Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Mild exercise along with limb blood-flow restriction modulates the electrocardiogram, angiotensin, and apelin receptors of the heart in aging rats

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ARTICLE INFO

Article type:
Original article

Article history:
Received: Jul 7, 2017
Accepted: Sep 28, 2017

Keywords:
Aging
Angiotensin II receptors
Apelin receptor
Blood flow restriction
Endurance exercise

ABSTRACT

Objective(s): Considering the lack of information, the effects of mild endurance exercise plus blood flow restriction (BFR) on electrocardiographic parameters, hypertrophy index, and expression of angiotensin II receptors type 1 (AT1R) and type 2 (AT2R) and apelin receptor (APJ) were assessed in hearts of old male rats.

Materials and Methods: Animal were grouped as control (CTL), Sham (Sh), lower extremities blood flow restriction (BFR), exercise (Ex), Sham + exercise (Sh + Ex), and blood flow restriction + exercise (BFR + Ex).

Results: Exercise plus BFR significantly decreased the corrected QT (QTc) interval (P<0.01 vs CTL and Sh groups) and increased the heart hypertrophy index (P<0.05 vs CTL and BFR groups). Exercise alone increased expression of the APJ (P<0.01, vs CTL, Sh, and BFR groups) and AT2 receptors (P<0.001, vs Sh, CTL, BFR, and BFR + exercise groups), whereas it reduced expression of AT1R (P<0.01 in comparison with CTL, Sh, and BFR groups). Exercise plus BFR caused a significant increase in APJ (P<0.05 vs Ex, Sh+Ex and P<0.001 vs CTL, Sh, and BFR groups) and also expression of AT1R (P<0.001 vs Ex, Sh + Ex, CTL, Sh, and P<0.01 vs BFR groups). Accompaniment of exercise with BFR destroyed the effect of exercise on the expression of AT2R.

Conclusion: Mild endurance exercise plus BFR can alter the expression of angiotensin II and apelin receptors that leads to cardiac hypertrophy and improves the ventricular conductivity of aging rats.

► *Please cite this article as:*
Raji-amirhasanig AR, Joukar S, Naderi- Boldaji V, Bejeshk MA. Mild exercise along with limb blood-flow restriction modulates the electrocardiogram, angiotensin, and apelin receptors of the heart in aging rats. Iran J Basic Med Sci 2018; 21:558-563. doi: 10.22038/IJBMS.2018.24796.6165

Introduction

The American College of Sports Medicine has suggested that sports exercises can lead to muscle growth only if exercise intensity is equal to or more than 60% of one repetition maximum (RM) (1). However, exercise along with blood flow restriction (BFR) has challenged the above theory: hypertrophic adaptations can occur with less intense exercise (2, 3). In this type of exercise, also known as Kaatsu, a pressure cuff or tourniquet that is tied to the proximal part of lower extremities (4, 5) or upper extremities is normally used (6), and the extremity is affected by ischemia during exercise. The safety of this type of exercise, especially for the cardiovascular system, has not yet been proven despite evidence on its effectiveness for inducing skeletal muscle hypertrophy (7-15). Increased blood pressure and myocardial oxygen demand may occur following BFR. Because BFR could be associated with enhanced systemic vascular resistance as well as accumulation of metabolites and hence stimulation of chemoreflex (6, 16). Furthermore, studies have shown that short periods of non-lethal ischemia and reperfusion in various organs such as the kidneys, intestines, limbs, and liver may induce ischemic tolerance in the same organ and other organs such as the heart (17, 18).

On the other hand, it is well- known that angiotensin (AngII) type 1 (AT1R) and type 2 (AT2R) receptors and apelin receptor (APJ), which are in the heart are involved both in hemodynamic activity and heart remodeling changes. Expression of the above receptors change in different physiological conditions (e.g. sports exercises) (19-21) and pathological conditions (e.g. cardiovascular diseases) (21-23) as well as aging (24, 25). Previous studies indicated that apelin engages in the cardiac contractility response, especially in the slow force response mechanism (26). In addition, there is a reverse adjustment between the apelin system and its receptor with the angiotensin system (27). Given the fact that in many countries the population is aging and many elderly people are not able to do heavy exercise, we decided to test the effect of this type of exercise on the hearts of elderly rats in order to evaluate its possible desired and undesired effects experimentally. Accordingly, the present study aimed to investigate

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the effect of light endurance exercise along with blood flow restriction of lower extremities on the expression of AT1R, AT2R, and APJ in the heart tissue followed by studying ECG changes and possible cardiac hypertrophy due to this exercise type as well.

