and immunosuppressive therapy were excluded. Also, patients with restrictive exercise conditions such as atopy, asthma, hypoxia, ischemic heart disease, history of myocardial infarction in the last three months, history of stroke or transient ischemic attacks in the previous three months, any disorders of the musculoskeletal system such as osteoarthritis, osteoporosis, joint problems and disabilities that prevent exercise were excluded. The inclusion criteria were Kt/V urea index of 1.2 to 1.6 per session, history of dialysis for at least one year and a maximum of 5 years, undergoing three sessions of HD per week and willing to exercise. Bicarbonate-buffered dialysate produced with ultrapure water (CWP 100 system, Gambro, Sweden) with high bacteriological quality (<0.005 endotoxin units/mL) was used for the dialysis procedure. In HD cases, 10 mL of blood sample was taken from the arteriovenous fistula just before the dialysis session and collected in the standard tubes containing 5 mM EDTA, as was the case for the venous blood drawn from controls. After centrifugation (600 g for 12 minutes at 4°C), plasma was kept at −80°C. Briefly, Tests were done both at the beginning and the end of the intervention, and sampling was performed before the dialysis session.

Laboratory methods

Plasma Alb levels, serum ferritin levels, and plasma levels of CRP were determined by an immuno-nephelometric procedure (Dade Behring, France), immunoassay (IMX; Abbott, Rungis, France), spectrophotometry (Coulter STKS; Coultronics, Margency, France), and immunoturbidimetric method (Roche, Meylan, France), respectively. Technically Some parameters in serum such as ferritin and some parameters including oxidative factors were measured in plasma. Also, oxidative and nitrosative factors were determined. MDA (One of the end products of lipid peroxidation) was determined by the TBARS fluorimetric method. Plasma levels of advanced oxidation protein products and protein carbonyls (PCs) were determined by 2, 4-dinitrophenylhydrazine spectrophotometric assay. TAC and thiol groups such as glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-Red) activities were measured using FRAP and colorimetric (5, 5'-dithiobis (2-nitrobenzoic acid), respectively. The BCA (bicinchoninic acid) protein assay was used to quantify the total protein in a sample.

Statistical analysis

Data were analyzed through SPSS software version 22, and using descriptive statistics, chi-square and independent *t* tests to compare the groups. The significance level was considered less than 0.05.

Results

Demographic and characteristic variables

For all patients in both groups, the frequency of dialysis sessions was three times a week, and there was no difference between them. In the case group (n = 19), 15.8% (n = 3), 10.5% (n = 2), 31.6% (n = 6), 36.8% (n = 7) and 5.3% (n = 1) of patients had A^+, A^-, B^+, $O^+$and O^- blood groups, respectively. But, in the control group (n = 18), 16.7% (n = 3), 5.6% (n = 1), 22.2% (n = 4), 5.6% (n = 1), 11.1% (n = 2), and 38.9% (n = 7) had A^+, A^-, B^+, B^-, $AB^+$and O^+blood groups. There was a significant difference between the case and control groups regarding dialysis access; that is, in the case group, it was mostly of the fistula and in the control group, of the permacath types. Only one patient in the control group was dialyzed via the peritoneal route too. In general, 48.6% (n = 18), 10.8% (n = 4) and 37.8% (n = 14) of patients were dialyzed via the fistula, graft and permacath routs, respectively. In the case group, 63.2% (n = 12), 10.5% (n = 2) and 26.3% (n = 5) of HD patients were dialyzed via fistula, graft, and permacath routs, respectively and there was no peritoneal dialysis route in the case group. In the control group, 50% (n = 9), 33.3% (n = 6), 11.1% (n = 2) and 5.5% (n = 1) were dialyzed via permcath, fistula, graft and peritoneal routs, respectively.

The mean age, height, weight, and body mass index in the case group were 56.5 years, 166.1 cm, 71 kg, and 25.7 kg/m^2 respectively, but in the control group, these values were 60.4 years, 162.5 cm, 65.2 kg, and 24.9 kg/m^2. None of the demographic variables of HD patients in the case and control groups were significantly different from each other ($P > 0.05$). Except for 2 HD patients in the cases group, all subjects in the control and case groups were married. There was no significant difference between the two groups in accompanying diseases (Table 1).

Comparison of quantitative variables between the case and control groups

Determination of CRP levels in the two groups at the first stage showed a significant difference in the case group compared to the control.

