

⇒ Research Article



The Effect of Short-term Beta-hydroxy Beta-methylbutyrate Supplementation on Serum Immunoglobulin A and G Levels in Male Wrestlers Following an Exhaustive Exercise: A Randomized Clinical Trial

Mona Madelat¹, Abbas Sadeghi^{2*} , Ali Hematti Afif²

¹Master of Sports Physiology, Imam Khomeini International University, Qazvin, Iran

²Department of Sports Sciences, Faculty of Social Sciences, Imam Khomeini International University, Qazvin, Iran

Abstract

Background: Exercise training has been shown to induce transient changes in immunity responses. Researchers have reported conflicting results about the effect of vigorous exercise training on immunoglobulins levels. Due to the lack of consistent existing findings as well as the lack of sufficient studies to clarify some of the ambiguities associated with scientific inconsistencies, this study aimed to examine the effect of short-term beta-hydroxy-beta-methyl butyrate (HMB) supplementation on serum immunoglobulin A (IgA) and immunoglobulin G (IgG) levels in male wrestlers after an exhaustive exercise.

Methods: In this randomized clinical trial conducted at the IKIU Sports Physiology Laboratory in Qazvin, Iran, January 2019, 16 wrestlers completed an informed consent form and, then, were randomly divided into two groups (i.e., the supplement group (n=8) and the control group (n=8)). The supplement group received daily 40 mg/kg body weight HMB supplement over two weeks (about 3 g/day). As for the control group, starch was used as a placebo. Blood samples were obtained from the subjects to measure the serum immunoglobulin in five phases, including the pre-supplementation, before, immediately after, 1 hour after, and 24 hours after the exercise protocol. "Bruce test until exhaustion" was adopted as the exercise protocol in this study. Data analysis was performed by using repeated measure analysis and SPSS 24 software.

Results: The results showed that there was no significant difference between the groups regarding the effects of supplementation of HMB on IgG ($P=0.75$) and IgA ($P=0.56$) levels. However, significant changes were observed in the levels of IgA ($P=0.049$) and IgG ($P=0.001$) in the groups.

Conclusion: According to the study results, It was suggested that the use of HMB supplementation at the dose used in this study may not have significantly affected the IgG and IgA levels after an exhaustive activity. However, it was recommended that further studies be carried out in this area to produce more consistent findings and clarify the ambiguities about the given issue.

Keywords: Beta-hydroxy-beta-methylbutyrate, Immunoglobulins, Immunity, Exercise

*Correspondence to

Abbas Sadeghi, Department of Sports Sciences, Faculty of Social Sciences, Imam Khomeini International University, Qazvin, Iran.
Tel: +982833901782,
Email: sadeghi@soc.ikiu.ac.ir



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Background

Recent researches have shown that stress from strenuous exercise can lead to dysfunction of the immune system and acute or chronic inflammation. Athletes are more susceptible to upper respiratory tract infections during strenuous physical activity (1). Immunoglobulins or antibodies, as glycoprotein molecules, are produced by plasma cells in response to an alien agent (2). Immunoglobulin G (IgG) as the most abundant antibody isotype is found in the circulation system (3). Immunoglobulin A (IgA) is the predominant immunoglobulin in mucosal fluids that inhibits pathogens' adhesion and neutralizes viruses and toxins (4). The relationship between heavy exercise and the disease's readiness has been confirmed (5). It is also a

common knowledge that physiological responses to acute and chronic adaptations of immunity to exercise training rely on the type or dose of exercise (6).

Researchers have found conflicting results about the effect of vigorous exercise training on immunoglobulins levels. Exercise training can cause a change in the concentration serum immunoglobulins levels (7, 8). McKune et al showed that performing exercise for 60 minutes with 75% of VO₂max resulted in significant increases in IgA, IgG, IgG₂, and IgG₃ concentrations (9). However, some other studies have produced different results. A study investigating the effects of exercise training time on serum immunoglobulin in male athlete students, for example, found no significant differences between the serum immunoglobulin levels of the subjects

in both groups in pre and post-test (10). Moreover, a study exploring the effects of optional exercise on A, M, and G serum immunoglobulins among professional semi-endurance runners demonstrated that prolonged and intensive activities may have suppressed the immune system, but moderate and short workouts may have strengthened this system (7).