Materials and Methods

Animals

The experiment was performed on 60 male rats aged 22–24 months with a weight range of 350–450 g. The animals had free access to food and water and were kept in 12-hr light and 12-hr dark cycles.

Ethical approval

All animals used in this study received humane care and the experimental procedures were reviewed and approved by the Ethics Committee of Kerman University of Medical Sciences (Ethic committee permission no: IR.KMU.RE.1395/197, Kerman University of Medical Sciences, Kerman, Iran).

Study Design

The rats were divided into six groups randomly:

(1) Control group (CTL): which was monitored for 11 weeks and no intervention was carried out. The animals were then anesthetized using sodium thiopental (50 mg/kg), which was recorded using a Power Lab device, Lead II ECG. Then, the hearts of the animals were collected to determine the extent of hypertrophy and AT1R, AT2R, and APJ levels of expression (28).

(2) Sham group (Sh): In this group, the animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg). Then the inner groin surfaces were incised to access femoral arteries. Thereafter, the femoral arteries were released from femoral sheaths and a small amount of penicillin powder was placed on the wounds, the incisions were stitched and the animals were taken care to recover from anesthesia and care was taken for 11 weeks.

(3) Limb Blood Flow Restriction group (BFR): Similar to the Sham group, all animals of this group were anesthetized and operated. In order to restrict the blood flow, after accessing the femoral artery, a 0.014-inch diameter steel wire was placed on the femoral artery and it was tightly knitted using a silk stitch thread (0-4); then carefully the wire was pulled out and incision sites were sutured. This condition did not restrict muscle blood flow in resting mode, but during contraction and training on the treadmill, the muscle encountered anemia (29-31).

(4) Exercise group (Ex): Animals in this group did light exercises for 10 weeks.

(5) Sham + Exercise group (Sh + Ex): There were sham operations in this group and a one-week recovery period was given to heal the wounds and then light exercises were done for 10 weeks.

(6) Limb Blood Flow Restriction + Exercise group (BFR + Ex): Similar to BFR group, surgery was performed in this group and a one-week recovery period was given to heal their wounds and then light exercises were done for 10 weeks. 24 hr after the last exercise session as noted in the control group, ECG recording and sampling were carried out.

Exercise protocol

Exercise consisted of 10 weeks of treadmill. Exercise sessions were 15 min long (speed: 7.5 m/min with a slope of 0°), 5 days a week. Speed and time of sessions were gradually increased until the final week (60 min per session with a speed of 15 m/min while keeping the treadmill incline at 0%). Also, besides the exercise total time from the second week, 5 min were spent to warm up, and 5 min to cool down at a speed of 7 meters per min. Non-exercised animals were familiarized with the turned off treadmill for 20 min in each session (32).

Measuring cardiac hypertrophy index

The ratio of heart weight (mg) to body weight (gr) was calculated as an index of cardiac hypertrophy (2, 33, 34).

Western blotting

Western blotting was used to measure AT1R, AT2R, and APJ levels of expression in the heart. In this method, heart tissue samples were homogenized in the Ripa buffer (Sigma Aldrich Company, USA; R0278) plus protease inhibitor (Sigma Aldrich Company, USA; S8820). Then the homogenized samples were centrifuged (12,000 rpm for 15 min at 4 °C) and the supernatant was collected. Protein concentration was determined by the Lowry method, and bovine serum albumin was used as standard (35, 36). An equal amount of protein (40 micrograms) of each sample were electrophoresed on 10% polyacrylamide gels, then proteins were transferred to nitrocellulose membrane (Thermo Fisher Scientific Inc; 88518) and incubated overnight at 4 °C in a solution of 5% non-fat dry milk (SigmaAldrich, St. Louis, MO, USA).