As shown in Table 2, uric acid, ferritin, Alb, lipid peroxidation (LPO), and carbonyl in the first and second stages did not differ significantly between the case and control groups. Before the intervention (stage 1), the variables of TAC, GSH, PC, FRAP, and TBARS showed no significant difference between the two groups, while after the intervention (aerobic exercise), the two groups showed significant differences in these parameters in a way that TAC, GSH, and FRAP in the case group were higher than the control, but PC and TBARS values were higher in the control group (Table 2).

Table 1. Comparison of the two study groups in terms of accompanying diseases

Accompanying disease	Case Group No. (%)	Control Group No. (%)	*P* value
Diabetes	11 (57.9%)	12 (66.7%)	0.582
Hypertension	14 (73.7%)	12 (66.7%)	0.641
Coronary artery bypass surgery	1 (5.3%)	2 (11.1%)	0.515
Diabetic foot	0 (0.0%)	1 (5.6%)	0.298
Hyperlipidemia	1 (5.3%)	1 (5.6%)	0.969
Ischemic heart disease	2 (10.5%)	2 (11.1%)	0.954
Hyperthyroidism	3 (15.8%)	1 (5.6%)	0.604
Lupus	0 (0.0%)	1 (5.6%)	0.486
diabetic retinopathy	1 (5.3%)	0 (0.0%)	0.324
Alport syndrome	1 (5.3%)	0 (0.0%)	0.324
Polycystic kidney disease	2 (10.5%)	0 (0.0%)	0.487
History of kidney disease	4 (21.1%)	3 (16.7%)	0.734

Comparison of variables under intervention in both groups separately

In both groups, after the intervention (second stage), the levels of Alb increased and ferritin and carbonyl levels decreased, but the differences were not significant. In other words, the intervention did not affect this variable. Uric acid levels decreased significantly after the intervention in both groups. In the control group, TAC, GSH, PC, LPO, FRAP, and TBARST variables showed no difference in the first and second stages, but all these variables were significantly different after the intervention in the case group, so after the intervention in the case group, TAC, GSH, FRAP increased but PC, LPO, and TBARST decreased (Table 3).

Discussion

Our study investigated the effect of aerobic exercise training on OS in HD patients. Findings showed that the case and control groups were completely matched for demographic characteristics and accompanying disease. It has been reported formerly that inflammation is related to increased cardiovascular risk and mortality in uremic cases (15). Raised plasma CRP levels are associated with acute phase response; though, more recent epidemiological researches suggest that even small changes in CRP levels, < 3 mg/L, are related to increased cardiovascular risk, at least in the overall population (15). No significant differences in CRP, Alb, Ferritin, and Uric acid levels were observed between the two groups in our study, and intervention did not affect these factors. Similar to our results, in the study of Torres et al, there were no significant changes in total protein levels, Alb levels, phosphate levels, or inflammatory parameters analyzed as CRP or ferritin. Contrary to our data, Afshar et al (15) showed that both endurance and aerobic exercise training in HD patients have a reducing effect

Table 2. Comparison of the two study groups for quantitative variables in the two stages

Variables	Group		Mean	*P* value
Uric acid	Step	Case	7.16	0.871
	S1	Control	7.07	
	S2	Case	6.07	0.805
		Control	5.98	
Ferritin	S1	Case	277.94	0.988⁺
		Control	211.77	
	S2	Case	168.31	0.957⁺
		Control	170.77	
Alb	S1	Case	4.23	0.661
		Control	4.16	
	S2	Case	4.30	0.489
		Control	4.17	
TAC	S1	Case	173.45	0.511
		Control	165.75	
	S2	Case	249.10	**0.0001***
		Control	170.72	
GSH	S1	Case	795.7	0.258
		Control	741.6	
	S2	Case	1185.26	**0.0001***
		Control	775.75	
PC	S1	Case	4.59	0.704
		Control	4.69	
	S2	Case	4.11	**0.006***
		Control	4.68	
LPO	S1	Case	4.79	0.546
		Control	4.67	
	S2	Case	4.37	0.052
		Control	4.69	
Carbonyl	S1	Case	4.52	0.519
		Control	4.76	
	S2	Case	4.35	0.209
		Control	4.7	
FRAP	S1	Case	173.4	0.511
		Control	165.7	
	S2	Case	249.1	**0.0001***
		Control	165.36	
TBARS	S1	Case	4.79	0.548
		Control	4.67	
		Case	4.35	
	S2	Control	4.81	**0.026***
		Control	755.18	

Abbreviations: S, step; Alb, albumin; TAC, total antioxidant capacity; GSH, glutathione; PC, protein carbonyl; LPO, lipid peroxidation; FRAP, ferric reducing ability of plasma; TBARST, and thiobarbituric acid reactive substances.
* Statistically significant; (+) : Due to the non-normality of ferritin, the Mann-Whitney U test was performed.

