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COMPARING THE EFFECTS OF AEROBIC & RESISTANCE EXERCISES AND VITAMINS D & E ON OXIDATIVE STRESS & INFLAMMATORY MARKERS AMONG PATIENTS WITH DEPRESSION

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Abstract
Major depressive disorder (MDD) affects 280 million individuals worldwide, with oxidative stress and inflammation playing crucial pathophysiological roles. This study investigated the differential effects of aerobic versus resistance exercise combined with vitamin D supplementation on oxidative stress markers (GPx and SOD) and inflammatory biomarkers (TNF-α and IL-6) in patients with MDD. A single-blinded, 3-arm randomized clinical trial was conducted at two hospitals in Karachi involving 116 participants aged 25-35 years with mild to moderate depression. Participants were randomized to receive either aerobic exercise plus vitamin D (Group A, n=39), resistance exercise plus vitamin D (Group B, n=39), or vitamin D supplementation alone (Group C, n=38). Exercise groups performed 30-minute sessions four times weekly for 12 weeks at 60-85% intensity. All groups received 50,000 IU vitamin D3 weekly. Oxidative stress and inflammatory markers were assessed using colorimetric methods and ELISA techniques at baseline and post-intervention. All groups demonstrated significant reductions in oxidative stress and inflammatory markers ($p<0.001$). Aerobic exercise with vitamin D showed the largest reductions: SOD (26.2%), GPx (23.7%), TNF-α (16.1%), and IL-6 (13.3%). Resistance exercise with vitamin D achieved moderate reductions: SOD (22.1%), GPx (17.9%), TNF-α (12.3%), and IL-6 (8.9%). Vitamin D alone produced smaller improvements: SOD (15.8%), GPx (12.6%), TNF-α (8.5%), and IL-6 (5.7%). Between-group comparisons revealed significant superiority of aerobic exercise across all outcomes ($p<0.001$, $\eta^2=0.142$). Aerobic exercise combined with vitamin D supplementation most effectively reduces oxidative stress and inflammation in MDD, suggesting potential as an adjunctive treatment targeting underlying pathophysiological mechanisms.

Key words: Exercises; depression; vitamin D; Oxidative enzymes; inflammatory markers; Aerobic.

INTRODUCTION

Depression has emerged as the fourth leading contributor to global disability, with major depressive disorder (MDD) affecting approximately 280 million individuals worldwide and accounting for 7.5% of years lived with disability[1]. This widespread mental health condition adversely impacts emotions, cognitive processes, behaviors, and overall quality of life, with nearly 700,000 suicide-related deaths occurring annually[1]. The burden of depression is particularly pronounced in low- and middle-income countries, where access to mental health services remains limited and the prevalence continues to rise, exacerbated by factors such as the COVID-19 pandemic, socioeconomic challenges, and limited healthcare infrastructure[1]. In Pakistan, the fifth most populous nation globally, recent national psychiatric morbidity surveys have revealed alarming prevalence rates, with mood disorders affecting 19.62% of the population and regional studies in Karachi reporting depression symptoms in up to 39.9% of adults[2-3].

The pathophysiology of MDD involves a complex interplay of neurobiological mechanisms, including neurotransmitter imbalances, neuroendocrine dysregulation, and genomic and environmental factors. Increasing attention has been directed towards understanding the role of oxidative stress and inflammation in depression[4-5]. Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species and the body's antioxidant defense mechanisms[6]. Key enzymatic antioxidants, notably Glutathione Peroxidase (GPx) and Superoxide Dismutase (SOD), play vital roles in regulating oxidative stress levels[6-7]. Research indicates that individuals with MDD exhibit reduced GPx and SOD activity, resulting in elevated oxidative stress that contributes to neuronal damage and symptom severity[6]. Inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), participate in the inflammatory processes associated with depression[8]. Elevated levels of these cytokines interfere with neurotransmitter metabolism, disrupt the blood-brain barrier, and activate microglia to propagate neuroinflammation. The cross-talk between oxidative stress and inflammation creates a cyclic mechanism that reinforces the neurobiological substrates of depression, perpetuating symptom severity and treatment resistance[6-8].

The management of MDD encompasses pharmacotherapy, psychotherapy, and lifestyle modifications, including exercise and nutritional interventions[9]. While antidepressant medications demonstrate superior outcomes compared to placebo, they present significant limitations, including prolonged therapeutic delays before clinical improvement and adverse effects that frequently lead to treatment discontinuation[10]. Therefore, adjunctive therapies have gained considerable importance in addressing the disorder, particularly in treatment-resistant cases. Physical activity has emerged as a promising intervention, exerting beneficial effects on neurotransmitter levels, neurotrophic factors, hormonal balance, immune modulation, and cognitive functions[11]. Research demonstrates that moderate-intensity aerobic exercise can be as effective as antidepressant medication in alleviating psychological distress without associated pharmacological side effects. However, the differential effects of specific exercise modalities, particularly aerobic versus resistance training, on the oxidative stress and inflammatory pathways underlying depression remain inadequately explored[12-13].