Leucine, a branched-chain amino acid, is believed to have immunomodulatory effects, mediated by its metabolites (11). Beta-hydroxy beta-methylbutyrate (HMB) is a metabolite derived from leucine (12). The dietary supplement HMB has been discovered to decrease muscle proteolysis during exercise and disease (13). A few clinical studies have revealed that HMB may contribute to a decline in muscle mass loss in diseases that have catabolic features, as well as to a reduction in sarcopenia and some other conditions characterized by loss of muscle mass and aging (14). It has been specified that HMB supplementation causes more time-up riding to exhaustion in a graded cycle ergometer test (15). Furthermore, HMB plays an essential role in reducing the inflammatory cytokines such as interleukin6 (IL-6) and tumor necrosis factor alpha (TNF- α) (16). Studies on strenuous physical activity have suggested that HMB consumption may reduce the detrimental effects of such physical stresses. These stresses depend on the rate at which cytokines increase as inflammatory agents (17). A recent study has shown that when soldiers supplement their diets with HMB for three weeks during intense training, the inflammatory response is diminished and accompanied by muscle integrity (16). These results are consistent with the findings from other researches, suggesting that short (e.g., four days) (18) and long duration (e.g., 12 weeks) (11) HMB supplementation can reduce the cytokine response to muscle-damaging training protocols. Since salivary IgA concentrations have been reported to be suppressed in response to intense exercise, they remain either unaltered or increased in response to moderate-low intensity physical training (19). As for IgG, and taking into account the facts that vigorous exercise reduces protein and causes muscle damage and immunoglobulins are protein molecules, it is assumed that HMB supplementation can positively contribute to proteinization and prevention of protein catabolism, as well as prevent IgG degradation and reduce immune system weakness during intense exercise (20).

Objectives

This study aimed to determine the effect of short-term HMB supplementation on serum IgA and IgG levels in male wrestlers following an exhaustive exercise, as well as to discuss some of the ambiguities surrounding the scientific inconsistencies and provide coaches, athletes, and practitioners of various sports disciplines with valuable information and suggestions in this regard. Our study results may have also been instrumental in

neutralizing or reducing the negative effects of strenuous physical activity on the body's immune system, especially following exhaustive exercise activities and the potential role of HMB supplementation in this field.

Materials and Methods

The present study was a randomized clinical trial of subjects whose pre-test and post-test design were compared with the control group.

Study Populations

The study was conducted at the Physiology Laboratory of the IKIU in Qazvin, Iran, January 2019. The statistical population included young male wrestlers aged between 17 and 27 from Qazvin province, with at least three years of professional experience.

Our study sample size was determined based on the sample size adopted in previous similar studies (21). Then 16 volunteer male wrestlers were asked to complete an informed consent form, and the candidates were selected through randomization (Figure 1). The subjects were given the necessary explanations about the training protocols and the applied interventions in the research before starting the examination. Next, the subjects were asked to complete a 24-hour food recall questionnaire as well as a health assessment questionnaire. Participants were strongly advised not to change their usual diet, and the researchers attempted to control this issue personally. The volunteers were randomly assigned into two homogeneous groups to receive either HMB (n=8) or placebo (n=8) based on some of the subjects' individual and physiological characteristics so that no significant difference was observed between the two groups regarding these features (Table 1). The groups were run in parallel, with each group receiving only one form of treatment.

Inclusion criteria for the study participants were those: aged 17-27 and were physically healthy, non-alcohol consumers, non-smokers, and not on drugs significantly contradicting with measurement parameters. Participants who were physically unhealthy, outside the age range, alcohol/drug addicts, especially addicts of steroid/hormonal substance interfering with the study parameters were excluded from this study. In all research stages, test conditions and potential risks were precisely explained in two thorough sessions. The subjects were requested to fill a personal info questionnaire to specify their exercise history, type of exercise, injury history, specific illness, and medication use. Written informed consent was obtained from all volunteers after explaining all procedures and risks of the participation in the study (e.g., about blood sampling, etc.). Subjects were also requested not to use any active ingredients during the study period, such as vitamins, dietary supplements, herbs, or other medications. We did a randomized double-blind trial method in the study. The participants in two groups were

