Effect of Sixteen Weeks Combined Training on FGF-23, Klotho, and Fetuin-A Levels in Patients on Maintenance Hemodialysis

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Original Paper

Introduction. Fibroblast growth factor 23 (FGF-23) and its cofactor alfa klotho, are one of the most important factors, directly and indirectly, involved in the process of calcification and atherosclerosis. This study aimed to evaluate the efficacy of a combination of regular exercise during dialysis on quality of life and markers including FGF-23, alfa klotho, and fetuin-A levels.

Methods. Forty-five hemodialysis patients aged 61 ± 9.02 years and weight 69 ± 11.25 kg were randomly divided into two training, EX (n = 24) and control groups, CON (n = 21). The EX group patients participated in a 16-week combined aerobic and resistance exercise program during dialysis. Bone markers including, FGF-23, klotho, fetuin-A, were measured before and at the end of the study in both groups. Statistical analysis for comparing data change during study by SPSS software and the *P* value was set at .05.

Results. In the control group in the secondary assessment, reduction in quality of life was observed ($P < .05$). Significant change in growth factor 23, CRP, and fetuin-A was not observed in exercise and control groups ($P > .05$), however significant rising of klotho was observed in treated patients ($P < .05$). Also, combined training reduced the amount of phosphorus, parathyroid hormone; significantly ($P < .05$).

Conclusion. This study showed that regular exercise during dialysis improves quality of life and physical functions. No significant change in FGF-23 and CRP were observed during the study. However significant rising of klotho and reduction of iPTH and phosphorous levels were observed in treated patients.

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INTRODUCTION

Cardiovascular disease (CVD) and bone disorders are very common in CKD patients on maintenance dialysis. Abnormal mineral metabolism resulting from renal dysfunction increases the loss of minerals from bones, causes mineral deposition and calcification. Fibroblast growth factor 23 (FGF-23) is one of the most important factors participate in the development of abnormal functions in patients with chronic kidney disease, which directly and indirectly involved in the process of calcification and atherosclerosis. FGF-23 is an approximately 30 KDA protein released by bone that requires the presence of the cofactor klotho for its classical

effects. FGF-23 promotes phosphate excretion with effect on its proximal reabsorption by reducing the expression of NPT2a and NPT2c mRNA, sodium/phosphate transporters.[1-3] Increased level of FGF-23 was associated with an increased risk for cardiovascular mortality in CKD patients. Increased levels of FGF-23 in outpatients with stable cardiovascular disease were also associated with mortality and cardiovascular events.[4] Numerous studies have highlighted the importance of FGF-23 on CVD risk.[5] Indeed, recent studies have shown that the expression and secretion of FGF-23 are increased in patients with coronary artery disease. This suggests that therapies that decrease the amount of FGF-23 may reduce CVD risk in chronic kidney patients.

On the other hand, klotho as a trans membrane protein appears to be involved in aging.[6] The klotho gene encodes a single-pass trans membrane protein that binds to multiple FGF receptors and affect as a co-receptor for FGF-23, as the main factor in bone metabolism. Investigators have been shown that klotho-deficient mice have increased vitamin D production, and altered mineral–ion homeostasis that may result in klotho-associated vitamin D metabolic abnormalities and aging.[7-9]

Besides, vascular calcification may also be enhanced by low serum levels of calcification inhibitors. Fetuin-A is one of the calcification inhibitors that inhibit the formation of hydroxyapatite crystals in circulation.[10] It has been observed that with decreasing renal function the amount of fetuin-A was reduced. Also, it has been shown that fetuin-A serum levels were significantly lower in patients whose glomerular filtration was low.[11] Various studies have shown that rats with diet enrich in mineral, fetuin-A knockout caused extensive vascular calcification in the lungs, heart, and kidneys.[9] On the other hand, in numerous articles, the high concentration of fetuin-A has been reported as a risk factor for metabolic syndrome.[12] In rodent subjects, fetuin-A binds to the insulin receptors, where it inhibits the auto-phosphorylation of tyrosine kinase to decrease insulin signaling.[13] Serum concentrations of fetuin-A are directly linked with insulin resistance and dyslipidemia.[14] Therefore, fetuin-A has now been recognized to be a pleiotropic molecule associated with diverse effects, sometimes even contradictory; in different systems across the body.[15] Moreover, CKD is a chronic inflammatory condition, accompanied by elevated C-reactive protein (CRP) and pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).[16-19] Elevated CRP is associated with increased vascular calcification in renal patients.[20] It is well established that exercise training helps CRP reduction in non-dialyzed patients, but there is significant controversy regarding whether or not it promotes CRP reduction in CKD patients.