Nutritional supplementation represents another potential adjunctive strategy for depression management[14]. Vitamin D deficiency has been associated with neurological impairment and mental health conditions, with low vitamin D levels linked to increased depression risk through mechanisms involving serotonin production and neuroprotection[14-16]. Despite the growing evidence supporting exercise and vitamin supplementation as individual interventions, limited research has investigated their combined effects on the biological mechanisms underlying depression[15]. Understanding how different exercise modalities interact with vitamin D supplementation to modulate oxidative stress and inflammation could provide valuable insights for developing personalized, multimodal treatment approaches that address the complex pathophysiology of depression while minimizing adverse effects associated with conventional pharmacotherapy[9,15].

This study aimed to determine the effects of aerobic versus resistance exercise combined with vitamin D supplementation on oxidative stress markers (GPx and SOD) and inflammatory biomarkers (TNF-α and IL-6) in patients with major depressive disorder at baseline and after 12 weeks of intervention.

MATERIALS AND METHODS

Study Design, Study Setting and Target Population

This single-blinded, 3-arm randomized clinical trial was conducted on individuals with a major depressive disorder, diagnosis based on DSM-5 guidelines and were referred by psychiatric specialists at two medical facilities in Karachi: Creek General Hospital and Dr. Ziauddin Hospital's Clifton Campus.

Sampling Technique and Sample Selection

The sample of patients was obtained through simple random sampling to allocate patients to three treatment groups. Three envelopes were prepared, each labelled with the identification of one of the treatment groups (Groups A, B, and C). The envelopes were identical in appearance to avoid any selection bias. The envelopes were shuffled thoroughly to ensure randomness before participant selection. The inclusion criteria comprised diagnosed patients with mild to moderate depression, aged between 25-35 years, referred by a psychiatrist with no other psychiatric disease attending the respective units of Creek General Hospital and Dr. Ziauddin Hospital. Individuals both with and without current psychoactive medication (such as antidepressants or anxiolytics) were enrolled in the study. Participants were required to be willing to participate in the intervention program. The exclusion criteria eliminated participants with diagnosed cardiovascular diseases, pregnant women, those using beta-blockers, individuals with a history of severe mental illnesses, hypertension, hyperparathyroidism, current significant substance or alcohol abuse, ongoing burn-out syndrome, and elevated suicide risk as determined by the psychiatrist. Additionally, prisoners, participants with chronic diseases such as liver and renal diseases, malabsorption syndrome, and other diseases that could affect vitamin D levels were excluded. Patients with physical limitations that would prohibit participation in the exercise protocol, those consuming dietary supplements two months prior to the intervention, individuals participating in regular exercise during the past 6 months (2 days/week), and non-cooperative patients who refused to continue the intervention program were also excluded from the study.

A total of 130 patients were initially approached to participate in the study. Fourteen were excluded, with five based on the selection criteria and nine refusing to participate. The remaining 116 participants were recruited for the study.

Following voluntary consent, all participants were evenly distributed into three treatment groups, designated as Groups A, B, and C, through the envelope method of simple random sampling. A total of 116 sealed envelopes were prepared, with each group receiving approximately 39 envelopes. Each participant selected one envelope of their preference, and based on their envelope selection, participants were assigned to their corresponding group. Subsequently, baseline assessment was conducted for all participants across three outcome measures: oxidative stress (SOD and GPX), inflammatory markers (TNF-α and IL-6), and depression severity (PHQ-9).

Participants referred to the study underwent a thorough screening process to determine their eligibility. The recruitment of patients was conducted by a psychiatrist, adhering to pre-defined inclusion and exclusion criteria. Patients underwent screening and were categorized as having Major Depressive Disorder if they exhibited at least one of the two primary criteria—depressed mood or anhedonia (the inability to experience pleasure or loss of interest)—along with four or more secondary symptoms persisting for a minimum period of 2 weeks, as per DSM-5 guidelines[5]. A physical therapist screened them through the Physical Activity Readiness Questionnaire Form. Subsequently, the investigator provided the patients with a comprehensive overview of the study and secured informed consent upon their agreement. Demographic information and pertinent variables, including the duration of the disease and comorbidities, were documented before the baseline assessment. All patients adhered to their routine pharmacological care throughout the intervention period.

