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ARYA Atherosclerosis

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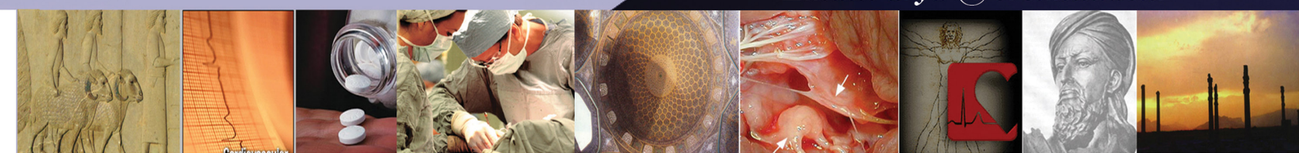

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* All the words of the article containing the references; each table is considered as 300 words.

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Table of Contents

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The effect of different digoxin concentrations on heart tissue and antioxidant status in iron-overloaded rats

Beydolah Shahouzehi[(1)], Hamid Reza Nasri[(2)], Yaser Masoumi-Ardakani[(3)]

Original Article

Abstract

BACKGROUND: Thalassaemia is a hereditary disorder and has an economic burden on patients and the government. The most prevalent complication in these patients is iron overload which is followed by cardiomyopathy. Digoxin is considered as a treatment against heart failure in thalassaemia. The present study evaluated the effect of two digoxin concentrations on iron content and antioxidative defense in cardiac tissue of iron-overloaded rats.

METHODS: The study was conducted on 48 rats which were divided into 6 groups. Group 1 was the control group and did not receive any treatment and group 2 was the iron overload group. In addition groups 3 and 4 were the digoxin control groups which received 1 and 5 mg/kg/day of digoxin, respectively. Groups 5 and 6 received 1 and 5 mg/kg/day of digoxin plus iron-dextran, respectively. After 1 month, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant status (TAS) were assessed in cardiac tissues.

RESULTS: Co-administration of iron-dextran and digoxin (1 and 5 mg/kg/day) significantly increased SOD and TAS levels ($P < 0.0010$) and reduced MDA ($P < 0.0010$) in heart tissue compared to control and iron overload groups. GPX levels significantly reduced in groups 5 and 6 (iron + digoxin 1 ($P < 0.0500$) and iron + digoxin 5) ($P < 0.0010$) compared to the iron control group.

CONCLUSION: Digoxin remarkably facilitates iron uptake by cardiomyocytes by affecting other channels such as L-type and T-type Ca^{2+} channels (LTCC and TTCC). Digoxin administration in the iron-overloaded rat model deteriorated antioxidative parameters and increased iron entry into heart tissue at higher doses. Therefore, in patients with beta thalassaemia major, digoxin must be administered with great care and serum iron and ferritin must be regularly monitored.

Keywords: Digoxin, Iron Overload, Superoxide Dismutase, Glutathione Peroxidase

Date of submission: 17 June 2017, ***Date of acceptance:*** 26 Jan. 2018

Introduction

Thalassaemia is a common hereditary disorder in which the synthesis of haemoglobin subunits is disturbed. There are about 15 million patients with thalassaemia all over the world and there are 240 million beta thalassaemiacarriers.[1] Iran is placed on the thalassaemia belt, and it has been reported that the prevalence of thalassaemia in Iran is about 3.6% and that there is a high population of carriers in Iran (about 4% of the population).[2] Iron is considered as an essential element and it contributes to the structure and function of proteins. Iron is required for hemoglobin and myoglobin production. Iron also has an important role in the formation of reactive oxygen species (ROS), including hydroxyl radical, superoxide radical, and hydrogen peroxide, which are toxic and cause profound damage to DNA, proteins, and lipids.[3-5] Since Iron has an important role in ROS generation and the human body has no mechanism for iron removal, iron accumulation in the body causes devastating damage to biological pathways and critical organs such as the heart, liver, bone marrow, and pancreas. Therefore, the chance of progression toward diseases such as diabetes, heart failure, atherosclerosis, and metabolic syndrome increases

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in patients with thalassaemia.[5-8]

Heart disease is still the leading cause of mortality and morbidity in patients with thalassaemia. Previous studies have reported the prevalence of death in thalassaemia due to cardiomyopathy as 63-71%.[8-12]

Cardiac glycosides are naturally derived compounds and their core structure is steroidal. Digoxin is a member of this family and binds and inhibits Na^+/K^+-ATPase (NKA) activity. NKA inhibition by digoxin induces the efflux of potassium and increases sodium entrance into cardiomyocytes. This sodium accumulation in cardiomyocytes promotes Na^+/Ca^{2+} exchangers (NCX) which results in free calcium elevation. This phenomenon explains the inotropic action of digoxin on cardiac muscle.[13,14] Data about iron entrance into cardiac cells is controversial and has indicated that L-type Ca^{2+} channels (LTCCs), T-type Ca^{2+} channels (TTCCs), divalent metal transporter1 (DMT1), and transferrin receptor protein 1 (TfR1) are involved in iron uptake into cardiomyocytes.[15-20] LTCCs transport Ca^{2+}, but have also been reported to be capable of transporting other divalent cations including Fe^{2+}, Zn^{2+}, and Mn^{2+} into cardiomyocytes. It seems that digoxin may indirectly activate LTCCs by limiting NKA activity, so other divalent cations can be taken up by cardiomyocytes. In our previous study, we showed that digoxin significantly increased the cardiac iron content of iron-overloaded rats.[21] In the present study, we evaluated the antioxidant status of heart tissue in iron-overloaded rats under different concentrations of digoxin to assess the effects of digoxin on iron entry into heart tissue and also its effects on antioxidative defense in cardiomyocytes in iron overload.

Materials and Methods

Iron was evaluated using an Iron assay kit (BioVision; Catalog #K390-100) and digoxin was assayed using the Digoxin enzyme-linked immunosorbent assay (ELISA) kit (Digoxin AccuBind ELISA Kits; code 925-300). Iron-dextran (Sigma; D8517) and digoxin were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). The measurement of superoxide dismutase (SOD), glutathione peroxidase (GPX), and total antioxidant status (TAS) in cardiac tissue was conducted using specific kits supplied by Randox laboratories Ltd. (Crumlin, UK) (TAS: Cat. No. NX2332; SOD: Cat. No. SD125; and GPX: Cat. No. RS505). Malondialdehyde (MDA), as representative of lipid peroxidation, was measured as a thiobarbituric acid-reactive substance (TBARS) at 534 nm, and the calibration graph was obtained using different concentrations of 1,1,3,3-tetramethoxypropane.[21,22]

The animals were purchased from Kerman Physiology Research Center, Iran, and after acclimatization, the animals were kept under controlled conditions (24 ± 1 °C, 12-hour light-dark cycle, and free access to rat chow and water). In the present study, 48 male Sprague Dawley rats weighing 200-230 g were selected and randomly divided into 6 groups. The study was approved by the ethic committee of Kerman University of Medical Sciences, Iran. In order to develop iron overload in rats, iron-dextran was used. Before beginning the main study, digoxin was administrated by intraperitoneal injection for a week to ensure high digoxin levels in the corresponding groups.

Group1 (control group): untreated group

Group2 (iron overload): received 12.5 mg/100g body weight iron-dextran every 5 days

Group3 (digoxin control 1): received 1 mg/kg/day digoxin

Group4 (digoxin control 5): received 5 mg/kg/day digoxin

Group5 (iron+ digoxin 1): received 12.5 mg/100g body weight iron dextran every 5 days + 1 mg/kg/day digoxin

Group6 (iron+ digoxin 5): received 12.5 mg/100g body weight iron dextran every 5 days + 5 mg/kg/day digoxin

At the end of the study (day 30), the animals were sacrificed under ether anesthesia. Subsequently, the abdominal part of the animal's body was incised, and the heart tissue was removed and quickly placed in cold saline to extract the remaining blood from tissues. Heart tissues were collected for the assessment of iron and other antioxidants; therefore, the tissues were placed in cold lysis buffer, and then, homogenized using an ultrasonic processor (UP200H, Hielscher Ultrasonics, Germany) on ice to reduce heat generation during sonication process. Finally, the homogenates were placed in new tubes and centrifuged at 15000 rpm and 4 °C for 15 minutes. The supernatants were collected and aliquoted in new collecting tubes and kept at -80 °C for further examinations.[21]

Statistical analyses were performed in SPSS software (version 21, IBM Corporation, Armonk, NY, USA). The results were presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test were performed for analysis and pair-wise group comparison. All P values < 0.0500 were considered statistically significant.

Results

The results showed that digoxin administration remarkably elevated heart iron content in the group that received a combination of iron-dextran and digoxin compared to control and digoxin control groups (P > 0.0001) (Figure 1).

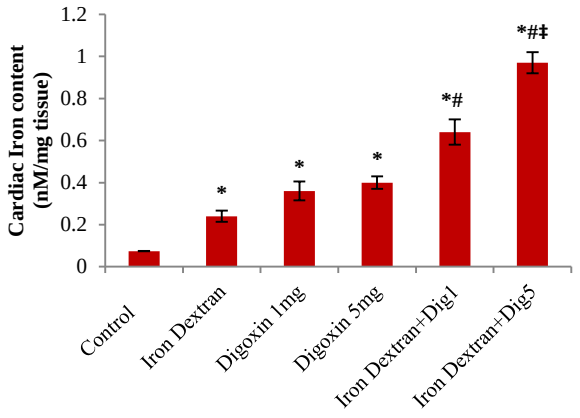

Figure 1. Cardiac iron contents in different studied groups.
Data is expressed as mean ± SEM.
SEM: Standard error of mean
* Statistically significant compared to control group, # Statistically significant compared to iron overload control group, ‡ statistically significant compared to iron-dextran + digoxin 1 group

Co-administration of iron-dextran and digoxin (1 and 5 mg/kg/day) significantly increased SOD and TAS levels (P < 0.0010), but reduced MDA levels (P < 0.0010) in the heart tissue compared to the control and iron overload control groups (Figures 2-4).

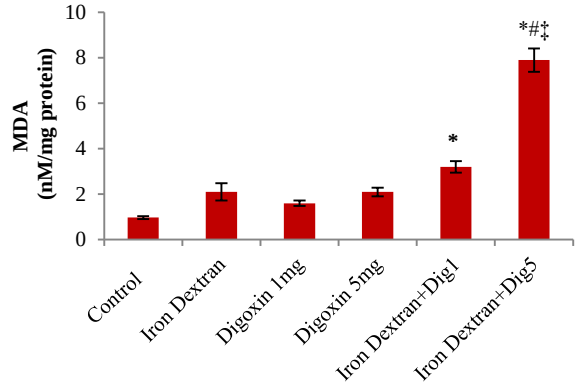

Figure 2. Malondialdehyde levels in heart tissue of rats in different studied groups
Data is expressed as mean ± SEM.
SEM: Standard error of mean
MDA: Malondialdehyde
* Statistically significant compared to control group, # Statistically significant compared to iron overload control group, ‡ statistically significant compared to iron-dextran + digoxin 1 group.