Thereafter, the membranes were incubated for 3 hr with primary antibody against AT1R (1:1000; Sc-579; Santa Cruz Biotechnology, USA), AT2 (1:1000; Sc-9040; Santa Cruz Biotechnology, USA), APJ (1:1000; Sc-33823; Santa Cruz Biotechnology, USA) or GAPDH (1:1000; Sc-47724; Santa Cruz Biotechnology, USA). After 3 times washing for 5 min with TBST buffer (Tris-buffered saline, 0.1% Tween 20), the membranes were incubated for one and half hr with horseradish peroxidase-conjugated secondary antibodies including goat anti-rabbit IgG-HRP (1:10,000; Sc-2004; Santa Cruz Biotechnology, USA) for AT1, AT2, and APJ and goat anti-mouse IgG-HRP (1:5,000; Sc-2005; Santa Cruz Biotechnology, USA) for GAPDH. Then, three times washing was performed for 5 min with TBST buffer, and the membrane was incubated for one min with chemiluminescence substrate (ECL) (Amersham ECL Prime Western Blotting Detection Reagent). In the next step, the membrane was placed adjacent to the sensitive X-ray film (Hyperfilm ECL, GE Healthcare) and then the film was emerged. The film was scanned and analyzed with Image J software. The amount of protein expression was normalized by dividing each receptor band density to the corresponding GAPDH band density (36).

Statistical methods

Results are expressed as mean±SEM. Statistical calculations were done by using the SPSS 20 software package. The normal distribution of data was evaluated

Table 1. The effects of low endurance exercise, blood flow restriction, and their combination on cardiac hypertrophy index, heart rate and PR, RR, QRS, and QTc (corrected QT) intervals of the electrocardiogram

Variables Groups	Hypertrophy index	Heart rate (BPM)	PR interval (ms)	RR interval (ms)	QRS interval (ms)	QTc interval
CTL	2.57±0.07	364±12	51±0.6	165±6	17±1.7	171.71±7
Sham	2.78±0.08	338±18	57±1.8	180±9	17±1	167.65±6
BFR	2.57±0.08	323±19	57±1.3	188±11	17±1	177.7±9
CTL+Ex	2.8±0.09	333±8	54±.9	180±4	17±1	157.16±4
Sham+Ex	2.8±0.09	345±9	53±1	174±4	16±1	162.6±4
BFR+Ex	3.31±0.19*	319±11	52±1	188±6	16.5±0.3	141.92±3**
P-value	*P=0.015*	*P=0.187*	*P=1*	*P=0.228*	*P=0.968*	*P=0.006*

Values are represented as mean ± SE. The number of animals in each group was 6–8. CTL: control group, Sh: sham group, BFR: Blood flow restriction group, Ex: Exercise group, Sh + Ex: Sham + exercise group, BFR + Ex: Blood flow restriction + exercise group.(*P*<0.05) vs CTL and BFR groups,(*P*<0.01) vs CTL, Sham, and BFR groups

by Shapiro test and statistical analysis was performed by using ANOVA and Tukey's tests. *P*<0.05 was taken as statistically significant.

Results

Percent of body weight changes

After eleven weeks, CTL and Sham groups showed five percent weight gain (*P*<0.01 vs their basal weights). However, the weight of the BFR group bore no change and other groups showed weight loss in comparison with their primary weight (Ex, Ex+ BFR (*P*<0.001), Ex+ Sham (*P*<0.01)). In addition, final weight of Ex, Sham+Ex, and BFR +Ex groups was significantly lower than CTl, Sham, and BFR groups (*P*<0.001) (Figure 1).

Cardiac hypertrophy index and electrocardiogram (ECG) parameters

Endurance exercise alone and along with BFR had no significant effect on heart rate and RR, PR, and intervals

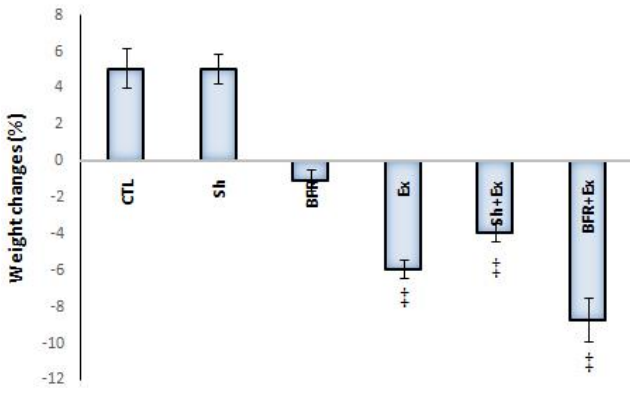

Figure 1. The effects of low endurance exercise, blood flow restriction, and their combination on body weight gain. Values are represented as mean±SEM. The number of animals in each group was 6–8. CTL: control group, Sh: sham group, BFR: Blood flow restriction group, Ex: Exercise group, Sh + Ex: Sham + exercise group, BFR + Ex: Blood flow restriction + exercise group. ‡ P<0.001 vs CTL, Sh, and BFR groups