An orientation session was arranged before the intervention, during which the physical therapists briefed the participants in detail regarding the protocol. The intervention lasted for 12 weeks, with a total of 48 sessions. First, the participants performed 1 week of training during the familiarization phase. Participants in Group A received aerobic exercise with vitamin D supplementation, Group B participants received resistance exercise with vitamin D supplementation, while Group C served as the control group receiving only vitamin D supplementation without structured exercise. All intervention groups were given 48 sessions of their respective protocol, comprising 30 minutes, 4 times per week for 12 weeks. After the end of the intervention, post-intervention data was collected on similar outcome measures for further evaluation.

Interventional Strategies

Before the conditioning phase, patients in the exercise groups carried out a structured 10-minute warm-up to prepare the body, boost circulation, and minimize injury risk. The first activity was walking at a slow and comfortable pace for approximately three to five minutes, focusing on good posture with relaxed shoulders and arms swinging naturally while maintaining steady breathing to allow muscles to loosen up. The second activity consisted of light stretches to increase flexibility, including neck-stretching motions by tilting the head slowly from side to side and forward, holding each position for about 10 seconds. Shoulder stretches were performed by bringing one arm across the chest and holding it with the opposite arm for 15 seconds, then repeating on the other side. Quadriceps and hamstring stretching exercises were included, wherein participants held one ankle behind for 15 seconds to stretch the front thigh and extended one leg forward while having the other knee slightly bent for back thigh stretching, again for a 15-second hold. Calf stretches were performed by placing one foot in front while keeping the back leg straight and leaning slightly forward. The final phase of the warm-up session involved deep breathing exercises emphasizing relaxation and oxygen intake, with participants seated or standing comfortably, taking deep breaths through their noses, holding for three seconds, and then exhaling slowly through their mouths. This sequence was repeated five times, emphasizing abdominal expansion during inhalation and contraction during exhalation.

For aerobic exercise, patients performed cycling on a stationary bike with an intensity of 60-85% of maximum heart rate (MHR) using the Karvonen formula for 30 minutes, 4 days per week[18]. The physical therapist advised participants to sit on a cycle ergometer with their back supported, hands holding the handlebar, and feet firmly placed on the pedals. The seat height was adjusted according to patient needs, and the pedals were kept at the middle position as a starting point. The exercise started slowly, and the speed was gradually increased to the targeted heart rate range. The quantification of exercise was based on the FITT protocol (frequency, intensity, time, and type) according to ACSM guidelines. The target heart rate (THR) was calculated using the formula: $THR = (MHR - RHR \times Training\ \%) + RHR$, where $MHR = 220 - Age$ and RHR = Resting Heart Rate[18].

The strength training protocol focused on 10 major muscle groups of the body. The sessions were performed for 4 days per week, lasting approximately 30 minutes. The training was divided systematically, with participants performing strength training of upper limb and abdominal muscles (biceps, triceps, pectoralis major, deltoid, and abdominal muscles) on the first and third days, and strength training of lower back and lower limb muscles (latissimus dorsi, abdominals, back extensors, hamstrings, quadriceps, and calf) on the second and fourth days[13]. The intensity of the weight-bearing exercises was calculated using the 1 Repetition Maximum (RM) method. Each muscle group underwent training with three sets, and each set involved 10 repetitions with a rest interval of 1 to 2 minutes between each set[13].

The upper limb and abdominal exercises included bicep curls, where patients stood with feet shoulder-width apart, holding dumbbells at arm's length, then bent the elbow towards the shoulder while breathing out, holding the position for 3 seconds before returning to the starting position while breathing in. Tricep exercises involved bent-over kickbacks, with patients standing with both hands overhead holding a dumbbell, lowering the weight until the elbow reached 90 degrees, holding for three seconds, then returning to the original position. Deltoid exercises consisted of side raises, where patients lifted dumbbells away from the body up to shoulder level while keeping the elbow straight. Pectoralis major exercises involved lying chest presses, with patients lying supine with shoulders at 90 degrees of abduction and elbows at 90 degrees of flexion, lifting towards the ceiling while exhaling. Abdominal exercises included crunches performed in a supine position with knees bent at 60 degrees, hands behind the head, bending the trunk towards the thigh while maintaining chin contact with the chest.
The lower limb and back exercises included quadricep exercises performed as squats, with patients standing holding dumbbells with feet shoulder-width apart, initiating knee movement while keeping the back straight and chest up. Hamstring exercises involved deadlifts using a barbell, extending the knees and hips simultaneously while maintaining proper form. Calf raises were performed by elevating the toes to maximum height while holding dumbbells, maintaining the position for 3 seconds. Latissimus dorsi exercises involved forward-leaning bent-over rows at 60 degrees, drawing dumbbells upward to waist height. Erector spinae exercises included the bird dog exercise, performed in a prone position with participants lifting one leg straight while extending the opposite arm, holding the position before repeating on the opposite side.
Following the conditioning phase, patients performed 10 minutes of cool-down exercises, including slow walks, gentle stretches, and breathing exercises to facilitate recovery and prevent muscle soreness.All three groups received vitamin D supplementation consisting of 50,000 IU of Vitamin D3 administered weekly for the entire 12-week intervention period. This dosage was selected based on established protocols for addressing vitamin D deficiency in clinical populations.
The exercise intervention were terminated for individual participants based on specific safety criteria[18]. Termination occurred if the patient reported any shortness of breath or dyspnea (rate of perceived exertion greater than 8), developed any severe or intolerable symptoms such as chest pain, dizziness, or lightheadedness, experienced a drop in oxygen saturation (SpO2) below 90% on pulse oximeter, showed signs of tissue damage such as bruising, excessive redness, or swelling, or refused to continue due to fatigue and discomfort during the procedure, following ACSM guidelines.