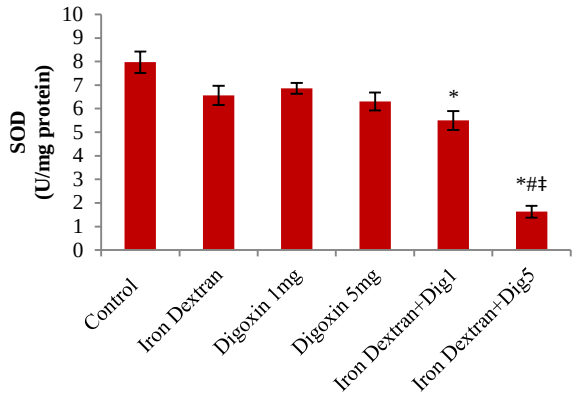

Figure 3. Superoxide dismutase levels in heart tissue of rats in different studied groups.
Data is expressed as mean ± SEM.
SEM: Standard error of mean
SOD: Superoxide dismutase
* Statistically significant compared to control group, # Statistically significant compared to iron overload control group, ‡ Statistically significant compared to iron-dextran + digoxin 1 group

GPX levels significantly reduced by iron + digoxin 1 (P < 0.0500) and iron + digoxin 5 (P < 0.0010) compared to the iron control group (Figure 5).

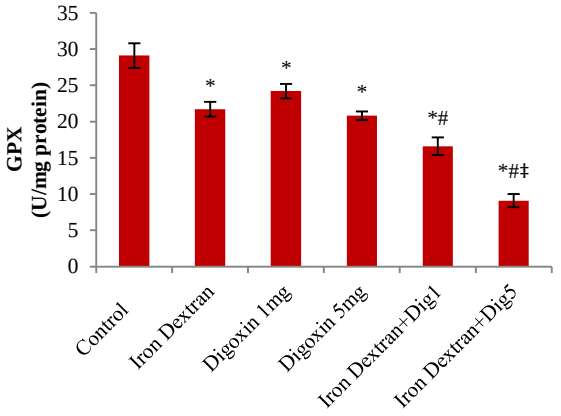

Figure 4. Glutathione peroxidase in heart tissue of rats in different studied groups
Data is expressed as mean ± SEM.
SEM: Standard error of mean
GPX: Glutathione peroxidase
* Statistically significant compared to control group, # Statistically significant compared to iron overload control group, ‡ Statistically significant compared to iron-dextran + digoxin 1 group

Significant changes were observed in total antioxidant capacity (TAC); TAC was significantly reduced in the group which received iron + digoxin 1 compared to the control group (P < 0.0010) (Figure 5).

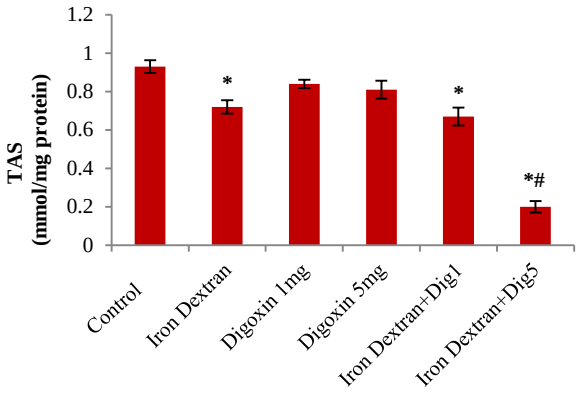

Figure 5. Total antioxidant status quantity in heart tissue of rats in different studied groups.
Data is expressed as mean ± SEM.
SEM: Standard error of mean
TAS: Total antioxidant status
* Statistically significant compared to control group,
# Statistically significant compared to iron overload control group

Moreover, a significant reduction was observed in TAC in the iron overload group and the group that received 5 mg of digoxin compared to the control and iron overload groups ($P < 0.0001$) (Figure 5).

Discussion

Thalassaemia is a group of inherited diseases in which globin chains which participates in haemoglobin formation are affected. In patients with thalassaemia, there is inadequate hematopoiesis, and patients with thalassemia major require regular transfusion. It has been shown that, in patients with thalassaemia, there is a high probability of hemochromatosis which results from repeated blood transfusion and entrance of high amounts of iron into their body.[6,12,23,24] Unfortunately, there is no known way for removal of excess iron from the body, so the entrance of iron into these patients' bodies cause a life threatening condition in these patients.[12,23] The main problem following transfusion and entry of large amounts of iron into the body of patients with thalassemia is iron deposition in critical organs such as the heart and liver, and bone marrow.[23,24] The most prevalent disease in patients with thalassemia is cardiomyopathy. This cardiomyopathy is the result of iron deposition and iron overload dependent ROS generation which is very harmful.[12] Thalassaemia is an expensive disease and its treatment places an economic burden on patients and the government.[25] Therefore, reduction of iron overload in these patients can reduce post-transfusion adverse effects.

The present study investigated lipid peroxidation and enzymatic antioxidative defense in the hearts of iron overloaded rats and effects of different concentrations of digoxin on these parameters. The iron contents of heart tissues were found to be significantly higher in digoxin and iron treated groups compared to the control group. These data demonstrated that serum iron and digoxin elevation increase iron uptake by cardiomyocytes. Iron uptake by cardiac muscle cells in groups 5-6, which received a combination of iron and digoxin, compared to the iron control group was significant. This shows that digoxin somehow increases iron uptake by cardiomyocytes. Furthermore, 5 mg compared to 1 mg digoxin showed more remarkable iron elevation in cardiomyocytes which showed the dose-dependent action of digoxin on iron uptake into cardiac muscle cells. Previously, it has been demonstrated that digoxin has an important role in iron entry into cardiomyocytes and deteriorates cardiac histological parameters.[21] Therefore, it can be concluded that digoxin administration in patients with thalassaemia must be reconsidered and performed with more care and serum iron levels of patients must be monitored. Digoxin administration in patients with thalassaemia helps them to recover their cardiac function and is vital for these patients to maintain their cardiac activity.[13,14] Nevertheless, it has been demonstrated that digoxin can indirectly affect other channels in cardiomyocytes such as LTCC and TTCC. These channels are involved in the uptake of Ca and other divalent cations by cardiac cells.[15,16,20]

In the present study, oxidative parameters of heart tissue of iron-overloaded rats were assessed after two different doses of digoxin administration in iron overload state. It was found that MDA, which is a lipid peroxidation marker, and cytotoxic aldehyde were significantly elevated and that 5 mg digoxin showed a much higher MDA elevation effect than 1 mg digoxin in the iron overload rat models. Administration of 1 mg digoxin elevates MDA only compared to the control group. Moreover, there were no differences between iron overload control and iron overload plus 1 mg digoxin in terms of MDA levels, which shows that 1 mg of digoxin is not as potent as 5 mg in increasing iron entry, and consequently, lipid peroxidation. The other factors evaluated included SOD and GPX in heart tissue. These two enzymes along with other antioxidants work together against

free radicals and protect cells from the destructive effects of these toxic agents. Another study reported that patients with thalassaemia are in an oxidative state.[4]

Data about iron uptake by cardiomyocytes are controversial and many studies have reported that LTCC, TTCC, DMT1, and TfR1 are involved in iron uptake into cardiac cells.[16,17,20,26,27] Kumfu et al. (2011) showed that only TTCCs are involved in iron uptake in thalassaemic cardiomyocytes in culture media, and that the inhibition of other channels and receptors had no effects on iron uptake by thalassaemic cardiomyocytes which refuse their role in iron entrance into cardiac muscle cells.[26] In another study, it was shown that LTCC, TTCC, and DMT1 blockers attenuated cardiac MDA and cardiac iron content, and that TTCC blockers are more beneficial in the reduction of iron accumulation in the heart than LTCC blockers.[17] Moreover, it was demonstrated that the entrance of Fe^{3+} into thalassaemic cardiomyocytes is not conducted by TfR1, DMT1, LTCC, and TTCC. Therefore, they proposed that there must be another way by which Fe^{3+} is taken up in thalassaemic cardiac muscle cells.[27]

Recently, it has been reported that a dual LTCC and TCC blocker, efonidipine, showed as much beneficial effect as other commercially available iron chelators, and this blocker is a new and strong remedy for iron overload in thalassaemia.[20] Mishra and Tiwari (2013) demonstrated that most patients with beta thalassaemia major showed increased serum ferritin levels that result from insufficient chelation. Poor chelation therapy causes iron overload development which results in downstream problems in these patients including cardiac complications.[24]

Arispe et al. (2008) indicated that digitoxin was able to form channels in cell culture which pass Ca over the lipid bilayer membrane, and also the same channels were formed by digoxin.[28] They explained that the digoxin calcium channel can be considered as the main way of Ca entrance into cardiomyocytes and it could account for the mechanism of toxicity of these glycosides in the heart. They also showed that cardiac glycosides promote Ca uptake in a dose-dependent manner.[28] According to these findings, it can be concluded that digoxin, in addition to affecting other calcium channels such as LTCC and TTCC, increases iron uptake into cardiomyocytes by developing a digoxin calcium channel. The present study results showed that a high dose of digoxin is more potent to increase iron uptake than low doses. A high dose of digoxin probably forms more calcium channels over the cardiac muscle cells membrane and facilitates iron entry into cardiomyocytes. Deterioration of the antioxidant status and elevation of MDA in cardiac tissue of iron overloaded rats after digoxin administration also confirms the effect of digoxin on iron entrance into cardiomyocytes.

Remarkably facilitates iron uptake by cardiomyocytes probably via affecting other channels such as TTCC and LTCC, or by forming newly described digoxin calcium channels. In addition, iron caused ROS production in the heart that is an important mechanism by which cardiac damage occurs and results in apoptosis, and finally, heart failure.[8] Moreover, chronic iron overload diseases result in mitochondrial DNA (mtDNA) damage and respiratory disorders which in heart tissue could result in ischemic heart failure.[5] Previous studies have reported a relationship between iron content in the body and atherosclerosis; thus, in the iron overload state, in addition to the progress toward heart failure, there is also the chance of atherosclerosis and coronary artery diseases development.[7,29,30] In the present study, it was demonstrated that digoxin administration at high and low doses in iron overload model deteriorate antioxidative parameters and increase iron entry into heart tissue. Hence, in patients with beta thalassaemia major, digoxin must be administered with care and serum iron and ferritin must be regularly monitored.

Conclusion

Digoxin significantly facilitates iron entrance into cardiomyocytes by affecting other channels such as LTCC, or by forming newly described digoxin calcium channels. The present study results showed that a high dose of digoxin in the iron overload rat model deteriorated antioxidative status and extremely elevated iron entrance into the heart tissue; therefore, digoxin administration in conditions which result in the iron overload state must be undertaken with care and serum iron and ferritin must be regularly monitored.

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Conflict of Interests

Authors have no conflict of interests.