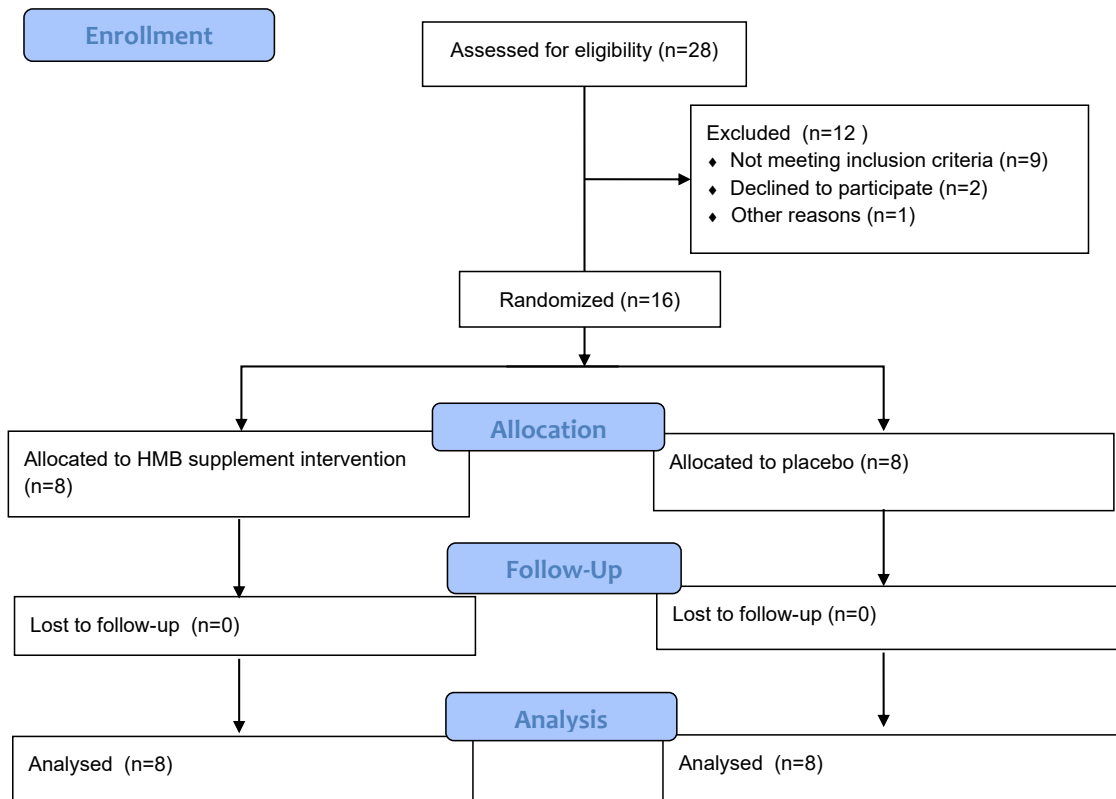


Figure 1. CONSORT Flow Diagram of the Study.

Table 1. General Characteristic of Participants

Variables	Groups	Mean \pm SD	P Value ^a
Age (y)	HMB	18.28 \pm 1.3	0.23
	Control	19.25 \pm 1.48	
Height (cm)	HMB	171.25 \pm 5.9	0.52
	Control	173.00 \pm 4.34	
Weight (kg)	HMB	70.83 \pm 5.97	0.34
	Control	74.46 \pm 8.35	
BMI (kg/m ²)	HMB	24.14 \pm 1.47	0.46
	Control	24.83 \pm 2.08	
Body fat (%)	HMB	17.6 \pm 4.22	0.23
	Control	15.32 \pm 2.9	

Abbreviations: BMI, body mass index; HMB, beta-hydroxy beta-methylbutyrate.
^a P value \leq 0.05 was considered statistically significant.

followed up and the data were collected in the same way.

Exercise Training Protocol

The training protocol followed in this study was the maximal Bruce test. The test which is one of the most common tests to assess the cardiovascular and respiratory systems (22) includes seven 3-minute stages, and the length of working time on the treadmill is regarded as the test score. The test starts at 2.74 km/h (1.7 mph) at 10% grade, and the incline rises by 2% in three minutes. In this study, subjects warmed up for 10 min before the test and then continued to run until they were exhausted.

Their heart rate and blood pressure were recorded before and immediately after the test. The treadmill used in this research was the HPCOSMOS brand made in Germany.