Data Collection Tools

Data was collected before the start of the first session and at the end of the last session based on the following assessment parameters and outcome measures. The DSM-5 (Diagnostic and Statistical Manual of Mental Disorders) was used as a screening tool for the diagnosis of mild to moderate major depressive disorder (MDD), with an Intraclass Correlation of 0.59 demonstrating acceptable reliability.
Oxidative stress was assessed in each group using glutathione peroxidase (GPx) and superoxide dismutase (SOD) through a colorimetric method with commercially available kits. This method involved measuring the optical density at 560 nm of the blue formazan dye produced during the interaction of nitroblue tetrazolium with superoxide radicals generated through the use of xanthine and xanthine oxidase[19]. The SOD present in the sample worked to neutralize the superoxide radicals in the medium, thereby inhibiting the formazan reaction. Specifically, one unit of SOD reduced the nitroblue tetrazolium at a rate of 50% under the specified assay conditions.
Inflammatory markers including TNF-α and IL-6 were assessed using commercially available ELISA kits. The procedures for preparing reagents, standards, and serum samples, as well as the overall assay process, were conducted per the manufacturer's instructions for each respective ELISA kit. Each ELISA kit plate consisted of 116 microtiter wells, all coated with antibodies targeting a specific antigenic site of the respective cytokine molecule. The concentrations of IL-6 and TNF-α were reported in units of pg/mL and mg/L, respectively.
The Patient Health Questionnaire (PHQ-9) was utilized as a subjective score for assessing depression severity level. The questionnaire contains nine questions with a maximum score of 27, and demonstrated good internal consistency with a Cronbach's alpha coefficient of 0.892[20].
Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 22. The demographic data of the participants was determined through descriptive statistics in terms of frequency, mean, and standard deviations. Test of normality was applied using skewness and kurtosis to determine the distribution of data. Depending on data distribution, the paired t-test or Wilcoxon signed rank test was applied for within-group analysis to compare baseline and post-intervention measurements. For between-groups analysis, one-way ANOVA was applied for

normally distributed data, while the Kruskal-Wallis test was used in cases where data deviated from a normal distribution. A p-value of less than 0.05 was considered statistically significant for all analyses.

Prior to the commencement of the study, the protocol was reviewed and approved by the Research Ethics Committee of Ziauddin University (ERC No. 8640424ETREH) and registered on ClinicalTrials.gov (NCT06775548). Before data collection, a participant information sheet was provided to each participant to ensure detailed information regarding the purpose, procedure, benefits, and risks associated with the study. After ensuring understanding of the details, participants were given a consent form for their voluntary participation. Participants' identities, information, and responses remained confidential throughout the study, with all data being appropriately coded.

RESULTS

The study enrolled 116 participants who were randomized equally into three experimental groups, with Group A and Group B, each comprising 39 participants and Group C comprising 38 participants. Throughout the twelve-week intervention period, 5 individuals discontinued participation, yielding an attrition rate of 4.31%. The distribution of withdrawals included two participants from Group A (Aerobic exercise + Vitamin D), two from Group B (Resistance exercise + Vitamin D), and one from Group C (Control + Vitamin D). Consequently, 111 participants completed the entire study protocol. Statistical analysis followed the intention-to-treat approach, including all randomized participants, and used the last observation carried forward (LOCF) method for early withdrawals.

Baseline demographic analysis revealed no significant differences between groups across all measured characteristics (p>0.05), confirming successful randomization. The mean age across groups ranged from 30.21 to 30.79 years, with a relatively balanced gender distribution (40.51% to 46.15% male participants). Body Mass Index (BMI) values were comparable across conditions, ranging from 24.68 to 25.09 kg/m². Depression duration at baseline showed similar patterns across groups, with means between 18.42 and 19.15 months, indicating comparable illness chronicity among participants. The details are depicted in Table-1.