References

1. Bhagat S, Sarkar PD, Suryakar AN, Hundekar PS. A study on the biomarkers of oxidative stress: The effects of oral therapeutic supplementation on the iron concentration and the product of lipid peroxidation in beta thalassemia major. J Clin Diagn Res 2012; 6(7): 1144-7.
2. Khodaei GH, Farbod N, Zarif B, Nateghi S, Saeidi M. Frequency of thalassemia in Iran and Khorasan Razavi. Int J Pediatr 2013; 1(1): 45-50.
3. Urbanski NK, Beresewicz A. Generation of *OH initiated by interaction of Fe^{2+} and Cu^{+} with dioxygen; Comparison with the Fenton chemistry. Acta Biochim Pol 2000; 47(4): 951-62.
4. Walter PB, Fung EB, Killilea DW, Jiang Q, Hudes M, Madden J, et al. Oxidative stress and inflammation in iron-overloaded patients with beta-thalassaemia or sickle cell disease. Br J Haematol 2006; 135(2): 254-63.
5. Gao X, Campian JL, Qian M, Sun XF, Eaton JW. Mitochondrial DNA damage in iron overload. J Biol Chem 2009; 284(8): 4767-75.
6. Handa P, Morgan-Stevenson V, Maliken BD, Nelson JE, Washington S, Westerman M, et al. Iron overload results in hepatic oxidative stress, immune cell activation, and hepatocellular ballooning injury, leading to nonalcoholic steatohepatitis in genetically obese mice. Am J Physiol Gastrointest Liver Physiol 2016; 310(2): G117-G127.
7. Kuo KL, Hung SC, Lee TS, Tarng DC. Iron sucrose accelerates early atherogenesis by increasing superoxide production and upregulating adhesion molecules in CKD. J Am Soc Nephrol 2014; 25(11): 2596-606.
8. Gammella E, Recalcati S, Rybinska I, Buratti P, Cairo G. Iron-induced damage in cardiomyopathy: Oxidative-dependent and independent mechanisms. Oxid Med Cell Longev 2015; 2015: 230182.
9. Engle MA, Erlandson M, Smith CH. Late cardiac complications of chronic, severe, refractory anemia with hemochromatosis. Circulation 1964; 30: 698-705.
10. Zurlo MG, De Stefano P, Borgna-Pignatti C, Di Palma A, Piga A, Melevendi C, et al. Survival and causes of death in thalassaemia major. Lancet 1989; 2(8653): 27-30.
11. Ladis V, Chouliaras G, Berdousi H, Kanavakis E, Kattamis C. Longitudinal study of survival and causes of death in patients with thalassemia major in Greece. Ann N Y Acad Sci 2005; 1054: 445-50.
12. Kremastinos DT, Farmakis D, Aessopos A, Hahalis G, Hamodraka E, Tsiapras D, et al. Beta-thalassemia cardiomyopathy: History, present considerations, and future perspectives. Circ Heart Fail 2010; 3(3): 451-8.
13. Lelie`vre LG, Lechat P. Mechanisms, manifestations, and management of digoxin toxicity. Heart Metab 2007; 35: 9-11.
14. Kurian M. The effect of digitalis on the heart-an update. J Pharm Sci Res 2015; 7(10): 861-3.
15. Tsushima RG, Wickenden AD, Bouchard RA, Oudit GY, Liu PP, Backx PH. Modulation of iron uptake in heart by L-type Ca^{2+} channel modifiers: Possible implications in iron overload. Circ Res 1999; 84(11): 1302-9.
16. Oudit GY, Sun H, Trivieri MG, Koch SE, Dawood F, Ackerley C, et al. L-type Ca^{2+} channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. Nat Med 2003; 9(9): 1187-94.
17. Kumfu S, Chattipakorn S, Chinda K, Fucharoen S, Chattipakorn N. T-type calcium channel blockade improves survival and cardiovascular function in thalassemic mice. Eur J Haematol 2012; 88(6): 535-48.
18. Chattipakorn N, Kumfu S, Fucharoen S, Chattipakorn S. Calcium channels and iron uptake into the heart. World J Cardiol 2011; 3(7): 215-8.
19. Chen MP, Cabantchik ZI, Chan S, Chan GC, Cheung YF. Iron overload and apoptosis of HL-1 cardiomyocytes: Effects of calcium channel blockade. PLoS One 2014; 9(11): e112915.
20. Kumfu S, Chattipakorn SC, Fucharoen S, Chattipakorn N. Dual T-type and L-type calcium channel blocker exerts beneficial effects in attenuating cardiovascular dysfunction in iron-overloaded thalassaemic mice. Exp Physiol 2016; 101(4): 521-39.
21. Nasri HR, Shahouzehi B, Masoumi-Ardakani Y, Iranpour M. Effects of digoxin on cardiac iron content in rat model of iron overload. ARYA Atheroscler 2016; 12(4): 180-4.
22. Lim CS, Vaziri ND. The effects of iron dextran on the oxidative stress in cardiovascular tissues of rats with chronic renal failure. Kidney Int 2004; 65(5): 1802-9.
23. Wood JC, Enriquez C, Ghugre N, Otto-Duessel M, Aguilar M, Nelson MD, et al. Physiology and pathophysiology of iron cardiomyopathy in thalassemia. Ann N Y Acad Sci 2005; 1054: 386-95.
24. Mishra AK, Tiwari A. Iron overload in Beta thalassaemia major and intermedia patients. Maedica (Buchar) 2013; 8(4): 328-32.
25. Sattari M, Sheykhi D, Nikanfar A, Pourfeizi AH, Nazari M, Dolatkhah R, et al. The financial and social impact of thalassemia and its treatment in Iran. Pharm Sci 2012; 18(3): 171-6.
26. Kumfu S, Chattipakorn S, Srichairatanakool S, Settakorn J, Fucharoen S, Chattipakorn N. T-type calcium channel as a portal of iron uptake into cardiomyocytes of beta-thalassemic mice. Eur J Haematol 2011; 86(2): 156-66.

27. Kumfu S, Chattipakorn S, Fucharoen S, Chattipakorn N. Ferric iron uptake into cardiomyocytes of b-thalassemic mice is not through calcium channels. Drug Chem Toxicol 2013; 36(3): 329-34.
28. Arispe N, Diaz JC, Simakova O, Pollard HB. Heart failure drug digitoxin induces calcium uptake into cells by forming transmembrane calcium channels. Proc Natl Acad Sci U S A 2008; 105(7): 2610-5.
29. de Valk B, Marx JJ. Iron, atherosclerosis, and ischemic heart disease. Arch Intern Med 1999; 159(14): 1542-8.
30. Lee TS, Shiao MS, Pan CC, Chau LY. Iron-deficient diet reduces atherosclerotic lesions in apoE-deficient mice. Circulation 1999; 99(9): 1222-9.

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Periopathogens in atherosclerotic plaques of patients with both cardiovascular disease and chronic periodontitis

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Original Article

Abstract

BACKGROUND: Atherosclerosis and periodontitis are both chronic inflammatory diseases. Although a strong relationship between the two has already been established, the underlying mechanism is unknown. The present study was conducted aiming to detect the deoxyribonucleic acid (DNA) of Aggregatibacter actinomycetemcomitans (A.a), Campylobacter rectus (C.r), and Porphyromonas gingivalis (P.g) in subgingival and atherosclerotic plaques of patients with both chronic periodontitis and cardiovascular disease (CVD).

METHODS: In this cross sectional study, patients with coronary artery disease (CAD) and moderate to severe periodontitis which were scheduled for coronary artery bypass grafting (CABG) were enrolled in the study. The subgingival plaques were collected before surgery. All samples were examined for the detection of selected periopathogens using polymerase chain reaction (PCR).

RESULTS: The subgingival and atherosclerotic plaque samples of 23 patients were examined. The DNA of P.g, A.a, and C.r were found to be positive in 43.47%, 43.47%, and 78.26% of subgingival plaques, and 13.04%, 17.39%, and 8.69% of atherosclerotic plaques, respectively. In all cases, the bacterial species found in atherosclerotic plaques were also found in the subgingival plaques of the same patient.

CONCLUSION: This study demonstrated the presence of periopathogens in atherosclerotic plaques of patients with chronic periodontitis. More studies are required to ascertain the exact role of these periopathogens in atherosclerotic plaque formation.

Keywords: Atherosclerosis, Coronary Artery Disease, Chronic Periodontitis, Porphyromonas Gingivalis, Aggregatibacter Actinomycetemcomitans, Campylobacter Rectus

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Introduction

Gingiva embraces teeth in a collar-like fashion. There is a space between the tooth and gingival tissue termed gingival sulcus, which is lined by sulcular epithelium.[1] Periodontitis is a chronic inflammatory disease in which microbial plaque is a causative factor. In periodontitis, the gingival sulcus is deepened and periodontal pocket is formed. The periodontal pocket is a preferable site for colonization of periopathogens. Bacterial challenge in this site aggravates the inflammatory process.[2] Due to inflammation, the inner wall of the periodontal pocket will usually become ulcerated. In this situation, the impeding role of the inner epithelium against systemic circulation is disrupted and microorganisms and their products and inflammatory mediators can enter the well-vascularized periodontal tissues and subsequently into the circulation.[3] Through the circulatory system, periodontal bacteria can reach distant organs like vascular cells.[2]

Atherosclerosis is a progressive chronic inflammatory process of the vessels, which may cause an increase in arterial wall thickness.[4] To prevent this condition, understanding the underlying pathomechanisms is vital.[5] The risk

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factors mentioned for this disease include genetic factors, hypercholesterolemia, hypertension, diabetes, obesity, smoking, and lack of physical activity.[4,6-8] These factors, however, account for only 50-70% of the atherosclerotic events[8,9] and atherosclerosis can develop in the absence of these factors.[6]

Apart from them, some studies mentioned that infectious pathogens are associated with atherosclerosis and prognosis of coronary artery disease (CAD).[10] Some studies investigated the deoxyribonucleic acid (DNA) of various periodontal pathogens in arterial atheromatous plaques removed in carotid endarterectomy (CEA), however, there is a significant inconsistency of available data.[1,5]

Considering the high incidence of periodontal and cardiovascular disease (CVD) worldwide, the present study was carried with the aim of assessing the presence of periopathogens [Porphyromonas gingivalis (P.g), Aggregatibacter actinomycetemcomitans (A.a) and Campylobacter rectus (C.r)] using polymerase chain reaction (PCR) in atherosclerotic plaques and subgingival plaques among patients with chronic periodontitis and CVD undergoing coronary artery bypass graft (CABG) in Isfahan, Iran.

Materials and Methods

This cross sectional study was performed in accordance with the World Medical Association (WMA) Declaration of Helsinki and subsequent revisions[11] and was approved by the ethics committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran, with the code P/17/1/21952. Written informed consents were obtained from patients before entering the study and after fully explaining the protocol. The patients admitted to Isfahan Shahid Chamran hospital from January to May 2013 and scheduled for CABG due to CAD were selected. Periodontal conditions of dentate patients (at least having 10 teeth) were examined and patients with signs of periodontal inflammation and at least two teeth with periodontal pocket ≥ 5 mm were enrolled in the study. The exclusion criteria comprised of history of periodontal treatments in the previous 6 months, suffering from other major systemic diseases including malignancies and antibiotic consumption. At least, 23 patients were enrolled in the study.

Subgingival plaque samples were collected one day prior to the CABG surgery. To perform this, selected teeth were carefully dried using sterile cotton swaps. Following supragingival plaque removal, subgingival plaques were obtained using a curette from two sites with the greatest pocket depth. The samples were placed in Stuart transport medium and sent to the laboratory for microbiologic analysis.