This study aimed to evaluate the efficacy of a combination of regular exercises during dialysis on quality of life, physical functions and bone markers including FGF-23, klotho among hemodialysis patients.

MATERIALS AND METHODS

Subjects

Forty-five patient (38 men and 7 women, aged 61 ± 9.02 and weight 69 ± 11.25) with end-stage renal failure on maintenance hemodialysis treatment (mean years on HD 7.1 ± 6.4) were entered in this study. Inclusion criteria were adult hemodialysis patients that can cooperate and tolerate the exercise program. These patients were on dialysis 3 sessions /week for more than 6 months. We exclude patients who had severe peripheral neuropathy, orthopedic limitations, symptomatic cardiovascular disease, or any other medical problem that prevented in an exercise training program during dialysis

The study protocol was approved by the Human Subjects Institutional Review Board at Tabriz University of Medical Sciences, Tabriz, Iran (IR. tbzmed. Rec5.4.12126) and conducted by the Declaration of Helsinki and registered on the Iranian Registry of Clinical Trials (IRCT 201106092858NS). Informed consent was obtained from all participants.

Study Design

Following recruitment, screening, and baseline testing, eligible subjects were randomly assigned to one of two groups: usual care/control (CON, n = 21); intradialytic exercise training (EX, n = 24).

Exercise Training Intervention

Before entering the rehabilitation program and soon after its end, all patients underwent an exercise one -repetition maximum (1RM). Subjects in the exercise group underwent a 4-month intradialytic

combined exercise training program. The program consisted of 3 days /week aerobic training and resistance exercise training. The aerobic training was stationary cycling of specialized cycle ergometers placed in front of each subject's dialysis bed. Subjects started the training program by cycling at a tolerable pace for 10 min during their first exercise session. The duration of exercise increased 5 to 10 min per session, depending on each subject's tolerance until they were able to cycle continuously for a total of 45 min per session at the intensity of 12 to 14 out of 20 according to the rate of perceived exertion (RPE) of Borg scale.

Resistance exercise training of the lower extremities was performed in three sets and under the supervision of a physician by applying foreleg weights in knee extension-flexion and hip abduction-flexion at the intensity of 9 to 15 out of 20 at the RPE scale. Starting weights were determined from one-repetition maximum (1RM) using foreleg weights. A 1RM is the maximum weight that can be lifted one time with a proper technique. Training started at approximately 20% of 1RM for one/two sets of 12 repetitions and was increased to three sets as tolerated. When patients could perform three sets successfully, the weights were increased. Training sections were continued with 40% of 1RM during the second month, with 55% in the third month, and with 65% in the fourth month of the program. All exercise sessions were attended by study staff to encourage the subjects and monitor their responses to exercise (e.g. heart rate and blood pressure). Blood pressure and heart rate of the participants were monitored every 5 min during exercise.

Clinical Testing and Measurements

At baseline and immediately following the 4-month intervention (final testing), all patients underwent a series of tests described below to evaluate the effects of the intradialytic exercise program on our primary outcomes. Blood was collected from patients before the first exercise section and 48 to 72 hours after the last exercise section. Serum was collected from blood samples by centrifugation and stored at −80 °C until analyzed. FGF-23, klotho, fetuin-A, hs-CRP, and PTH were measured using commercially available ELISA kits (FGF-23 ELISA, mybiosource ELISA kit (USA);, Fetuin-A ELISA, diametra ELISA kit (Italy); hs-CRP ELISA, monobind Inc ELISA kit (USA); PTH, IBL ELISA kit (Germany). Serum soluble klotho was measured by enzyme-linked immunosorbent assay (ELISA) kit (Immuno Biological Laboratories Co., Ltd, Tokyo, Japan). Serum potassium, phosphate, calcium, alkaline phosphatase (ALP), were measured using an auto-analyzer (Olympus, Inc.). To assess the quality of life and efficacy of practice the dialysis patients' quality of life questionnaire was used and physical performance was measured by the 1RM test.