Table-1: Baseline Participant Demographics

Variable	**Group A (Aerobic + Vit D) n=39**	**Group B (Resistance + Vit D) n=39**	**Group C (Control + Vit D) n=38**	**p-value**
Age (years)	30.48±3.12	30.79±2.87	30.21±3.05	0.789
Gender				0.891
Male	18 (46.15%)	17 (43.59%)	16 (40.51%)	
Female	21 (53.85%)	22 (56.41%)	22 (59.49%)	
Body Mass Index (kg/m²)	24.89±2.79	25.09±2.91	24.68±2.82	0.851
Depression duration (months)	18.67±8.38	19.15±8.81	18.42±8.52	0.912

n (%): Frequency (Percentage); Mean±Standard Deviation

SOD concentrations decreased significantly across all treatment conditions following the intervention period. The aerobic exercise plus vitamin D group demonstrated the largest reduction (68.42±37.15 pg/mL, p<0.001), representing a 26.2% decrease from baseline values of 261.08±39.21 pg/mL to 192.66±21.38 pg/mL. The resistance exercise plus vitamin D group showed a 22.1% reduction (56.83±34.28 pg/mL, p<0.001), declining from baseline levels of 257.15±37.46 pg/mL to 200.32±24.87 pg/mL. The control group receiving only vitamin D supplementation exhibited a significant but smaller reduction of 15.8% (41.27±29.54 pg/mL, p<0.001), decreasing from 260.94±38.65 pg/mL to 219.67±28.93 pg/mL. Effect sizes for SOD changes ranged from 1.42 to 1.97, indicating large clinical effects across all interventions, with the aerobic exercise plus vitamin D group demonstrating the largest effect size.

GPX levels similarly declined in all treatment groups. The aerobic exercise plus vitamin D combination produced the greatest reduction (42.15±22.47 pg/mL, p<0.001), representing a 23.7% decrease from baseline concentrations of 177.92±12.18 pg/mL to 135.77±15.32 pg/mL. The resistance exercise plus vitamin D group achieved a 17.9% reduction (31.58±20.14 pg/mL, p<0.001), declining from 176.52±12.84 pg/mL to 144.94±17.05 pg/mL. The control group demonstrated the smallest reduction at 12.6% (22.18±17.92 pg/mL, p<0.001), decreasing from 176.08±11.95 pg/mL to 153.90±16.78 pg/mL. Effect sizes for GPX changes were exceptionally large, ranging from 1.98 to 2.84 across all conditions, with the aerobic exercise plus vitamin D group showing the most substantial effect (Table-2).

Table-2: Pre-post Changes in Oxidative Stress Biomarkers

Biomarker	Group	Baseline	Post-treatment	Change	t-statistic	p-value	Effect Size (d)
SOD (pg/mL)	A	261.08±39.21	192.66±21.38	68.42±37.15	11.51	<0.001***	1.97
	B	257.15±37.46	200.32±24.87	56.83±34.28	10.36	<0.001***	1.73
	C	260.94±38.65	219.67±28.93	41.27±29.54	8.62	<0.001***	1.42
GPX (pg/mL)	A	177.92±12.18	135.77±15.32	42.15±22.47	11.73	<0.001***	2.84
	B	176.52±12.84	144.94±17.05	31.58±20.14	9.80	<0.001***	2.46
	C	176.08±11.95	153.90±16.78	22.18±17.92	7.64	<0.001***	1.98

*Statistical significance: *p<0.05, **p<0.01, ***p<0.001

TNF-α concentrations declined significantly in all treatment groups. The aerobic exercise plus vitamin D group achieved the most substantial reduction (67.94±40.28 pg/mL, p<0.001), corresponding to a 16.1% decrease from baseline levels of 420.87±44.15 pg/mL to 352.93±42.67 pg/mL. The resistance exercise plus vitamin D group showed a 12.3% reduction (51.46±38.92 pg/mL, p<0.001), declining from 423.64±45.38 pg/mL to 372.18±47.25 pg/mL. The control group exhibited a smaller but significant reduction of 8.5% (35.89±33.76 pg/mL, p<0.001), decreasing from 422.15±43.82 pg/mL to 386.26±46.94 pg/mL. Effect sizes for TNF-α changes ranged from 0.78 to 1.31, representing moderate to large clinical effects, with the aerobic exercise plus vitamin D group demonstrating the largest effect.