The surgeon provided a biopsy from the coronary atherosclerotic plaque during the CABG procedure. A 0.5-1 mm tissue from the periphery of the coronary plaque was resected during arteriotomy. The samples were then soaked in saline with sulfate buffer in order to eliminate blood contamination and were then placed in Stuart transport medium. The atherosclerotic plaques were homogenized before the PCR procedure. In all cases, the atherosclerotic plaque and the subgingival plaque were obtained from the same patient.

Following DNA isolation, PCR was performed on all atherosclerotic and subgingival plaque samples and the products were then sequenced for further analysis as described in the study by Mahendra et al.[12] The primers utilized were described in table 1.

The PCR protocol for all microorganisms included the following: 100 ng of DNA template of sample was added to 50 µl of working stock reaction mixture (containing 10 mM of PCR buffer, 1.25 unit of Taq DNA polymerase, 0.2 mM of each deoxyribonucleotides (dNTPs), primers, 0.3 mM and $Mgcl_2$ 1.5 mM). Biometra Thermocycler was used to perform PCR which comprised of initial denaturation (95 °C for 3 min) stage followed by 35 cycles of denaturation (94 °C for 30 s), annealing (60 °C for 1 min), extension (72 °C for 1 min) with a final extension of 72 °C for 5 min. After amplification, 10 µl of PCR product was subjected to electrophoresis in a 1% agarose gel containing 0.5 mg/ml ethidium bromide in 1x Tris-Borate electrophoresis buffer (TBE).

Table 1. Bacterial target and primer sequences used in the polymerase chain reaction (PCR) detection

Bacteria	Primers
Aggregatibacter actinomycetemcomitans	Forward primer: CTT ACC TAC TCT TGA CAT CCG AA Reverse primer: ATG CAG CAC CTG TCT CAA AGC
Porphyromonas gingivalis	Forward primer: AGG CAG CTT GCC ATA CTG C Reverse primer: ACT CTT AGC AAC TAC CGA TGT
Campylobacter rectus	Forward primer: TTT CGG AGC GTA AAC TCC TTT TC Reverse primer: TTT CTG CAA GCA GAC ACT CTT

Table 2. Data on number, age, and gender of participants and the presence of periopathogens in subgingival and atherosclerotic plaques

No	Age (year)	Gender (M/W)	Subgingival plaque			Atherosclerotic plaque		
			P.g	A.a	C.r	P.g	A.a	C.r
1	60	M	-	-	+	-	-	-
2	71	W	-	-	+	-	-	-
3	59	M	-	-	+	-	-	-
4	64	M	-	-	+	-	-	-
5	60	M	+	+	+	+	+	+
6	60	M	-	+	+	-	+	-
7	67	M	-	+	-	-	-	-
8	60	M	+	-	+	-	-	-
9	58	M	-	-	+	-	-	-
10	57	W	+	-	+	-	-	-
11	63	M	-	-	+	-	-	-
12	61	M	+	-	-	-	-	-
13	58	M	-	-	+	-	-	-
14	63	M	+	-	-	-	-	-
15	64	M	-	+	+	-	-	-
16	59	M	-	-	+	-	-	-
17	53	W	-	+	-	-	-	-
18	65	M	+	-	+	-	-	-
19	65	M	-	+	+	-	-	-
20	61	M	+	+	-	+	+	-
21	58	M	+	+	+	-	-	-
22	60	M	+	+	+	-	-	-
23	59	M	+	+	+	+	+	+
Total positive case (%)			43.47	43.47	78.26	13.04	17.39	8.69

M: Man; W: Woman; P.g: Porphyromonas gingivalis; A.a: Aggregatibacter actinomycetemcomitans; C.r: Campylobacter rectus

Results

A total of 23 patients including 20 men and 3 women with mean age of 61.00 ± 4.01 years participated in this study. Totally, 23 atherosclerotic plaque samples and 23 subgingival plaque samples were examined and compared for the incidence rate of three periopathogens (P.g, A.a, C.r). All 23 (100%) subgingival plaques and only 4 (17.39%) atherosclerotic plaques tested were positive for at least one periopathogen. Table 2 shows the rate of periopathogens in subgingival and atherosclerotic plaques of each patients. The DNA of P.g was found positive in 10 subgingival samples (43.47%), from which 3 (13.04%) atherosclerotic samples were positive as well. The DNA of A.a was found in 10 (43.47%) and 4 (17.39%) subgingival and atherosclerotic plaque samples, respectively. In addition, the DNA of C.r was positive in 18 (78.26%) and 2 (8.69%) subgingival and atherosclerotic samples, respectively. McNemar analysis showed the significant differences between subgingival plaque and atherosclerotic samples for all the three microorganisms ($P = 0.016$, $P = 0.031$, and $P < 0.001$ for P.g, A.a, and Cr, respectively).

In overall, 2 patients tested were positive for all the three bacteria and one patient had P.g and A.a in both atherosclerotic and subgingival samples. All patients who tested positive for a bacterium in the atherosclerotic plaque sample also had the same bacterium in the subgingival plaque sample, although, the presence of a bacterium in subgingival plaque was not necessarily associated with its presence in the atherosclerotic plaque sample.

Discussion

Several epidemiologic studies have reported a statistical correlation between periodontitis and CAD.[13] Microorganisms have been proposed to contribute to CVD by direct and indirect mechanisms. Direct invasion of microorganisms to arterial wall or atherosclerotic plaques was assessed with evaluation of bacterial DNA. Microorganisms can produce infectious agents and also exacerbate and maintain inflammation and hence affect CVDs indirectly.[6,14]

Studies aiming at detecting DNA of periopathogens in atherosclerotic and subgingival plaques have yielded different results. Totally, in the present study, 23 subgingival and 23 atherosclerotic

plaques isolated from patients with chronic periodontitis who were scheduled for CABG surgery were assessed. Contrary to the findings in the studies by Cairo et al.,[15] Aimetti et al.,[16] and Aquino et al.,[17] which did not report periodontal pathogens in atherosclerotic plaques, the present study indicated the presence of periopathogens in atherosclerotic plaques only in 17.39% of the samples.

In the present study, the DNA of P.g was found in 43.47% of the subgingival plaques, and 13.04% of both samples (subgingival and atherosclerotic plaques) were found to be positive. The presence of P.g DNA in the studies by Pucar et al.,[18] Marcelino et al.,[19] Toyofuku et al.,[20] Mahendra et al.,[12] and Szulc et al.[5] was reported as 53.33%, 50%, 51%, 45.1%, and 23% of atherosclerotic samples, respectively. The fimbria of P.g has a capacity to adhere to endothelium and to invade it. This was the suggested mechanism by which P.g may contribute to the initiation or acceleration of atherosclerosis.[20]

The DNA of A.a in the current study was found to be present in subgingival and atherosclerotic plaques with a rate of 43.47% and 17.39%, respectively. Other studies including Pucar et al.,[18] Marcelino et al.,[19] Toyofuku et al.,[20] found the DNA of A.a in 26.67%, 7.10%, and 0% [20] of both samples, respectively.

Schenkein et al. showed that phosphorylcholine-bearing phenotype of A.a can invade the endothelial cells via the receptor for platelet-activating factor. This mechanism may aid this phenotype of A.a to access to the systemic circulation.[21]

The rate of Cr DNA in the present study was 78.26% and 8.69% in subgingival plaque and atherosclerotic plaque samples, respectively. The investigations by Marcelino et al.[19] and Mahendra et al.[12] found the DNA of Cr in 7.10% and 11.76% of both samples, respectively.

The presence of periopathogens in atherosclerotic plaques in the current study is in agreement with investigations performed in the studies by Szulc et al.,[5] Mahendra et al.,[12] Aquino et al.,[17] and Marcelino et al.,[19] which may be caused by bacteremia. Surprisingly, patients who tested positive for a given microorganisms in the atherosclerotic plaque sample also had the same bacteria in the subgingival plaque sample. None of the available studies could reveal the real invasion and colonization of these microorganisms in atherosclerotic plaques.[19]

Differences in the results of existing studies may be due to differences in subgingival microbial plaque species, host immune responses, study populations, and sampling volume techniques.[16] Szulc et al.[5] concluded that the method of sample collection may affect the results and isolation of microorganisms, and sampling the atherosclerotic plaque is more predictable compared to using paper points which contact with atherosclerotic plaque.

Limitation to this study was the rather small sample size which could be addressed in future studies.

Conclusion

In conclusion, this study demonstrates the presence of periopathogens in atherosclerotic plaques of patients with chronic periodontitis. These periopathogens may contribute to the pathogenesis of atherosclerosis directly or indirectly. Further studies are required to ascertain the underlying mechanism of these periopathogens in the atherosclerotic process.

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Conflict of Interests

Authors have no conflict of interests.

References

1. Ainamo J, Loe H. Anatomical characteristics of gingiva. A clinical and microscopic study of the free and attached gingiva. J Periodontol 1966; 37(1): 5-13.
2. Chistiakov DA, Orekhov AN, Bobryshev YV. Links between atherosclerotic and periodontal disease. Exp Mol Pathol 2016; 100(1): 220-35.
3. Kjellstrom B, Ryden L, Klinge B, Norhammar A. Periodontal disease-important to consider in cardiovascular disease prevention. Expert Rev Cardiovasc Ther 2016; 14(9): 987-9.
4. Gaetti-Jardim E Jr, Marcelino SL, Feitosa AC, Romito GA, Avila-Campos MJ. Quantitative detection of periodontopathic bacteria in atherosclerotic plaques from coronary arteries. J Med Microbiol 2009; 58(Pt 12): 1568-75.
5. Szulc M, Kustrzycki W, Janczak D, Michalowska D, Baczynska D, Radwan-Oczko M. Presence of Periodontopathic Bacteria DNA in Atheromatous Plaques from Coronary and Carotid Arteries. Biomed Res Int 2015; 2015: 825397.
6. Campbell LA, Rosenfeld ME. Infection and atherosclerosis development. Arch Med Res 2015; 46(5): 339-50.