Statistical Analysis

All statistical analyses were performed using the SPSS software and significance was based on a two-tailed alpha value of .05. Data were checked for normality using the Kolmogorov–Smirnov test. To investigate the effect of the independent variable on dependent variables the independent t-test and for examining the relationship between the variables Pearson correlation test were used. The quality of life scores was calculated by using a Likert scale.

RESULTS

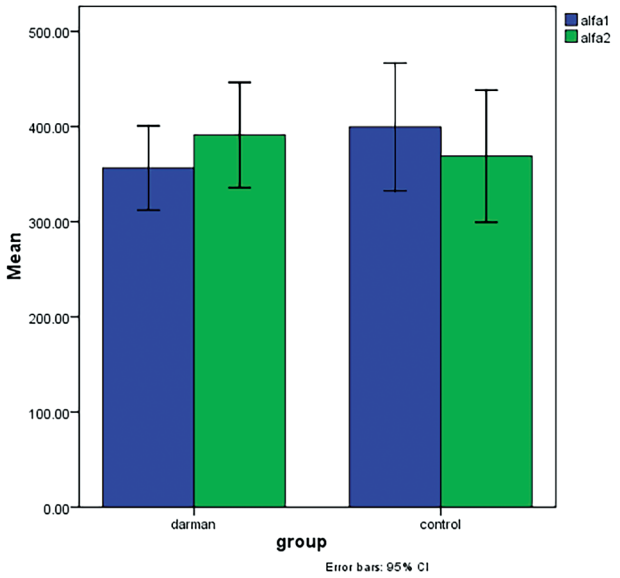
The characteristics of subjects at baseline and final testing are listed in Table 1. A total of 45 patients were recruited for the study, 24 in EX and 21 in CON. At baseline, the two groups did not differ significantly regarding age, body weight, years on dialysis, and Blood pressure of dialysis sessions.

Four months of combined training improved the quality of life and physical functions of dialysis patients. In the CON group in the secondary assessment, reduction in quality of life was observed ($P < .05$). There was no significant change in the amount of serum FGF-23, fetuin-A, and CRP in both EX and CON groups during the study ($P > .05$). The serum level of klotho increased significantly in the EX group at the end of the study ($P < .05$) while

Table 1. Participants' Characteristics at Baseline

Factors	Groups		*P*
	Exercise	Control	
Age, y	61.33 ± 9.54	60 ± 8.40	> .05
Weight, kg	67.47 ± 11.27	70.75 ± 9.90	> .05
Dialysis duration, years	6.50 ± 5.09	8.07 ± 5.79	> .05
Diabetic, %	37.5	47.5	> .05
SBP, mmHg	126.25 ± 21.48	125.95 ± 22.61	> .05
DBP, mmHg	70.63 ± 9.92	75 ± 12.34	> .05

it reduced in CON patients significantly ($P < .05$). Between-group results were also showed significant change in serum Klotho level ($P < .05$, Figure and Table 2). Also, there was a significant reduction in the amount of phosphorus, parathyroid hormone and alkaline phosphatase (ALP) ($P < .05$) in the EX group, but serum levels of calcium was not changed significantly ($P > .05$). There was also no correlation between plasma FGF-23, fetuin-A, and CRP concentration in both groups ($P > .05$, Table 2). Besides, there was a significant difference between EX and CON groups in physical performance, as 1RM test increased in EX, but did not change in CON group.



It shows comparison of alpha klotho levels in exercise and control patients (alpha 1: before, alpha 2: after study, darman: exercise).