IL-6 levels decreased across all intervention groups, with the aerobic exercise plus vitamin D combination producing the largest reduction (12.48±5.52 pg/mL, p<0.001). This represented a 13.3% decrease from baseline concentrations of 93.72±4.78 pg/mL to 81.24±3.85 pg/mL. The resistance exercise plus vitamin D group achieved an 8.9% reduction (8.42±5.18 pg/mL, p<0.001), declining from 94.38±5.12 pg/mL to 85.96±4.46 pg/mL. The control group demonstrated the smallest reduction at 5.7% (5.36±4.89 pg/mL, p<0.001), decreasing from 93.94±4.91 pg/mL to 88.58±4.37 pg/mL. Effect sizes for IL-6 changes were large across all conditions, ranging from 1.12 to 2.27, with the aerobic exercise plus vitamin D group showing the most substantial effect (Table-3).

Table-3: Pre-post Changes in Inflammatory Biomarkers

Biomarker	Group	Baseline	Post-treatment	Change	t-statistic	p-value	Effect Size (d)
TNF-α (pg/mL)	A	420.87±44.15	352.93±42.67	67.94±40.28	10.54	<0.001***	1.31
	B	423.64±45.38	372.18±47.25	51.46±38.92	8.27	<0.001***	1.09

	C	422.15±43.82	386.26±46.94	35.89±33.76	6.56	<0.001***	0.78
IL-6 (pg/mL)	A	93.72±4.78	81.24±3.85	12.48±5.52	14.14	<0.001***	2.27
	B	94.38±5.12	85.96±4.46	8.42±5.18	10.16	<0.001***	1.73
	C	93.94±4.91	88.58±4.37	5.36±4.89	6.77	<0.001***	1.12

*Statistical significance: *p<0.05, **p<0.01, ***p<0.001

ANOVA revealed significant between-group differences in final oxidative stress and inflammatory marker concentrations. For SOD, the aerobic exercise plus vitamin D group achieved significantly lower post-intervention levels (192.66±21.38 pg/mL) compared to both the resistance exercise plus vitamin D group (200.32±24.87 pg/mL) and the control group (219.67±28.93 pg/mL), with F=15.82, $p<0.001$, $\eta^2=0.221$. Post-hoc testing confirmed that the aerobic exercise plus vitamin D group differed significantly from all other conditions, while the resistance exercise plus vitamin D group also showed significantly lower SOD levels compared to the control group.

GPX concentrations also showed significant between-group differences (F=18.47, $p<0.001$, $\eta^2=0.249$). The aerobic exercise plus vitamin D group demonstrated the lowest final concentrations (135.77±15.32 pg/mL), significantly different from both the resistance exercise plus vitamin D group (144.94±17.05 pg/mL) and the control group (153.90±16.78 pg/mL). The control group showed the highest post-intervention levels, while the resistance exercise plus vitamin D group achieved intermediate reductions that were significantly different from both other groups.

Tumor necrosis factor-alpha levels differed significantly across groups (F=8.94, $p<0.001$, $\eta^2=0.142$), with the aerobic exercise plus vitamin D group achieving the lowest concentrations (352.93±42.67 pg/mL). The control group demonstrated the highest final levels (386.26±46.94 pg/mL), while the resistance exercise plus vitamin D group (372.18±47.25 pg/mL) showed intermediate outcomes. Post-hoc analysis confirmed that the aerobic exercise plus vitamin D group was significantly different from both other groups, and the resistance exercise plus vitamin D group was significantly lower than the control group.

Interleukin-6 concentrations exhibited substantial between-group differences (F=21.36, $p<0.001$, $\eta^2=0.276$). The aerobic exercise plus vitamin D group achieved significantly lower final levels (81.24±3.85 pg/mL) compared to both the resistance exercise plus vitamin D group (85.96±4.46 pg/mL) and the control group (88.58±4.37 pg/mL). The control group showed the highest post-intervention concentrations, while the resistance exercise plus vitamin D group demonstrated intermediate reductions. All pairwise comparisons were statistically significant, indicating distinct treatment effects across all three groups (Table-4).

Table-4: Post-intervention Oxidative Stress and Inflammatory Biomarker Comparison

Biomarker	**Group A**	**Group B**	**Group C**	**F-ratio**	**p-value**	**η^2**
SOD (pg/mL)	$192.66±21.38^a$	$200.32±24.87^b$	$219.67±28.93^c$	15.82	<0.001***	0.221
GPX (pg/mL)	$135.77±15.32^a$	$144.94±17.05^b$	$153.90±16.78^c$	18.47	<0.001***	0.249
TNF-α (pg/mL)	$352.93±42.67^a$	$372.18±47.25^b$	$386.26±46.94^c$	8.94	<0.001***	0.142

IL-6 (pg/mL)	81.24±3.85[a]	85.96±4.46[b]	88.58±4.37[c]	21.36	<0.001***	0.276

*Statistical significance: *p<0.05, **p<0.01, ***p<0.001

DISSCUSSION

This research examined how aerobic versus resistance training protocols, when paired with Vitamin D supplementation, influenced oxidative stress indicators, inflammation markers, and depressive symptom severity among adults diagnosed with major depressive disorder. The results offer important understanding regarding how these different therapeutic approaches affect the biological processes associated with depression and their possible therapeutic utility.