7. Pourmoghaddas Z, Sadeghi M, Hekmatnia A, Sanei H, Tavakoli B, et al. Different Measurements of the obesity, adiponectin and coronary heart disease: A single-center study from Isfahan. J ResMed Sci 2012; 17(Spec 2): S218-S222.
8. Nesarhoseini V, Khosravi M. Periodontitis as a risk factor in non-diabetic patients with coronary artery disease. ARYA Atheroscler 2010; 6(3): 106-11.
9. Haynes WG, Stanford C. Periodontal disease and atherosclerosis: From dental to arterial plaque. Arterioscler Thromb Vasc Biol 2003; 23(8): 1309-11.
10. Georges JL, Rupprecht HJ, Blankenberg S, Poirier O, Bickel C, Hafner G, et al. Impact of pathogen burden in patients with coronary artery disease in relation to systemic inflammation and variation in genes encoding cytokines. Am J Cardiol 2003; 92(5): 515-21.
11. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA 2013; 310(20): 2191-4.
12. Mahendra J, Mahendra L, Kurian VM, Jaishankar K, Mythilli R. 16S rRNA-based detection of oral pathogens in coronary atherosclerotic plaque. Indian J Dent Res 2010; 21(2): 248-52.
13. Howell TH, Ridker PM, Ajani UA, Hennekens CH, Christen WG. Periodontal disease and risk of subsequent cardiovascular disease in U.S. male physicians. J Am Coll Cardiol 2001; 37(2): 445-50.
14. Hansen GM, Egeberg A, Holmstrup P, Hansen PR. Relation of Periodontitis to risk of cardiovascular and all-cause mortality (from a Danish Nationwide Cohort Study). Am J Cardiol 2016; 118(4): 489-93.
15. Cairo F, Gaeta C, Dorigo W, Oggioni MR, Pratesi C, Pini Prato GP, et al. Periodontal pathogens in atheromatous plaques. A controlled clinical and laboratory trial. J Periodontal Res 2004; 39(6): 442-6.
16. Aimetti M, Romano F, Nessi F. Microbiologic analysis of periodontal pockets and carotid atheromatous plaques in advanced chronic periodontitis patients. J Periodontol 2007; 78(9): 1718-23.
17. Aquino AR, Lima KC, Paiva MS, Rocas IN, Siqueira JF Jr. Molecular survey of atheromatous plaques for the presence of DNA from periodontal bacterial pathogens, archaea and fungi. J Periodontal Res 2011; 46(3): 303-9.
18. Pucar A, Milasin J, Lekovic V, Vukadinovic M, Ristic M, Putnik S, et al. Correlation between atherosclerosis and periodontal putative pathogenic bacterial infections in coronary and internal mammary arteries. J Periodontol 2007; 78(4): 677-82.
19. Marcelino SL, Gaetti-Jardim E Jr, Nakano V, Canonico LA, Nunes FD, Lotufo RF, et al. Presence of periodontopathic bacteria in coronary arteries from patients with chronic periodontitis. Anaerobe 2010; 16(6): 629-32.
20. Toyofuku T, Inoue Y, Kurihara N, Kudo T, Jibiki M, Sugano N, et al. Differential detection rate of periodontopathic bacteria in atherosclerosis. Surg Today 2011; 41(10): 1395-400.
21. Schenkein HA, Barbour SE, Berry CR, Kipps B, Tew JG. Invasion of human vascular endothelial cells by Actinobacillus actinomycetemcomitans via the receptor for platelet-activating factor. Infect Immun 2000; 68(9): 5416-9.

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Methods of sampling and sample size determination of a comprehensive integrated community-based interventional trial: Isfahan Healthy Heart Program

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Original Article

Abstract

BACKGROUND: The aim of this study was describing the sampling methods and sample size of the Isfahan Healthy Heart Program (IHHP) and its sub-studies in focus.

METHODS: The IHHP was carried out between 2000 and 2007 in urban and rural areas in 3 districts, namely Isfahan and Najafabad (as the intervention areas), and Arak (as the reference area), Iran. It consisted of the 3 phases of baseline surveys during 2000-2001, interventions between 2002 and 2005, and post-intervention surveys during 2006-2007 on 4 target groups (adults, health professionals, cardiac patients, children, and adolescents). During 2002 to 2005, 4 evaluation studies were conducted to evaluate short-term results. An ongoing cohort study entitled the Isfahan Cohort Study was performed on those aged ≥ 35 years at baseline in 2001 to access the risk of cardiovascular disease (CVD) occurrence.

RESULTS: Using stratified random cluster methods, 12514, 5891, 4793, 6096, 3012, and 9572 adults and 1946, 1999, 1427, 1223, 389, and 1992 adolescents were chosen in the 1st to 3rd phases. Furthermore, simple random sampling was used for selecting 923, 694, 1000, and 2015 health professionals and 814, 452, 420, and 502 cardiac patients. A multistage sampling method was adopted for the collection of samples from parents of preschoolers and primary school children aged 2-10 years, adolescents' parents, and some teachers. A prospective cohort study was started on 6504 eligible individuals.

CONCLUSION: The IHHP, as a comprehensive community-based interventional trial in Iran, among the few population-based studies around the world, has reasonable sampling methods and sample size.

Keywords: Cardiovascular Disease, Sample Size, Sampling Design, Isfahan Healthy Heart Program

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Introduction

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity all over the world. More than 80% of deaths due to CVD take place in low- and middle-income countries.[1] Moreover, the CVD health burden is increasing in almost all countries, particularly in developing nations.[2]

CVD is responsible for a considerable proportion of the mortality and morbidity among the Iranian population. According to reports by the Iranian Ministry of Health and Medical Education, death from CVD is the first leading cause of mortality and morbidity in Iran.[3]

Epidemiologic transition from infectious to chronic diseases, as well as the aging of the population, in developing countries is exacerbating the burden of chronic non-communicable diseases (CNCDs). Such diseases account for 80% of the total burden of mortality due to CNCDs in these countries. The prevention and control of CNCDs would avert 24 million deaths in low-income and middle-income countries over the next decades.[4]

Prevention of CVDs is the most effective way of combating the CVDs epidemic in less developed and developing nations.[5] Earlier studies have revealed that interventional population-based programs were effective on improving health promotion knowledge and behaviors. The successful

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development and implementation of a health promotion program depends upon the identification of the scope and breadth of baseline knowledge among the targeted population members.[1,6-12] Lack of a reliable design, well defined target population, good sampling design, and adequate sample size may impede all administrative efforts targeted at the promotion of prevention as well as adoption of healthy lifestyle changes.

Numerous community-based trials have been conducted in developed countries including the US and Finland and have resulted in significant decreases in the risk of CNCDs and deaths due to coronary heart disease (CHD). However, there are few reports of such trials in the developing world.[8-10,12] Due to the increasing prevalence of CVD and its large burden in Iran in recent years, conducting such comprehensive interventions can provide a good pilot or feasibility study to implement large-scale preventive programs at a national level.

The Isfahan Healthy Heart Program (IHHP) was designed and launched in 2000. This study was aimed to modify population lifestyle and risk factors of the disease and was also intended to enhance the knowledge, attitude, and practice of individuals (adults, adolescents and their parents, children and their parents, school teachers, health professionals, and cardiac patients) in relation to the risk factors of CVD. Furthermore, it introduced ways to prevent and control these diseases and their risk factors. This program provided an opportunity to assess whether lifestyle interventions are effective or not in a developing country. The program targeted individuals, the community, and environmental changes to support health behavior modification. Many report papers have been published on the whole design, methods, and evaluation components and on assessing the feasibility and outcomes of IHHP with brief referral to sample size and sampling methods.[13-35] However, no comprehensive report is available regarding the sampling strategies and sample size of IHHP and its sub-studies. The current paper describes the sampling strategies, and sample size for IHHP and its sub-studies in detail.

Materials and Methods

Sampling methods and sample size

The IHHP was carried out between 2000 and 2007 in urban and rural areas in 3 districts, namely Isfahan and Najafabad (as the intervention areas) and Arak (as the reference area), Iran.[14,15] The IHHP consisted of 3 phases: assessing the current situation in intervention and reference areas (baseline survey), implementing intervention activities only in the intervention areas, and assessing the post-intervention situation in the intervention and reference areas (post-intervention survey).

Phase 1: Baseline survey in 2000-2001

It was a cross-sectional survey on the current status of community in relation to knowledge, attitude, and practice (KAP) in a variety of fields, including nutrition, smoking, and physical activity, on each of the 4 target groups including adult, health professionals like physicians, nurses, health workers, and health volunteers, adolescents aged 11–18 years in intermediate and high schools, their parents and some school teachers, and cardiac patients. The study also investigated the status of cardiometabolic risk factors such as high lipid levels, hypertension, diabetes, and obesity. Blood samples were only collected from adults and adolescents.

Phase 2: Intervention activities between 2002 and 2005

The IHHP intervention phase consisted of 10 projects targeting different groups and various interventions. These projects were implemented for a period of 4 years. By using both population and high risk strategies for CVD prevention, these projects integrate the interventions and policies that address the promotion of healthy lifestyle behaviors, as major determinants of CNCDs, such as healthy diet, tobacco control, physical activity, and stress management among participants in the intervention areas. During 2002 to 2005, 4 independent evaluation studies were performed to evaluate short-term results like knowledge and behavior changes in each of the 4 target groups. In addition, random samples of parents of preschoolers and primary school children aged 2-10 years were evaluated from this phase onwards.

Phase 3: Post-intervention surveys in 2006-2007

Similar to phase 1, a study was conducted on independent random samples to determine the effects of interventions on each target group.[14,15]

The remainder of this section is devoted to a detailed description of the target population (adult, health professionals, adolescents aged 11–18 years in junior high school and high school, their parents and some teachers and school staff, as well as parents of preschoolers and primary school children aged 2-10 years, and cardiac patients) and sample size, sampling methods, and evaluated variables for each of the 4 target groups.

1. Adults group

Target population: adults (over 19 years old)

Sample size and sampling methods: In the adult

group, a multistage sampling was conducted. Initially, quota sampling of the population of Isfahan to Najafabad was considered (i.e., 3:1). Then, stratified sampling was conducted based on age, sex, and urban and rural areas. The sample size in different age groups (19-24, 25-34, 35-44, 45-54, 55-64, and 65 years and older) were determined based on the age distribution of the population in baseline and post-intervention surveys (in evaluation studies in 2002-2005, similar to MONICA method, an equal number was considered for each age group). Furthermore, by considering the equal gender distribution of the population in Iran, an equal sample size was selected from among men and women. In addition, the number of subjects in urban and rural areas of each district was determined based on population distribution. Then, multistage cluster sampling was used based on clusters of the provincial health center. Accordingly, the clusters of each of the urban and rural areas in Isfahan, Najafabad, and Arak were randomly selected from different health centers. Then, the sample was assigned to every cluster based on the number of households covered. Households were then selected in each cluster using systematic sampling and the questionnaire was randomly completed for a person over 19 years of age in the family.

Considering p1 = 20%, p2 = 15%, d = 0.05, and β = 10%, about 12514 and 9572 people were selected based on the proportional to size estimation formula from intervention and reference areas, in the 1st and 3rd phase, respectively. In the selection process, equal ratios in sex, accuracy of cluster sampling in both phases, and the missing rate of samples (about 30%) for cohort surveys in the 1st phase were also taken into account. Sex-based and age-based CINDI protocol sampling methods were used for sample size determination in the annual intervention phases.[14,15] The total sample size and details of sampling methods in different phases of the IHHP study are presented in table 1. In addition, table 2 presents the distribution of the studied sample sizes in the adult group.

All participants aged over 35 years (n = 6504) living in both areas who took part in the 1st phase of the IHHP were asked to participate in a 10-year longitudinal study named Isfahan Cohort Study (ICS).[26] This study was aimed to determine the individual impact of interventions, the incidence of cardiovascular events, and the Iranian risk assessment chart. It is essential to mention that a telephone follow-up interview was performed every 2 years. CVD events such as fatal and nonfatal myocardial function (MI) and stroke, stable and unstable angina, and sudden cardiac death were expressed in telephone interviews and were confirmed by performing verbal autopsy. In addition to the initial questionnaire of the adults' study of the IHHP in 2000-2001 which was completed for ICS participants, a questionnaire similar to the one used at the beginning of the study was completed for them in 2006-2007 and 2013-2014. The sampling method of this study was similar to the adult sub-study of IHHP. Details of the follow-up phone calls and interviews as well as the number of people followed-up in the ICS were published elsewhere.[26] The new version of the diagram of this cohort study is presented in figure 1.