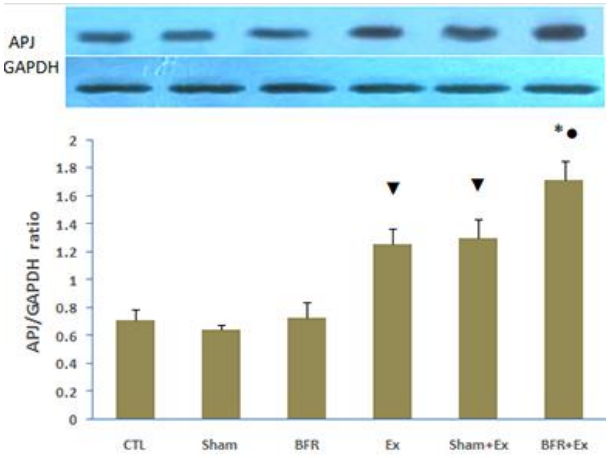

Figure 2. The expression of cardiac apelin receptor (APJ) protein in different experimental groups at the end of the study. Values are presented as mean±SE. The number of animals in each group was 6–8. (*P*<0.01) vs CTL, Sham, and BFR groups; * (*P*<0.05) vs Ex and Sham+ Ex groups; (*P*<0.001) vs CTL, Sham, and BFR groups. CTL: control group, Sh: sham group, BFR: Blood flow restriction group, Ex: Exercise group, Sh + Ex: Sham + exercise group, BFR + Ex: Blood flow restriction + exercise group

of ECG (Table 1) whereas, exercise plus BFR significantly decreased the QTc interval (*P*<0.01 vs CTL, Sham, and BFR groups) and increased cardiac hypertrophy index (*P*<0.05compared to CTL and BFR groups) (Table 1).

Amount of AT1, AT2, and APJ receptors expression in the heart tissue

Endurance exercise caused a significant increase in APJ expression of animal hearts in Ex and sham+Ex groups compared to CTL, Sham, and BFR groups (*P*<0.01). Also, combination of endurance exercise with BFR in lower extremities increased APJ expression of heart significantly compared to Ex, Ex+ sham (*P*<0.05), CTL, Sham, and BFR (*P*<0.001) groups (Figure 2).

Regarding the AngII receptors of animal hearts, 11-week endurance exercise training significantly reduced expression of the AT1 receptor in Ex and Sham+ Ex groups in comparison with CTL, Sham, and BFR groups

(P<0.01). Also, endurance exercise plus BFR of lower extremities significantly increased expression of AT1R, when compared with Ex, Sham+Ex (P<0.001), CTL, Sham, and BFR (P<0.01) groups (Figure 3).

On the other hand, BFR of the lower extremities was associated with a significant reduction in AT2R expression in comparison with Sham and CTL groups (P<0.05). However, the heart- AT2R expression in Ex and Sham+Ex groups increased significantly when compared to CTL, Sham, BFR, and BFR+Ex groups (P<0.001). BFR with endurance exercise eliminated the effect of exercise on the expression of this receptor (Figure 4).