Substantial decreases in oxidative stress indicators were observed across all treatment groups, with SOD levels declining 26.2% among participants receiving aerobic training plus Vitamin D (Group A), 22.1% in those assigned resistance training with Vitamin D (Group B), and 15.8% in the control group receiving only Vitamin D. GPX concentrations similarly diminished by 23.7%, 17.9%, and 12.6% in Groups A, B, and C respectively. These marked improvements indicate that exercise approaches, when supplemented with Vitamin D, successfully reduce oxidative burden in depressed individuals more effectively than vitamin supplementation alone. The magnitude of these changes, reflected in large effect sizes (Cohen's d ranging from 1.42 to 1.97), demonstrates the meaningful clinical impact of these combined interventions on oxidative stress measures[6-7]. Inter-group analysis showed that participants in Group A (aerobic training combined with Vitamin D) exhibited significantly reduced SOD and GPX levels following treatment compared to both Group B and the control group. This outcome indicates that aerobic exercise protocols may provide enhanced effectiveness for lowering oxidative stress biomarkers when paired with Vitamin D therapy[11]. The biological basis for this distinction could relate to aerobic training's ability to enhance mitochondrial biogenesis and improve peripheral circulation, potentially contributing to improved antioxidant function via enhanced cellular pathways that neutralize free radicals[21-22].

Significant decreases in inflammation biomarkers were noted across all treatment groups, with TNF-alpha levels dropping 16.1% in Group A, 12.3% in Group B, and 8.5% in the control group. IL-6 concentrations fell by 13.3%, 8.9%, and 5.7% in Groups A, B, and C respectively. These results demonstrate that aerobic and resistance training protocols, when supplemented with Vitamin D, successfully lower systemic inflammatory responses linked to depressive disorders more effectively than vitamin D alone[8]. The substantial effect sizes (Cohen's d ranging from 0.78 to 2.27) observed across groups support the clinical meaningfulness of these anti-inflammatory outcomes. Between-group comparisons indicated that Group A participants (aerobic training with Vitamin D) achieved significantly reduced TNF-alpha and IL-6 concentrations post-treatment relative to both Group B and the control group. This pattern suggests aerobic exercise protocols offer superior anti-inflammatory benefits compared to resistance training when paired with Vitamin D therapy. The enhanced inflammatory reduction associated with aerobic activity is consistent with research demonstrating that continuous moderate-intensity exercise creates a stronger anti-inflammatory response by influencing cytokine regulation and promoting myokine release[11,22]. Aerobic training has additionally been documented to more directly impact inflammation within adipose tissue, representing an important source of systemic inflammatory burden in depressive conditions.

Both exercise interventions demonstrated remarkable improvements in depression severity, with PHQ-9 scores decreasing by 49.1% in Group A, 38.2% in Group B, and 22.4% in the control group. The exceptionally large effect sizes (Cohen's d ranging from 1.98 to 6.24) for all groups highlight the substantial clinical impact of these interventions on depressive symptoms, with exercise groups showing considerably larger effects than the control group. These reductions far exceed the established minimally important clinical difference of 5 points on the PHQ-9 scale, indicating that all interventions led to clinically meaningful improvements in depression symptoms[20]. Between-group analysis revealed significantly lower post-intervention PHQ-9 scores in Group A compared to both Group B and the control group, suggesting that aerobic exercise with Vitamin D may be more effective than resistance exercise with Vitamin D or vitamin D supplementation alone in reducing depression symptoms. This finding may be explained by the more potent anti-inflammatory and antioxidant effects observed in Group A, as inflammation and oxidative stress are increasingly recognized as key pathophysiological mechanisms in depression[6,8]. Additionally, aerobic exercise has been associated with greater increases in brain-derived neurotrophic factor (BDNF), which plays a crucial role in neuroplasticity and has been implicated in the therapeutic effects of exercise on depression[11,23].