Treatment Supplementation

Subjects were randomly assigned to either placebo or HMB groups. Supplementation consisted of 40 mg daily HMB per kg body weight of the subjects, provided in three servings, that is, 30–60 minutes before daily exercise, immediately after exercise, and before bedtime (23). This plan was followed by the supplement group for two weeks. The consumable supplements were manufactured by Karen-Iran Pharmaceutical Company (PNC). Placebos (i.e., starch-containing capsules similar in amount, size, and weight to those containing HMB supplement) were obtained in identical plain packaging, which kept the subjects blind to the study procedure.

Blood Sampling

IgG and IgA levels were measured during five stages: 1) Baseline (Pre1), before starting supplementation or placebo consumption; 2) Baseline (Pre2), on the day of exercise intervention, after two weeks of supplementation or placebo consumption before the starting exercise protocol. In this stage, blood samples, resting blood pressure, resting heart rate, and subjects' body composition were collected using the ZEUS 9.9 PLUS Body Composition Analyzer. All blood samples were

taken 5 minutes after resting in a sitting position from an antecubital arm vein. After one hour, the exercise protocol was started; 3) Immediately after the exercise protocol (Post); 4) One hour after exercise protocol (Post1h); 5) 24 hours after exercise protocol (Post24h). All measurements and protocols were conducted from 9 to 12 am to avoid circadian variations.

All blood samples were immediately centrifuged at 3000 r.min⁻¹ for 15 minutes. The serum was separated and frozen at -80°C for later analysis. Serum immunoglobulin levels were measured using the US-made Selectra ProXL analyzer and immunoturbidimetric method. The kit used in this study was the Pars Test Kit whose serial number was 97001. All blood samples collected from each subject during all stages were analyzed in the same batch by an experienced technician blinded to the group (placebo vs. HMB) and sampling stage (pre vs. post-training stage).

Statistical Analysis

After collecting the data, the normal distribution was evaluated using Shapiro-Wilk, and homogeneity of the variances was determined using Levene's tests. Then repeated measures ANOVA and Bonferroni post hoc tests were used. All data were analyzed by SPSS 24 software at a significant level of 0.05.

Results

According to the results obtained from Shapiro-Wilk and Levene's tests, the significance level recorded at five various time points for each variable was higher than 0.05 ($P > 0.05$); therefore, we assumed that data are normally distributed with the same spread (homogeneity). Then, Repeated measures ANOVA test was used. Table 1 shows the general characteristic of participants. As shown in the given table, no significant difference was detected between two groups in terms of the subjects' general characteristics.

Table 2 compares IgG and IgA concentrations between HMB and control groups at five stages of blood sampling. According to the test results, intra-group changes in IgG were less than 0.05, and were significant ($P = 0.001$); a significant change was observed in IgA in the study groups at five stages of blood sampling ($P = 0.049$). This

table also shows that inter-group variations produced the most significant results. As shown in the table, significant levels were greater than 0.05, indicating no significant difference between groups regarding the changes in variables IgG ($P = 0.75$) and IgA ($P = 0.56$) at five blood sampling stages (Figure 2).

Discussion

The present study investigated the effects of short-term HMB supplementation on IgA and IgG levels following