Table 3. Paired sample *t* test comparison of variables in both groups

Variables	Group	Mean S1	Mean S2	*P* value
Uric acid	Case	7.2	6.1	0.006*
	Control	7.1	5.9	0.004*
Ferritin	Case	277.9	168.3	0.052
	Control	211.8	170.7	0.19
Alb	Case	4.2	4.3	0.407
	Control	4.2	4.2	0.867
TAC	Case	173.5	249.1	0.0001*
	Control	165.6	170.7	0.495
GSH	Case	795.7	1185.3	0.0001*
	Control	741.6	775.7	0.181
PC	Case	4.6	4.11	0.0001*
	Control	4.7	4.7	0.924
LPO	Case	4.8	4.4	0.032*
	Control	4.7	4.7	0.917
Carbonyl	Case	4.5	4.4	0.471
	Control	4.7	4.7	0.496
FRAP	Case	173.4	249.1	0.0001*
	Control	165.7	165.4	0.906
TBARS	Case	4.8	4.4	0.028*
	Control	4.7	4.8	0.358

Abbreviations: S, step; Alb, albumin; TAC, total antioxidant capacity; GSH, glutathione; PC, protein carbonyl; LPO, lipid peroxidation; FRAP, ferric reducing ability of plasma; TBARST, and thiobarbituric acid reactive substances.
*Statistically significant.

on inflammatory factors (CRP, serum creatinine, serum Alb levels, urea, hemoglobin, and fat status), but have no impact on dialysis adequacy.

In our study, there was no significant difference between the two groups in TAC, GSH, PC, FRAP, TBARS, and GSH, while after the intervention, a significant difference was observed in the TAC, GSH, FRAP variables. Similar to our study, Mohkam (16) declares the positive effect of 30 minutes of daily Yoga exercise on increasing antioxidant factors, and reducing OS (including OS markers such as MDA, POX, PLA_2 activity, superoxide dismutase (SOD) oxidative status, and catalase activity). On the other hand, following our findings, in Wilund et al study conducted in the USA (17), the serum thiobarbituric acid levels, alkaline phosphatase levels, and the epicardial fat layer reduced by endurance exercise, but an exercise in HD patients had no effect on serum lipids and inflammatory markers such as interleukin 6, and CRP (18).

Previous studies have shown that people on HD have higher levels of OS, both at rest and after exercise, than the control group. A study in Jamaica showed that Hatha yoga significantly reduced OS (MDA, POX, and PLA_2), oxidative status (SOD), and catalase activities in patients (19) which is similar to our findings.

Contrary to our study, Esgalhado et al (20) found that exercise during dialysis cannot reduce inflammatory factors (such as CRP) and OS (antioxidant enzyme activity such as SOD, catalase, and GSH-Px, and MAD) (20). This discrepancy may be due to the type of dialysis, duration and type of exercise, demographic information (including accompanied illnesses), and markers examined.

Our study showed that doing aerobic exercise training programs during HD can improve some laboratory parameters and reduce OS factors. These results can have clinical uses to reduce the complications of HD.

Limitations

One limitation of this study was the small number of patients in each group. The observed results may not be generalizable to other centers considering that this was a single-center study design. Further studies with a larger sample size and more extended exercise period are suggested. It is also recommended to evaluate the relationship between other exercises such as stretching exercises and yoga with oxidative factors in HD patients.

Conclusion

Aerobic exercise training programs during HD can reduce OS factors. These results can have clinical uses to minimize the complications of HD.

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Author Contributions

Conception and design: AS; Experiments performance: RGH; Manuscript writing: AB and RGH; Final approval of manuscript: SKM and AS.

Conflict of Interests

The authors declare that they have no financial interest or conflict of interests regarding the study.

Ethical Approval

The entire procedure was approved by the Ethics Committee of Kerman University of Medical Sciences (IR.KMU.REC.1395.588) and carried out after obtaining the written informed consent of the donors.

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