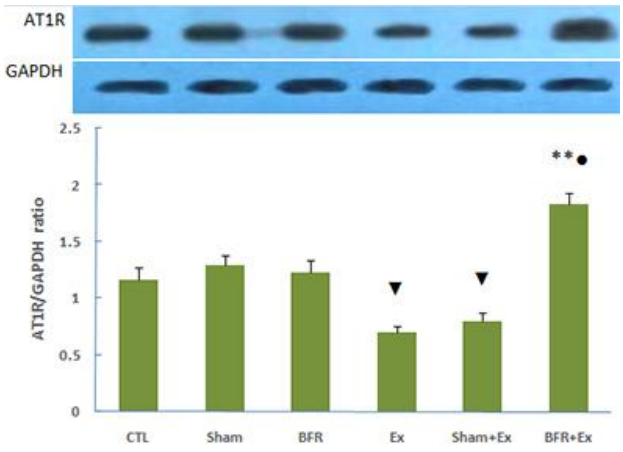

Figure 3. The expression of cardiac angiotensin II receptor1 (AT1R) protein in different experimental groups at the end of the study. Values are presented as mean±SD. The number of animals in each group was 6–8. (P<0.01) vs CTL, Sham, and BFR groups; ** (P<0.01) vs CTL, Sham, and BFR groups; (P<0.001) vs Ex and Sham + Ex groups. CTL: control group, Sh: sham group, BFR: Blood flow restriction group, Ex: Exercise group, Sh + Ex: Sham + exercise group, BFR + Ex: Blood flow restriction + exercise group

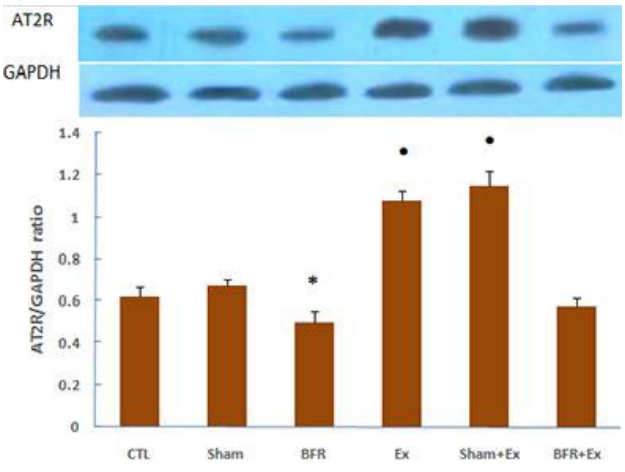

Figure 4. The expression of cardiac angiotensin II receptor 2 (AT2R) protein in different experimental groups at the end of the study. Values are presented as mean ± SE. The number of animals in each group was 6–8. (P<0.001) vs CTL, Sham, BFR, and BFR+Ex groups; * (P<0.05) vs CTL and Sh groups. CTL: control group, Sh: sham group, BFR: Blood flow restriction group, Ex: Exercise group, Sh + Ex: Sham + exercise group, BFR + Ex: Blood flow restriction + exercise group

Discussion

The present study aimed to assess the effect of combining low-intensity endurance exercise and BFR on ECG, cardiac hypertrophy index and cardiac AT1R, AT2R, and APJ of old rats. The results showed that combination of endurance exercise and BFR was associated with animal weight loss, reduction of QTc interval, and increased cardiac hypertrophy index, APJ, and AT1R level of expression.

In line with our findings, many studies have shown that exercise decreases body weight (37, 38). In the present study, we revealed that this effect of exercise can be continued in the presence of BFR. Weight loss can result from reduction in fat mass and increased metabolism following sports activities (39).

Regarding the ECG parameters, our findings are consistent with other previous studies that have shown exercise alone or together with BFR does not affect heart rate and RR, PR, and QRS intervals (7, 14, 15, 40). In the present study, light endurance exercise alone reduced the QT corrected (QTc) interval, which was statistically insignificant, but endurance exercise with BFR in lower extremities caused a significant reduction in the QT corrected (QTc) interval compared with untrained groups. The ineffectiveness of light, moderate, and severe exercises on QTc interval has been reported in previous studies (2, 33, 34). However, in some cases such as patients with type 2 diabetes, aerobic exercise can significantly decrease the QTc interval, which is probably due to the improvement of autonomic nervous system function (41). Furthermore, hypertensive patients treated by enalapril as an ACE (AngII converting enzyme) inhibitor for several years showed a significant reduction in QTc, which is likely due to its anti-fibrotic effect and reduction of cardiac muscle remodeling (42). It is reported that the QTc interval can increase with age (38); however, so far, there is no study in the literature that has considered the effect of exercise on the QTc interval in senescence directly. The modulatory effect of light exercise plus BFR on cardio-AngII axis and perhaps the improvement of autonomic nervous system balance may explain the reduction of the QTc interval in aging rats in our study.