DISCUSSION

The initial amount of the FGF-23 in the exercise group and the controls were 273.1 and 274.06 pg/mL, respectively which is eight times higher than the basic amount of FGF-23 normal state. Thus, any treatment which reduces FGF-23 and prevents its increasing can be useful for CKD patients. The results showed that the amount of serum FGF-23 between the two groups had no significant changes after 16 weeks of combined training. This research is one of the first studies about the effects of exercise on FGF- 23 levels in dialysis patients, so the previous information that could be cited in the conclusion is very limited. In a study by Yung SJ on an obese woman there was a modest decrease in FGF-21 levels after 3 months of training. However, fetuin-A levels were not changed significantly in this study.[21] The only study in this field was the research of Lombardi and *et al.* (2013) which was on the professional cyclists in the Italy tour. They found that a period of cycling increased secretion of FGF-23,[22] but as the results belonged to a serious activity and healthy persons, so could not apply to this study.

By the adaptations which occur in the body as effects of long-term training, and by increasing the FGF-23 secretion inhibitor proteins (such as DMP1),[23] it is expected the amount of FGF-23 decrease. Recent reports have shown that Leptin directly stimulates the expression of FGF-23 in bone[24,25] and considering phosphorus reduction as an adjustment due to long-term practice[26] the FGF-23 reduction hypothesis is emphasized. In addition, with the adaptations that occur within cells in calcium homeostasis by exercise, and also

Table 2. Subject Characteristics at Baseline and Final Testing

Laboratory Tests	Exercise		Control		*P*
	Before	After	Before	After	
FGF23, Pg/mL	273.11 ± 82.28	294.67 ± 88.11	274.04 ± 88.24	276.49 ± 66.56	> .05
Fetuin-A, ug/mL	123.65 ± 24.04	110.23 ± 19.19	116.24 ± 21.74	105.66 ± 22.74	> .05
Alpha Klotho	360.59 ± 107.55	397.25 ±133.36	392.75 ± 143.22	362.93 ± 147.05	< .05
CRP, mg/mL	8.68 ± 5.87	7.25 ± 5.45	7.36 ± 4.64	7.66 ± 4.86	> .05
PTH, pg/dL	431.48 ± 282.26	371.79 ± 319.6	382.10 ± 295.95	415.49 ± 380.03	< .05
ALP, U/L	309.03 ± 119.20	298.05 ± 138.83	330.85 ± 151.55	354 ± 149.48	> .05
Calcium, mg/dL	8.69 ± 0.72	8.13 ± 1.08	8.63 ± 0.51	8.45 ± 1.09	> .05
Phosphorous, mg/d	6.27 ± 1.79	5.17 ± 1.25	4.45 ± 0.75	4.67 ± 0.71	< .05
Hemoglobin, g/dL	10.77 ± 1.44	12.14 ± 1.50	11.60 ± 2.09	11.33 ± 1.64	> .05
Ferritin, ng/mL	418.36 ± 290.53	364.15 ± 294.92	276.64 ± 254.42	473.14 ± 198.98	> .05
Cr, mg/dL	10.19 ± 3.24	10.1 ± 2.51	9.87 ± 4.44	9.69 ± 2.35	> .05
1RM, kg	3.19 ± 1.16	6.38 ± 1.84	4.38 ± 0.74	4.22 ± 1.09	< .05

Parathyroid hormone reduction by exercise, which can hurt bone cells in the production of FGF-23,[27] it is expected that the FGF-23 amount is reduced. Non-significant changes in serum levels of FGF-23 in dialysis patients may be due to the exercises performed in the supine position, in which the hip bone cells and associated factors such as FGF-23 were not adequately under the exercise and gravity pressure. Studies have shown that there is a strong correlation between changes in hip bone density and levels of FGF-23.[28,29] Meanwhile, it is necessary to state that this study just was focused on factors that influence metabolism and changes in vascular and bone calcification levels in kidney patients and any type evaluation was not implemented for the subjects' vascular diseases and bone structural measurements.