Evaluated variables in the adults group: In this study, the questionnaires were developed based on the World Health Organization (WHO) STEPwise approach (STEPS) and measurements were designed based on WHO standards. In some parts of the questionnaire, besides maintaining the structure of STEPS, additional questions have been completed for respondents. In general, the questionnaires included demographic data (i.e., age, sex, education, occupation, and marital status), questions related to KAP on nutrition, physical activity, smoking, stress levels, and how to cope with stress as well as quality of life (QOL) and other behavioral risk factors.[14,15] To determine the nutritional practice of individuals, a standard food frequency questionnaire with appropriate reliability and validity was completed.[36] Furthermore, a 24-hour recall was completed for a subsample of about 2000 participants for each of the 1st and 2nd phases.[37,38] Past history and physical and paraclinical examination that included questions about the history of CVD risk factors, such as diabetes, hypertension, hyperlipidemia, and family history of heart diseases, as well as Rose's questionnaire for chest pain were also considered. For those who were over 35 years of age, an electrocardiogram (ECG) was performed and standard Minnesota codes were used for ECG interpretation and analysis. These 2 were performed in the 1st and last phases. Additionally, all cardiometabolic factors such as weight, height, waist and hip circumferences, systolic and diastolic blood pressure, cholesterol (total, LDL, and HDL), apolipoproteins A and B, triglycerides, 2-hour and fasting blood glucose, complete blood count (CBC), and C-reactive protein (CRP) were measured for all adults aged over 19 years in the 1st and last phases.[14,15]

Table 1. Sampling in the Isfahan Healthy Heart Program: Size and Method

Target Population	Population Definition	Sample Frame	First Phase (Baseline) 2001			Second Phase (Impact evaluation) 2002-2005			Third Phase (Outcome) 2006-2007		
			Sampling Method	Sample Size	Details	Sampling Method	Sample Size	Details	Sampling Method	Sample Size	Details
Adults Population	Age ≥ 19 years	Adult population in interventional and reference communities	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, systematic, random	12514	Sampling based on age, sex, and residency (Urban/Rural) distribution in population	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, systematic, random	5891 4793 6096 3012	- The 4th phase was performed only in the interventional area - Sampling based on sex and residency (Urban/Rural) distribution in population - Similar to Monika's method, an equal number was considered for each age-group	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, systematic, random	9572	Sampling based on age, sex, and residency (Urban/Rural) distribution in population
Pediatrics surveys											
Adolescents	Age: 11-18 years	Intermediate and high schools in interventional and reference communities	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	1946	Sampling based on grade, sex, and residency (Urban/Rural) distribution in population	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	1999 1427 1223 389	-The 4th phase was performed only in the interventional area - Sampling based on sex, and residency (Urban/Rural) distribution in population -- similar to Monika's method, an equal number was considered for each age-group	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	1992	Sampling based on grade, sex, and residency (Urban/Rural) distribution in population

Table 1. Sampling in the Isfahan Healthy Heart Program: Size and Method (continue)

Target Population	Population Definition	Sample Frame	First Phase (Baseline) 2001			Second Phase (Impact evaluation) 2002-2005			Third Phase (Outcome) 2006-2007		
			Sampling Method	Sample Size	Details	Sampling Method	Sample Size	Details	Sampling Method	Sample Size	Details
Pediatrics surveys											
Children	Age: 2-10 years	Health Centers/ kindergarten/ primary schools in interventional and reference communities	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	0	Sampling based on grade, sex, and residency (Urban/Rural) distribution in population	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	1998 1754 1542 431	-The 4th phase was performed only in the interventional area - Sampling based on sex, and residency (Urban/Rural) distribution in population - Similar to Monika's method, an equal number was considered for each age-group	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	1914	Sampling based on grade, sex, and residency (Urban/Rural) distribution in population
Adolescents Parents	No limitation	Intermediate / high school in interventional and reference communities	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	1946	-	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	1999 1303 1163 342	-The 4th phase was performed only in the interventional area	Multistage Sampling: quota, stratified, cluster, random, probability proportionate to size, random ,random	1984	-
Teachers	No limitation	Kindergarten/primary/ intermediate / high school in interventional and reference communities	Multistage Sampling: quota, stratified, cluster, random	398	-	Multistage Sampling: quota, stratified, cluster, random	201 327 436 181	-The 4th phase was performed only in the interventional area	Multistage Sampling: quota, stratified, cluster, random	425	-

Table 1. Sampling in the Isfahan Healthy Heart Program: Size and Method (continue)

Target Population	Population Definition	Sample Frame	First Phase (Baseline) 2001			Second Phase (Impact evaluation) 2002-2005			Third Phase (Outcome) 2006-2007		
			Sampling Method	Sample Size	Details	Sampling Method	Sample Size	Details	Sampling Method	Sample Size	Details
Health Professionals	General Physician/Nurses/Health workers	Health centers/ hospitals/ educational courses in interventional and reference communities	Random Sampling	923	-	Random Sampling	694 1000	-	Random Sampling	2015	-
Patients	Patients hospitalized during 9-12 months before the survey (IHD/CeVD)	Surveillance Data File for CAD and CeVD in ICRC Surveillance Department in interventional and reference communities	Random Sampling	814	-	Random Sampling	452 420	-	Random Sampling	502	-

IHD: Ischemic heart disease; CeVD: Cerebrovascular disease; ICRC: International Committee of the Red Cross; CAD: Coronary artery disease

Table 2. Distribution of the sample size in adult group of Isfahan Healthy Heart Program

Isfahan Healthy Heart Program Adults Population		First Phase (Baseline) sample size 2001	Second Phase (Impact Evaluation) sample size 2002-2005				Third Phase (Outcome) sample size 2006-2007
			1st	2nd	3rd	4th	
Area	Intervention	6175 (49.3)	2994 (50.8)	2400 (50.1)	3014 (49.4)	3012 (100.0)	4719 (49.3)
	Reference	6339 (50.7)	2897 (49.2)	2393 (49.9)	3082 (50.6)	0(0)	4853 (50.7)
Residency	Urban	9093 (72.7)	4370 (74.2)	3506 (73.1)	4521 (74.2)	2652 (88.0)	6692 (69.9)
	Rural	3421 (27.3)	1521 (25.8)	1287 (26.9)	1575 (25.8)	360 (12.0)	2880 (30.1)
Sex	Female	6391 (51.1)	2993 (50.8)	2424 (50.6)	3112 (51.2)	1558 (51.7)	4786 (50.0)
	Male	6123 (48.9)	2898 (49.2)	2369 (49.4)	2971 (48.8)	1453 (48.3)	4786 (50.0)
Age groups (year)	19-24	2310 (18.5)	1175 (19.9)	967 (20.2)	971 (16.0)	427 (14.2)	1886 (19.7)
	25-34	3662 (29.3)	1202 (20.4)	969 (20.2)	1042 (17.1)	540 (17.9)	2913 (30.5)
	35-44	2717 (21.7)	1201 (20.4)	958 (20.0)	1025 (16.9)	512 (17.0)	1898 (19.8)
	45-54	1628 (13.0)	1137 (19.3)	946 (19.7)	1030 (16.9)	509 (16.9)	1174 (12.3)
	55-64	1130 (9.0)	671 (11.4)	501 (10.5)	986 (16.2)	492 (16.3)	757 (7.9)
	> 65	1067 (8.5)	505 (8.6)	452 (9.4)	1029 (16.9)	530 (17.6)	937 (9.8)

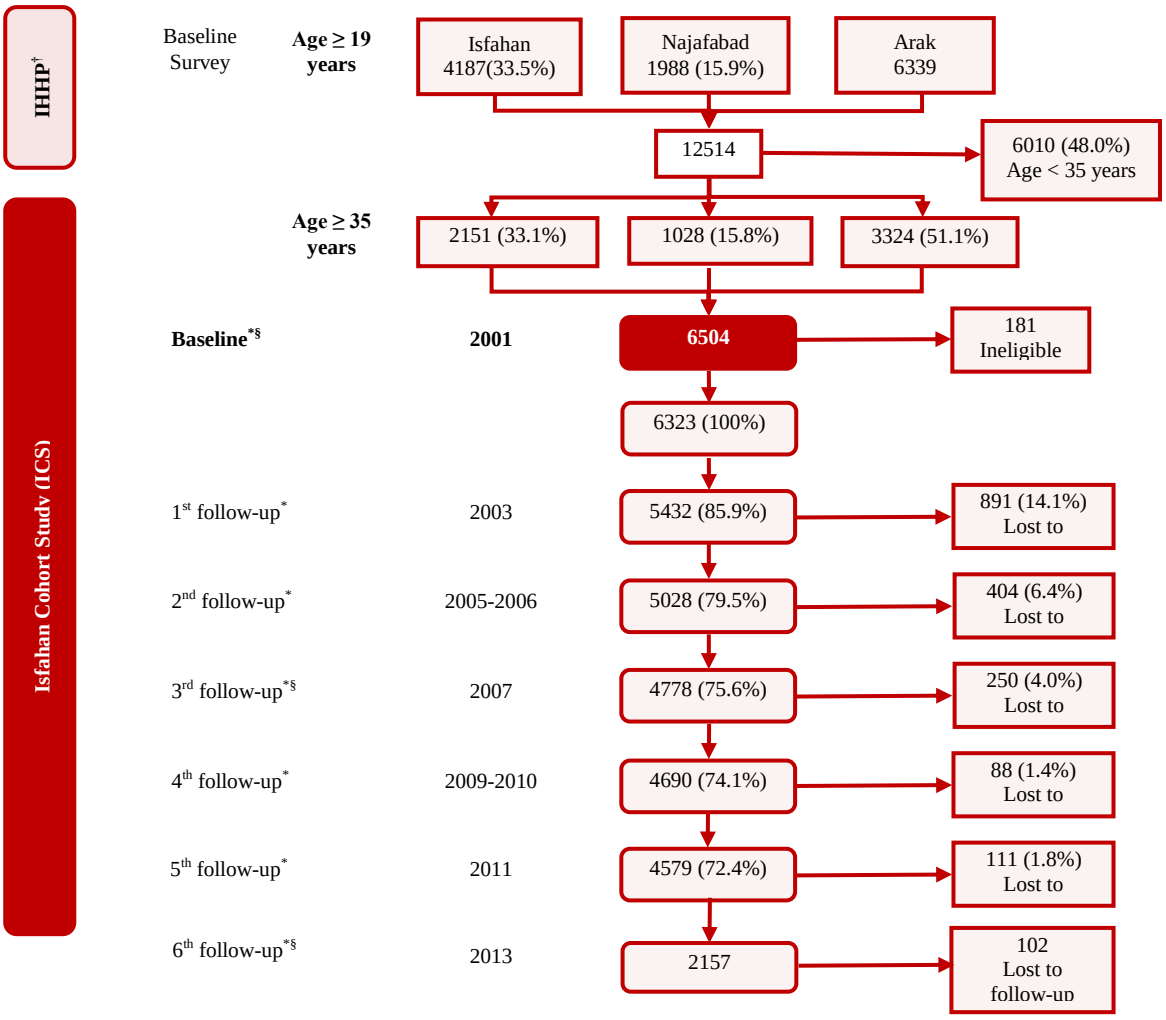

Figure 1. Isfahan Cohort Study
† Isfahan Healthy Heart Program
* Telephone interview asking for (CVD) events (being confirmed with medical records).
§ Full Interview including demographics, socioeconomic status, lifestyle and biochemical examinations were carried out.