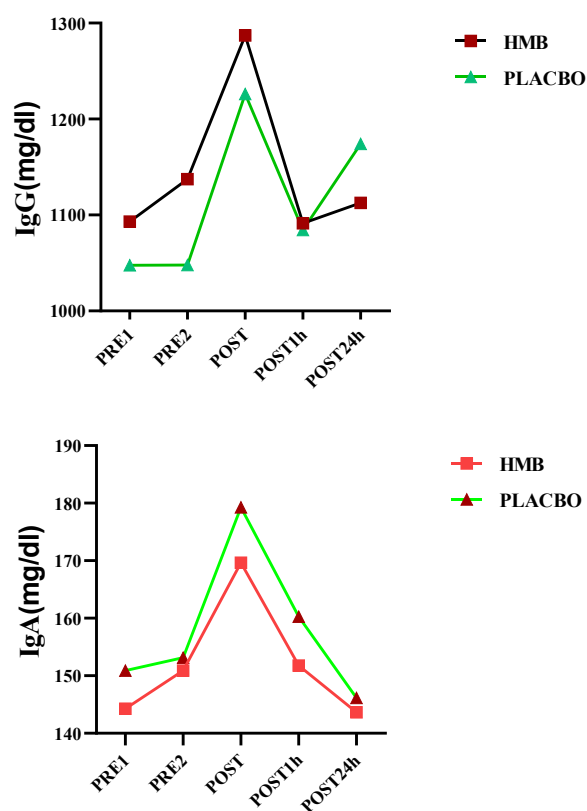


Figure 2. The Concentration of Serum IgG and IgA in the Control and HMB Groups at Five Stages of Blood Sampling. A significant difference is seen in Intra-group, and no significant difference is observed Inter-group at $P < 0.05$. Abbreviation: IgA, Immunoglobulin A; IgG, Immunoglobulin G; HMB, beta-hydroxy-beta-methyl butyrate; Pre1, Baseline before starting supplementation or placebo consumption; Pre2, Baseline on the day of exercise intervention, after two weeks of supplementation or placebo consumption before the starting exercise protocol; Post, Immediately after the exercise protocol; Post1h, one hour after exercise protocol; Post24h, 24 hours after exercise protocol.

Table 2. Comparison of IgG and IgA Concentration Between HMB and Control Groups at Five Stages of Blood Sampling

Variables	Groups	PRE1 Mean ± SD	PRE2 Mean ± SD	POST Mean ± SD	POST1h Mean ± SD	POST24h Mean ± SD	Inter-group Changes P Value ^a	Intra-group Changes P Value
IgG (mg/dL)	HMB	1093.13 ± 141	1137.38 ± 124	1287.13 ± 152	1091.38 ± 166	1112.5 ± 139	0.75	0.001
	Control	1047.63 ± 223	1047.75 ± 225	1226 ± 258	1084.25 ± 190	1174 ± 159		
IgA (mg/dL)	HMB	144.25 ± 43	150.88 ± 42	169.63 ± 52	151.75 ± 51	143.63 ± 52	0.56	0.049
	Control	150.88 ± 69	153.13 ± 68	179.25 ± 84	160.25 ± 81	146.13 ± 69		

Abbreviations: IgA, Immunoglobulin A; IgG, Immunoglobulin G; Pre1, Baseline before starting supplementation or placebo consumption; Pre2, Baseline on the day of exercise intervention, after two weeks of supplementation or placebo consumption before the starting exercise protocol; Post, Immediately after the exercise protocol; Post1h, one hour after exercise protocol; Post24h, 24 hours after exercise protocol.

^a P value ≤ 0.05 was considered statistically significant.

an exhaustive exercise in young male wrestlers. A novel aspect of this investigation was its measurement of IgG and IgA as immunity markers. The results showed that there was no significant difference between the groups regarding the effect of HMB supplementation on IgG and IgA levels. However, the levels of IgA and IgG changed significantly in the groups ($P < 0.05$).

The mechanisms associated with alterations in serum immunoglobulin levels in response to exercise among athletes have not been entirely and accurately identified yet, and the changes related to these factors need to be well-considered (24). An increase in serum immunoglobulin concentration of less than 10% has been found attributable to its daily changes and extracellular circulation as well as to the lymphatic storage of immunoglobulin (25). Cells and various factors are involved in regulating the production of IgG by B cells. These factors include the release of immune regulatory elements such as cytokines, the number and sensitivity of lymphocyte receptors to these molecules, the number and proportion of circulating lymphoid cells and lymphoid tissues, neurohormonal changes circulating hormone levels, the subject sensitivity, and the effect of psychological stress. It is worth mentioning that the given factors may work in parallel (26). The acute effects of exercise may persist or may interfere/overlap with the chronic effects of exercise (27). A study by Gunga et al found that IgA, IgG, and IgM plasma levels increased in the early hours of a marathon race, but returned to normal levels afterward (8). Since monocytes increase during exercise and prostaglandins are produced from these cells, the soluble factors such as prostaglandins released during exercise indirectly affect immunoglobulin productions (28).