As a first step, Schefer *et al.* evaluated the effect of physical activity on klotho to find any connection between physical activity and klotho levels in mice. They found that acute aerobic exercise significantly increased the circulating klotho level.[30] Also, Avin *et al.* found that circulating Klotho levels is up regulated in response to acute exercise.[31] In another study, Shive *et al.* evaluated s-klotho and IGF-I serum levels in patients with coronary artery disease following aerobic exercise training. They found that s-klotho levels were significantly higher while IGF-I lever was significantly lower in aerobically trained patients when compared with control patients.[32] Our result showed that sixteen weeks of combined training significant raising in the serum level of klotho in the EX group when compared to CON patients. In our knowledge, this is the first case-control study regarding the effect on combined exercise training on s-klotho level inpatient during hemodialysis. In other studies on dialysis patients klotho levels were lower in patients with PD than in healthy controls.[33] The klotho-FGF-23 complex induces phosphorus and vitamin D homeostasis, and the soluble form of klotho is described to reduce oxidative state. In a study by Izquierdo MC *et al.* circulating klotho levels are inversely correlated with phosphorus and PTH levels in patients with CKD before dialysis.[34] We also showed that serum phosphorus and PTH reduced significantly and there was an inverse correlation with klotho change that is comfortable with the previous study in CKD patients.

In this study, the serum levels of phosphorous and alkaline phosphatase, the putative risk factors for vascular calcification, were reduced in the EX group, but they did not change in the CON group. These results are in line with Vaithilingam *et al.*[26] and Makhloughi *et al.*[35] findings, which indicated that physical activity reduces phosphate levels in hemodialysis patients. Disturbance of minerals metabolism such as phosphorus has the main role in bone lesions and vascular calcification after hyper parathyroid. Hyperphosphatemia with increased inflammatory cytokines led to the deformation of vascular smooth muscle cells toward mineralization.[36] Therefore, in this study, control of hyper phosphatemia by exercise can help to prevent vascular calcification. Also, the decrease of phosphate after exercise shows that regular exercise reduces the consumption of phosphate-binding drugs in these patients.

According to the findings, fetuin-A levels did not have significant changes after 16 weeks of combined training among the two groups. These findings coincide with results of Wilund *et al.*[1] which found no change in fetuin-A serum levels after endurance training in dialysis patients, Yang *et al.*[24] and Reinehr *et al.*[37] indicating that physical activity cannot change fetuin-A levels in hemodialysis patients. But it conflicts with some previous studies such as Wilund *et al.*[38] which demonstrated that fetuin-A levels after the interval training increases in older men, Blumenthal *et al.*[13] reported an increase in plasma fetuin-A levels following a 6-month exercise protocol in overweight-to-obese older men. Fetuin-A is a multifunctional protein that has been assigned multiple tasks and it is said that fetuin-A is a protein that low levels of it can be useful or harmful.[11,39] In this study, fetuin-A levels had not changed which may be due to, no changing in other markers of inflammatory factors such as CRP. Also, it should mention that the basic level of fetuin-A in these patients is much lower than normal. It has been reported that fetuin-A low levels can be used as a marker of malnutrition, particularly in dialysis patients.[40] Because the subjects of this study are also confounding factors such as diabetes and renal dysfunction, the relationship between Fetuin-A and clinical parameters is in doubt.[41] This study had multiple strengths including a relatively large sample size; concurrent availability of FGF-23, klotho and fetuin-A values. Despite these and

other strengths, several limitations of the current study must be acknowledged. `It is proposed that variability in the measured biomarkers may be related to the state of calcification in the vasculature, but the direct measures of calcification have not be done to corroborate it.

CONCLUSION

In this study, it was seen that regular exercise increases muscle strength and physical performance among hemodialysis patients and thereby improve their quality of life. Changes in cardio-renal syndrome risk factors (such as alkaline phosphatase and phosphate) as well as improving their quality of life reflect the positive impact of activities on these patients. Findings emphasize the importance of promoting and maintaining physical fitness for the prevention of cardiovascular risk in hemodialysis patients. Also, this study adds considerable evidence providing support for the adoption of intradialytic exercise as a standard component of care for hemodialysis patients.

Changes in some factors related to vascular calcification and also the improvement in the quality of life show the positive effects of physical activity on these patients. As the initial levels of CRP in these patients are high, the anti-inflammatory effects of exercise training, which is seen among the normal population, may not found in these dialysis patients with extensive inflammation. As the subjects of this study were also had interfering factors such as diabetes and kidney disorders, fetuin-A and other clinical parameters relations may be affected. Further studies are needed to clarify the dynamics of FGF-23, klotho and fetuin-A production and secretion, and to clarify the mechanisms of exercise-induced secretion of these factors.

CONFLICT OF INTEREST

None to declare.

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