2. Children, adolescents, parents, and teachers groups

Target population: Parents of preschoolers and primary school children aged 2-10 years, adolescents aged 11–18 years in intermediate and high school, adolescents' parents, and some teachers

Sample size and sampling methods: A multistage sampling method was adopted for the collection of samples from kindergartens, preschools, primary schools, intermediate schools, and high schools, reported by the Department of Education based on the urban and rural areas of Isfahan, Najafabad, and Arak. We determined the sampling quota of Isfahan population to Najafabad (two-thirds to one third) and stratified samples based on gender distribution, level of education, and urban and rural population distribution. Then, clusters were randomly selected from schools of urban and rural areas of the intervention and reference areas. Sampling size in each school was determined with probability proportional to the size of each school. Finally, the random selections of classes from each school as well as students from the classes included in the random sampling process were conducted.

Considering the proportional sample size estimation formula and sampling accuracy of 10%, the minimum proportion of 0.3, and type I error of 5%, sample size was estimated. The sample sizes from the 1st to the 3rd phases (totally in the intervention and reference areas), were 1946, 1999, 1427, 1223, 389, and 1992 students aged 11-18 years, 1946, 1999, 1303, 1163, 342, and 1984 parents of students, and 398, 201, 327, 436, 181, and 425 teachers including managers, schoolmasters, health teachers, school counselors, and biology teachers, respectively. It should be noted that, random samples

Table 3. Distribution of the sample size in adolescents, children, and teachers of Isfahan Healthy Heart Program

Isfahan Healthy Heart Program			First Phase (Baseline) sample size 2001	Second Phase (Impact Evaluation) sample size 2002-2005				Third Phase (Outcome) sample size 2006-2007
				1st	2nd	3rd	4th	
Adolescents	Area	Intervention	969 (49.8)	1000 (50.03)	629 (44.1)	516 (42.2)	389 (100.0)	972 (48.8)
		Reference	977 (50.2)	999 (50.00)	798 (55.9)	707 (57.8)	0 (0)	1020 (51.2)
	Residency	Urban	1251 (64.3)	1320 (66.00)	1085 (76.0)	837 (68.4)	321 (82.5)	1532 (76.9)
		Rural	695 (35.7)	679 (34.00)	342 (24.0)	386 (31.6)	68 (17.5)	460 (23.1)
	Gender	Girl	1001 (51.4)	999 (50.00)	732 (51.7)	658 (57.7)	193 (54.8)	978 (49.1)
		Boy	945 (48.6)	1000 (50.00)	683 (48.3)	482 (42.3)	159 (45.2)	1014 (50.9)
	Grade	Middle school	986 (50.7)	1000 (50.02)	774 (54.7)	618 (50.5)	203 (52.2)	1039 (52.2)
		High school	960 (49.3)	999 (49.98)	641 (45.3)	605 (49.5)	186 (47.8)	953 (47.8)
Children	Area	Intervention	0 (0)	1000 (50.10)	671 (38.3)	584 (37.9)	431 (100.0)	972 (50.8)
		Reference		998 (49.90)	1083 (61.7)	958 (62.1)	0(0)	942 (49.2)
	Residency	Urban		1998 (100.00)	1477 (84.2)	1206 (78.2)	399 (92.6)	1475 (77.1)
		Rural			277 (15.8)	336 (21.8)	32 (7.4)	439 (22.9)
	Gender	Girl		998 (49.90)	919 (52.6)	774 (51.1)	194 (45.8)	933 (48.7)
		Boy		1000 (50.10)	829 (47.4)	740 (48.9)	230 (54.2)	981 (51.3)
	Grade	Preschool		987 (49.70)	831 (47.5)	649 (42.1)	155 (36.0)	922 (48.2)
		Elementary school		1000 (50.30)	920 (52.5)	892 (57.9)	275 (64.0)	989 (51.8)
Teachers	Area	Intervention	201 (50.5)	200 (100.00)	209 (63.9)	306 (70.2)	181 (100.0)	193 (45.4)
		Reference	197 (49.5)	0 (0)	118 (36.1)	130 (29.8)	0 (0)	232 (54.6)
	Headmaster		398 (100)	201 (100.00)	30 (9.2)	53 (14.2)	23(15.1)	64 (15.1)
	Schoolmaster				53 (16.2)	55 (14.7)	36 (23.7)	72 (16.9)
	School consultant				10 (3.1)	42 (11.2)	20 (13.2)	51 (12.0)
	Biology teacher				9 (2.8)	17 (4.5)	5 (3.3)	14 (3.3)
	Sport coach				216 (66.1)	26 (7.0)	11 (7.2)	16 (3.8)
	Health coach				3 (0.9)	13 (3.5)	2 (1.3)	5 (1.2)
	Other teachers				0 (0)	168 (44.9)	55 (36.2)	192 (45.2)

were also drawn from among 2-10-year-old children in preschools and primary schools along with one of their parents in the intervention and post intervention surveys.

In the second and third phases (totally in the intervention and reference areas), there were 1998, 1754, 1542, 431, 1914 parents of children.[13-15,27,29,30,32] The total sample size and sampling details of all phases of the IHHP in the target population are presented in table 1. In addition, the distribution of the sample size in the study structure is shown in table 3; this was done based on the items involved in the sampling.

Evaluated variables in Children, adolescents, parents, and teachers groups: Due to the diversity of the studied groups, 4 types of questionnaires were designed for the following groups: parents of preschool and primary school children, intermediate and high school students, parents of intermediate school and high school students, and school teachers. After obtaining written consent from parents of children and adolescents, a questionnaire which consist of demographic questions, knowledge, attitude and practice about risk factors, as well as the importance, prevention, and control of these risk factors from childhood were completed. Then, specific questions about food frequency for all children and adolescents were completed. Measurements of weight, height, waist and hip circumferences, systolic and diastolic blood pressure, total cholesterol, LDL, HDL, triglycerides, and fasting blood glucose in the 1st and 3rd phases as well as apolipoproteins A and B in the third phase were taken from 11-18-year-old students.[13-15,27,29,30,32]

3. Health professionals group

Target population: Health professionals including physicians, nurses, health workers, and primary health workers

Sample size and sampling methods in health professionals group: By simple random-sampling method from among health professionals, the sample sizes for the 1st and 3rd phases were estimated as 923 and 2015 individuals, respectively (totally for both the intervention and reference areas).[14,15,18,34] The total sample size and sampling details of all phases for the IHHP in this target group is presented in table 1. Similarly, the distribution of sample size in the study structure is presented in table 4.

Table 4. Distribution of the sample size in patients and health professionals of the Isfahan Healthy Heart Program

Isfahan Healthy Heart Program			First Phase (Baseline) sample size 2001	Second Phase (Impact Evaluation) sample size 2002-2005				Third Phase (Outcome) sample size 2006-2007
				1st	2nd	3rd	4th	
Patients	Area	Intervention	269 (33.0)	0 (0)	0 (0)	252 (55.8)	249 (59.3)	250 (49.8)
		Reference	545 (67.0)			200 (44.2)	171 (40.7)	252 (50.2)
	Sex	Female	379 (46.6)			177 (41.5)	169 (41.1)	235 (47.2)
		Male	435 (53.4)			250 (58.5)	242 (58.9)	263 (52.8)
Health Professionals	Area	Intervention	512 (55.5)			376 (54.2)	500 (50.0)	1482 (73.5)
		Reference	411 (44.5)			318 (45.8)	500 (50.0)	533 (26.5)
	Primary health workers		262 (28.4)	191 (27.5)	0 (0)	0 (0)	200 (20.0)	993 (49.3)
	Junior health workers		200 (21.7)	94(13.6)			0 (0)	172 (8.5)
	Senior health workers		149 (16.1)	218 (31.4)			200 (20.0)	128 (6.3)
	Health volunteers		312 (33.8)	0 (0)			0 (0)	180 (8.9)
	Nurses		0(0)	0 (0)			200 (20.0)	542 (27.0)
	Physicians		0 (0)	191 (27.5)			400 (40.0)	0 (0)

Evaluated variables in health professionals group: To gather data, specific questionnaires, with more than 50 questions for physicians, 40 for nurses, and 50 questions for primary health workers, were used.

The questionnaires contained questions about the KAP of health care providers regarding suitable nutrition, smoking cessation, physical activity, prevention and control of CVD, and pharmacological and non-pharmacological treatments.[14,15,18,34]

4. Cardiac patients group

Target population: Patients with a history of myocardial infarction (MI), stroke, coronary artery bypass grafting (CABG), percutaneous coronary angioplasty (PCI), and patients with a history of hospitalization due to cardiovascular attacks

Sample size and sampling methods in cardiac patients group: In this study, simple random sampling was implemented among cardiac patients referred to medical centers. Based on the proportional to size estimation formula and the sampling accuracy of 10%, the minimum proportion of 0.3, and type I error of 5%, the sample size was estimated. The sample sizes for the 1st and 3rd phases were estimated to be 814 and 502 (totally for the intervention and reference areas), and 452 and 420 patients in the annual intervention phases, respectively.[14,15,22] The total sample size and sampling details of all phases of the IHHP in the mentioned target group are provided in table 1. In addition, the distribution of sample size in the study structure is presented in table 4.

Evaluated variables in cardiac patients group: In this study, the questions regarding KAP, measurement of height, weight, systolic and diastolic blood pressure, total cholesterol, LDL, HDL, triglycerides, fasting blood glucose, apolipoproteins A and B, and CRP, as well as questions on morbidity including the number of visits to a doctor, re-hospitalization, medication, diagnostic procedures, days of absenteeism from work due to disability, open-heart surgery, and PCI were answered for each participant.[14,15,22]

Results

We chose 12514, 5891, 4793, 6096, 3012, and 9572 adults and 1946, 1999, 1427, 1223, 389, and 1992 adolescents using stratified random cluster method in the 1st to 3rd phases. Moreover, simple random sampling was used to select 923, 694, 1000, 2015 health professionals and 814, 452, 420, 502 cardiac patients. A multistage sampling method was adopted for collection of samples from parents of preschoolers and primary school children aged 2-10 years, adolescents' parents and some teachers. Prospective cohort study was started on 6504 eligible people.