The comparative analysis of the current findings with existing literature reveals several important patterns. Previous researches demonstrated significant reductions in oxidative stress markers with aerobic exercise alone, while studies examining vitamin D supplementation in isolation, such as showed more modest improvements[6,16]. The current study's superior outcomes suggest synergistic effects between exercise and vitamin D supplementation[10]. Exercise increases endogenous antioxidant production while creating an acute oxidative challenge that may prime cellular defenses, and vitamin D complements this process by providing additional anti-inflammatory and antioxidant support[10,14]. Similarly, research on inflammatory markers showed that exercise and vitamin D individually reduce inflammation in depression, but the current study's larger effect sizes indicate that combined interventions offer enhanced benefits through complementary mechanisms[8]. Exercise reduces adipose-derived inflammation, increases anti-inflammatory myokine production, and improves immune regulation, while vitamin D directly modulates inflammatory pathways and supports cellular antioxidant systems[10,14,22].

Regarding depression severity outcomes, the current study's findings align with and extend previous research that demonstrated aerobic exercise alone produced a 47% reduction in depression severity, comparable to antidepressant medication. The current study's finding of a 49.1% reduction with aerobic exercise plus vitamin D suggests that the addition of vitamin D supplementation may offer modest additional benefits. The control group's 22.4% improvement with vitamin D alone is comparable to findings that reported a reduction with vitamin D supplementation in deficient patients[16]. The substantially greater improvements observed in the exercise groups underscore the crucial role of physical activity in depression treatment[11,23]. The finding that aerobic exercise with vitamin D achieved lower absolute post-intervention depression scores than resistance exercise with vitamin D, despite both showing substantial improvements, suggests that aerobic exercise may interact more favorably with vitamin D's mechanisms through enhanced neuroplasticity, improved mitochondrial function, or more efficient redox regulation in neural tissues[21-24].

Several limitations must be considered when interpreting these findings. The relatively homogeneous age range (25-35 years) limits generalizability to older or younger populations. Future studies should include broader age ranges to examine potential age-dependent effects of these interventions. Although key oxidative stress and inflammatory markers were assessed, more extensive biomarker panels including BDNF, cortisol, and additional cytokines would help elucidate the mechanisms underlying the differential effects observed. The 12-week intervention duration does not provide insights into long-term sustainability of the observed benefits, and longitudinal studies with extended follow-up periods are needed to determine whether improvements persist beyond the intervention period. The absence of exercise-only control groups limits the ability to isolate the specific contributions of each component to the observed effects, and future factorial designs could help identify potential synergistic interactions. Finally, potential moderators of treatment response, such as genetic factors, baseline fitness levels, or psychosocial variables, were not assessed, and identifying such moderators could help personalize interventions based on individual characteristics and needs.

The findings have important clinical implications. Both aerobic and resistance exercise combined with vitamin D supplementation represent effective interventions for addressing multiple pathophysiological mechanisms in depression, including oxidative stress, inflammation, and clinical symptoms. Healthcare providers should consider incorporating these interventions into comprehensive treatment plans for individuals with depression. The differential effects of the two exercise modalities suggest the potential for personalized approaches based on individual needs and priorities. Aerobic exercise with vitamin D may be preferable for patients with more pronounced inflammatory profiles or greater symptom severity, while resistance exercise with vitamin D might yield favorable outcomes for those seeking structured strength training protocols. The favorable safety profile observed in the study suggests that these interventions are well-tolerated and feasible for implementation in clinical practice, though proper supervision and gradual progression remain essential. The substantial clinical improvements highlight the potential of these interventions as adjunctive or even alternative approaches to conventional pharmacotherapy for depression, particularly for individuals who prefer non-pharmacological options or experience adverse effects from medications.

CONCLUSION

This study demonstrates that aerobic and resistance exercise combined with vitamin D supplementation significantly reduces oxidative stress, inflammation, and depression severity in adults with major depressive disorder, with all interventions producing superior outcomes compared to vitamin D supplementation alone. Aerobic exercise with vitamin D appears most effective across all measured outcomes, demonstrating the largest reductions in oxidative stress markers (26.2% for SOD, 23.7% for GPX), inflammatory markers (16.1% for TNF-α, 13.3% for IL-6), and depression severity (49.1% reduction in PHQ-9 scores). Resistance exercise with vitamin D also produced substantial benefits, though to a lesser degree than aerobic exercise, while vitamin D supplementation alone showed modest but clinically meaningful improvements. These findings enhance our understanding of the differential effects of exercise modalities combined with vitamin supplementation on the pathophysiological mechanisms underlying depression and provide evidence-based guidance for the development of personalized exercise and supplementation interventions for individuals with depression. The exceptionally large effect sizes observed, particularly for the aerobic exercise group, suggest that combined exercise and vitamin D interventions may represent a highly effective treatment approach that warrants consideration as part of comprehensive depression management strategies. Future research should focus on further elucidating the mechanisms underlying these differential effects, identifying moderators of treatment response, evaluating the long-term sustainability of these interventions, and exploring optimal combinations and dosing strategies to maximize therapeutic benefits for individuals with depression.

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