According to our results, exhaustive physical activity and HMB supplementation had no significant effects on serum IgG and IgA level changes in male wrestlers. However, supplementation with HMB may have slightly improved serum IgG and IgA levels in them. Our study results regarding the effect of short-term HMB supplementation on serum IgG and IgA before an exhaustive physical activity were not consistent with the findings from the studies by Jalili et al (29) and Shirvani and Sobhani (30). Jalili et al investigating the effect of an intensive anaerobic training session on serum levels of IgA, and IgG in handball, volleyball, and mountaineering athletes reported no significant changes in serum IgG levels before, after, and two hours after exercise (29). Shirvani and Sobhani also observed no significant change in the levels of IgG in soccer players before and after two consecutive matches, following their intake of vitamin C supplement (30). Karampour et al reported that both high-intensity intermittent activity and one session of resistance exercise significantly decreased IgG serum levels (31).

This finding was in agreement with the results from

the studies by McKune et al (9), Aghaee et al (32), and Karampour et al (11). McKune et al detected a significant increase in total serum IgG immediately after a session of ultra-marathon 90 km among elite runners (33). Aghaee et al suggested that probiotic consumption following an exhaustive activity may have increased some immune factors such as lymphocytes, monocytes, and granulocytes (32). The response of each immunoglobulin to the duration and intensity of an activity as well as the type of supplementation seemed to be different. The results were completely contradictory and varied depending on the increase or decrease in duration and intensity of the activity.

Our study results concerning the effect of short term HMB supplementation before a specific activity on serum IgA levels of young men athletes were not in line with the findings of Thomas et al (34), Shirvani and Sobhani (30), and Mir et al (35). Thomas et al demonstrated that high-intensity short-term anaerobic exercise did not significantly alter the salivary IgA levels (34). Shirvani and Sobhani showed that the levels of IgA did not change significantly in soccer players before and after two consecutive matches, following their intake of vitamin C supplement (30). Mir et al found no significant change in IgA levels after the combined exercise (35).

However, our study results were in good agreement with the study findings of Karacabey et al (36), Allgrove et al (37), and Safaei et al (38). The results of the study by Karacabey et al revealed that moderate-intensity exercise exerted a favorable effect on the immune system and increased immunoglobulin levels (36). Allgrove et al studied the effects of exercise intensity on salivary antimicrobial proteins and stress markers in inactive men, and concluded that short-duration, high-intensity exercise increased the secretion rate of s-IgA, although no change occurred in the saliva flow rate (37). Safaei et al also determined that IgA levels were decreased after six intermittent exercises (38). Our study argued that HMB supplementation duration may have been an essential factor in serum IgA and IgG levels in male wrestlers after an exhaustive exercise.

To the best of our knowledge, this study was the first investigation to examine the effect of HMB supplementation on serum IgA and IgG levels following an exhaustive exercise. Our study faced two limitations. First, only 40 mg daily HMB per kg body weight of the subjects in three servings was investigated in this study and, as the result, significant uncertainty remained about the optimal dosage of HMB intake required to improve the serum IgA and IgG levels following an exhaustive exercise. Therefore, it was recommended that further studies be carried out to examine the relationship between lower/upper dosage of HMB and serum IgA/IgG levels following an exhaustive exercise. Second, based on our study results, probably more than two weeks of HMB

supplementation needs to affect serum IgA and IgG levels positively. However, it is unclear about the period of two weeks of HMB supplementation.

It was also suggested that further studies be conducted to clarify the relationship between optimal periods in this regard. This study also had some other limitation common among many studies, including the short research period compared to a more extended period, high costs of assessment resulting in a failure to assess other immune system factors related to the research topic, and a failure to thoroughly investigate the effects of intense short-term or long-term anaerobic exercise on immunoglobulins and other factors as well as the functions of the immune system and the response of skeletal muscle to the exhausting activities.

Conclusion

In this study, short-term HMB supplementation effects on serum IgA and IgG levels of young men athletes before an exhaustive activity were investigated. It was found that HMB supplementation in the given dose had no significant effects on the levels of IgG and IgA after an exhaustive activity. IgA and IgG were discovered to be significantly affected by the Intra-group results; however, inter-group changes were not significant. Therefore, it was concluded that two weeks of HMB supplementation does not affect improving immune function after exhaustive physical activity compared to the placebo group. However, more detailed studies are recommended due to inconsistent results and the small number of initial investigations.

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Conflict of Interests

The authors declare that they have no conflict of interests.

Ethical Approval

The study was approved by the Ethics Committee of the Imam Khomeini International University (IKIU) (Code: IR.IKIU.1397.17682), and the principles and codes of the ethics were all followed when conducting it.

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