Type, length, and intensity of exercise can also affect the heart hypertrophy index. Consistent with the present study, others demonstrated that 8-week (33, 37) and 10-week (40) mild to moderate endurance exercises have no significant effect on heart hypertrophy index; however, more long-term exercise training (19, 43) can increase the ratio of heart to body weight.

Increased heart hypertrophy index can result from body weight loss (44) or increased heart weight (7). In this study, body weight decreased significantly after exercise. On the other hand, it is reported that exercise along with BFR increases cardiac work that in turn can induce cardiac hypertrophy (7). Therefore, both weight loss and cardiac hypertrophy can explain the significant increase of hypertrophy index in animals subjected to exercise plus BFR. Further investigation is required to determine whether this model of hypertrophy is physiological or pathological.

The results showed that endurance exercise reduced

the level of heart AT1R expression significantly in comparison with non-trained groups, while endurance exercise with BFR increased AT1R expression significantly compared to all other groups. Aging is associated with increased AT1R (21) but no AT2R expression (24) in the heart, which in turn can cause increased activity of oxidative stress pathways and expand the pathological cardiac hypertrophy and cardiac fibrosis (21, 24). Lee *et. al.* have shown that treadmill exercise reduces the AT1R expression in old rat hearts, which confirms our findings (21).

In this study, the expression of AT2 receptor increased significantly in animals subjected to light exercise. However, BFR prevented the positive effect of exercise on the expression of this receptor in aging rat hearts. Fernandes *et. al.* reported that high-intensity swimming exercise can increase AT1R, AT2R, ACE2, and Ang (1-7) levels of expression and can decrease the ACE enzyme and AngII in female rats heart tissue with normal blood pressure and caused physiological cardiac hypertrophy (19).

Activation of AT1R has both physiological and pathological effects. Pathological effects were revealed in many diseases and also due to aging. On the other hand, stimulation of AT2R alone has a minor role in heart remodeling and its remodeling effects are performed mainly through interactions of other proteins, membrane enzymes, and autacoids (such as AT1R, sodium-hydrogen exchanger, sodium bicarbonate simport, ACE, TGF-β1, adrenaline, and bradykinin) (45-47). It seems that exercise probably by creating a balance between various factors of cardiac-AngII system and other signaling pathways can cause physiological effects of this system. In the BFR group the ratio of AT1R to AT2R in exercise was increased. This may explain cardiac hypertrophy in this group.

Increased expression of APJ in trained animals and strengthening this effect in the presence of BFR was the other finding of the present study. Previous studies have shown that exercise has increased Apelin and APJ receptors' levels of expression in heart tissue of rats with hypertension, which leads to improvement of function in the cardiovascular system (20). It is likely that positive effect of exercise on apelin and APJ levels of expression in hypertensive rats inhibits pathological effects of the RAS system and thus, improves cardiovascular performance (22). Moreover, there is much evidence that apelin plays an important role in angiogenesis and development of heart tissue (48, 49). It is known that the APJ receptor has a dual function, so that it prevents hypertrophy if stimulated by their ligands, and leads to hypertrophy if activated by mechanical stretch (50). According to these findings, it can be assumed that BFR is able to increase peripheral and cardiac resistance after load. On the other hand, increased venous return to the heart and increased wall tension and strong stimulation of APJ during exercise can be considered as a part of hypertrophy effect of exercise plus BFR. Additionally, APJ receptors are able to neutralize Ang II-AT1R signaling through physical interaction with AT1 receptors. Moreover, apelin can increase AngII converting enzyme 2 (ACE2) promoter activity and ACE2 expression via APJ activation, which improves cardiac functions independently of AT1R signaling (51).

Therefore, it seems that this type of exercise leads to a new balance between Apline- APJ and the AngII systems of heart in aging rats.

Conclusion

In summary, light exercise plus BFR induces a desirable change in ECG by reducing QTc interval, stimulating cardiac hypertrophy, and leading to a new balance in the cardiac AngII receptors and the APJ-apline system. Further research is needed to clarify the physiological or pathological nature of these alterations.

Acknowledgment

The authors express their thanks to Kerman University of Medical Sciences, Kerman, Iran for the financial support of this study. The results presented in this paper were derived from an M.Sc. student thesis (Alireza Raji-Amirhasani).

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