Discussion

CVD is a leading cause of death throughout the world, which is increasingly growing not only in industrialized, but also developing countries that are affected by significant changes in lifestyle due to rapid industrialization.[39] A number of community CVD prevention programs have been implemented over the last 40 years. A systematic review reported that there were a number of well-known community CVD prevention programs such as Stanford Three Community Study, Stanford Five-City Project, The Minnesota Heart Health Program, and The North Karelia Project; but this systematic review identified an unexpectedly large number of additional

programs, and many of these have not been featured in previous systematic reviews.[6]

The Stanford Three Community Study was conducted in 1972 to investigate the risk factors of CVD through a health promotion program in 2 cities of the State of California as the intervention and in 1 city as the reference area. In 1978, in the framework of the Stanford Three Community Study in California, a quasi-experimental study was carried out on the general population aged 12-74 years in Northern California, called the Stanford Five-City Project. This study was aimed to assess changes in the prevalence of risk factors for CVDs, and morbidity and mortality due to CVDs based on the implementation of a major community-based intervention program. This program was carried out in the form of 4 annual cross-sectional studies with independent samples, and a longitudinal study with repeated measurements using a 6-year educational intervention (1980-1986) on all people of the intervention area as well as follow-up until 1992. In this study, with random assignment, the 2 cities of Monterey (n = 43400) and Salinas (n = 80500) were considered as the intervention areas and the 3 cities of Modesto (n = 132400), San Luis Obispo (n = 343000), and Santa Maria (n = 39700) were chosen as the reference areas. In this study, households of intervention and reference areas were randomly selected, and then, all members of the households aged 12-74 years were invited to participate in the study.[7,11]

The Minnesota Heart Health Program is another intervention program that was conducted as a non-randomized community-based study for primary prevention of CVDs and stroke from 1980 to 1990 on the general population aged 25-74 years, children in 6th-10th grade, and employees of 119 participating companies. This 5-6-year quasi-experimental intervention program at the community and individual levels was conducted on 400 thousand people in 6 selected communities from Minnesota and North and South Dakota in Northwestern United States. In this program, the intervention and reference areas were matched in terms of population size (25,000 to 110,000), the type of community (small and medium rural and urban areas), and the distance to a metropolitan area. However, the allocation of communities to the intervention and reference areas was non-random. The sample sizes throughout the study were about 231,222 people in the intervention area and 181,149 in the reference area. In this program, risk factors and behaviors were measured before starting the intervention program; furthermore, they were annually followed-up for 6-7 years in all 6 communities. Some cross-sectional studies in the framework of the Minnesota Heart Health Program were conducted periodically with a random selection of people in each community using a 2-stage cluster sampling method. The cohort study of this program consisted of participants randomly selected from all cross-sectional studies (before the beginning of the intervention).[8,10]

The North Karelia Project started in 1972, as one of the world's most successful intervention studies in Finland, it aimed to change people's lifestyles and control and prevent the major risk factors for CVDs. After the implementation of the original project and obtaining preliminary results during 1972-1977, the experiences of this program were used to implement a national action and preventive activities throughout the country. The cross-sectional studies of this project were carried out in the province of North Karelia as the intervention area and in the province of Kuopio as a reference area, once every 5 years in 1972, 1977, 1982, 1987, and 1992. In each cross-sectional study, a random sample was selected for each province from the national population register system. In studies during 1972 and 1977, each sample contained a population of 6.6% born between the years 1913 and 1947; however, the other 3 cross-sectional studies in 1982, 1987, and 1992 consisted of people of 25-64 years of age. These samples were classified so that in each area at least 250 people were selected from each gender and each 10-year age group of 25-34, 35-44, 45-54, and 55-64. The investigated sample sizes in the 2 provinces of intervention and reference in 1972, 1977, 1982, 1987, and 1992 were 9882, 10012, 5712, 4512, and 3012, respectively.[9,12]

In Iran, few intervention studies have been conducted to prevent heart diseases and their risk factors. The Tehran Lipid and Glucose Study was developed to determine the risk factors for atherosclerosis; in addition, it was aimed to modify people's lifestyle and prevent the growing trend of diabetes, dyslipidemia, and other risk factors for CVDs. The design of this study consisted of 2 main phases: the 1st phase was a cross-sectional study to determine the prevalence of risk factors for CVDs and the 2nd phase consisted of a cohort, prospective, intervention which began in January 2002. Both of the intervention and reference areas were selected from the same district in Tehran, Iran. A total of 15,005 residents of Tehran aged 3-69 years with the same age and sex distribution were selected using a multistage cluster sampling from district 13 (1 out of

22 districts) of Tehran. The participants were covered by 3 primary health care centers (out of 20 health care centers in this district) and entered the baseline cross-sectional study. People, who participated in the 1st phase of this cross-sectional study, were followed up in the intervention phase in order to improve the lifestyle of the population and prevent non-communicable diseases (NCD) risk factors. The target groups of these interventions included 5630 participants from students, housewives, and people at risk covered by 1 of the 3 sampling health care centers. The reference group included 9375 people who were covered by the other 2 health centers.[39]

The IHHP is the most comprehensive integrated community-based interventional study in Iran and is similar to many well-known studies specifically to the North Karelia Project in Finland. It was launched in 1999 and started in 2000 with a quasi-experimental design, a control area, and multiple levels of evaluation. The intervention areas were both urban and rural areas within Isfahan and Najafabad, while the control area was Arak located in Markazi Province which is at a distance of 375 km from the intervention areas. A notable feature of this study was the performance of various interventions on the whole population of more than 2 million in the intervention areas through 10 different projects in terms of target groups including the general population, students of primary, intermediate, and high schools (aged 2-18 years), their parents, teachers, and school staff, and cardiac patients and health professionals, including physicians, nurses, health workers, and primary health workers. Another feature was its emphasis on suitable nutrition, smoking cessation, physical activity, and copying strategies. The third notable feature was that both cross-sectional studies with independent samples were performed in the 2 pre-intervention and post-intervention phases and 4 cross-sectional studies within the intervention phase during a period of 6 years, and a 20-year longitudinal study was started in 2001 with repeated measurements to calculate the risk assessment chart for CVD occurrence. The other features were its intervention and reference areas, and collection of an immense amount of information regarding demographic characteristics, socioeconomic conditions, knowledge, attitude and practice regarding CVD risk factors, lifestyle, stress and ways to cope with it, QOL, medical and family history, physical and paraclinic examinations, and physical and biochemical measurements.

According to the established criteria about well-designed interventional population-based CVD studies in the literature review, the IHHP could be considered a reasonable study in terms of the aspect of sample size in its sub-studies as shown in table 1. Furthermore, the IHHP had reliable multistage sampling methods taking into account the structure of each sub-community. These were quota, stratified, cluster, random, probability proportionate to size, systematic and simple random in adults. They were quotas, stratified, cluster, random, probability proportionate to size, simple random, and simple random in adolescents and their parents and teachers. Moreover, simple random sampling was used in health professionals and patients with cardiac diseases. It is worth noting that the IHHP had full coverage of the included study areas in sampling and reliable sample size from each age and sex group, and residential (urban or rural) area through a well-designed sampling process.[14,15,19,40-42] However, in a study such as the Minnesota Heart Health Program on adults, surveys were conducted periodically in each community with a 2-stage cluster sampling design. Initially, census blocks were randomly selected from each city, with the probability of selection proportional to the expected number of households. Geographically adjacent groups of 5 households were randomly selected from within the selected blocks. Within households, a single age-eligible adult was selected at random.[8,10] In the North Karelia Project, for each cross-sectional survey in the included province, a random sample was drawn from the national population registry. The sample selection was performed in a stratified framework in which at least 250 subjects of each sex and 10 year age group (25-34, 35-44, 45-54, and 55-64 years) were chosen in each area.[9,12] In the Stanford study, after considering each included city as intervention or control areas, random samples from among residents aged 18-74 years who lived in the 4 central cities in California were selected.[7,11]

Conclusion

The experience and results of the IHHP in Iran will support the idea that a well-planned community-based program is practical and feasible, and can have a major impact on lifestyle and risk factors to reduce CVD rates in the community.

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Conflict of Interests

Authors have no conflict of interests.

References

1. Gaziano T, Reddy KS, Paccaud F, Horton S, Chaturvedi V. Cardiovascular Disease. In: Jamison DT, Breman JG, Measham AR, et al., Editors. Disease Control Priorities in Developing Countries. 2nd ed. Washington, DC: World Bank Publications; 2006.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 2006; 3(11): e442.
3. Khosravi A, Aghamohamadi S, Kazemi E, Pour Malek F, Shariati M. Mortality profile in Iran (29 provinces) over the years 2006 to 2010. Tehran, Iran: Ministry of Health and Medical Education; 2013. [In Persian].
4. Abegunde DO, Mathers CD, Adam T, Ortegon M, Strong K. The burden and costs of chronic diseases in low-income and middle-income countries. Lancet 2007; 370(9603): 1929-38.
5. Talaei M, Sarrafzadegan N, Sadeghi M, Oveisgharan S, Marshall T, Thomas GN, et al. Incidence of cardiovascular diseases in an Iranian population: The Isfahan Cohort Study. Arch Iran Med 2013; 16(3): 138-44.
6. Pennant M, Davenport C, Bayliss S, Greenheld W, Marshall T, Hyde C. Community programs for the prevention of cardiovascular disease: a systematic review. Am J Epidemiol 2010; 172(5): 501-16.
7. Farquhar JW, Fortmann SP, Maccoby N, Haskell WL, Williams PT, Flora JA, et al. The stanford five-city project: Design and methods. Am J Epidemiol 1985; 122(2): 323-34.
8. Luepker RV, Murray DM, Jacobs DR Jr, Mittelmark MB, Bracht N, Carlaw R, et al. Community education for cardiovascular disease prevention: Risk factor changes in the Minnesota Heart Health Program. Am J Public Health 1994; 84(9): 1383-93.
9. Vartiainen E, Puska P, Pekkanen J, Tuomilehto J, Jousilahti P. Changes in risk factors explain changes in mortality from ischaemic heart disease in Finland. BMJ 1994; 309(6946): 23-7.
10. Luepker RV, Rastam L, Hannan PJ, Murray DM, Gray C, Baker WL, et al. Community education for cardiovascular disease prevention. Morbidity and mortality results from the Minnesota Heart Health Program. Am J Epidemiol 1996; 144(4): 351-62.
11. Fortmann SP, Varady AN. Effects of a community-wide health education program on cardiovascular disease morbidity and mortality: The Stanford Five-City Project. Am J Epidemiol 2000; 152(4): 316-23.
12. Nissinen A, Berrios X, Puska P. Community-based noncommunicable disease interventions: Lessons from developed countries for developing ones. Bull World Health Organ 2001; 79(10): 963-70.
13. Kelishadi R, Pashmi M, Ahmadi R, Mohammad Zadeh M, Sadry G, Sarrafzadegan N. Healthy Heart Program: Heart health promotion from childhood. J Qazvin Univ Med Sci 2003; 7(2): 15-26.
14. Sarraf-Zadegan N, Sadri G, Malek AH, Baghaei M, Mohammadi FN, Shahrokhi S, et al. Isfahan Healthy Heart Programme: A comprehensive integrated community-based programme for cardiovascular disease prevention and control. Design, methods and initial experience. Acta Cardiol 2003; 58(4): 309-20.
15. Sarrafzadegan N, Baghaei A, Sadri G, Kelishadi R, Malekafzali H, Boshtam M, et al. Isfahan healthy heart program: Evaluation of comprehensive, community-based interventions for non-communicable disease prevention. Prevention and Control 2006; 2(2): 73-84.
16. Sadeghi M, Roohafza H, Shirani S, Poormoghadas M, Kelishadi R, Baghaii A, et al. Diabetes and associated cardiovascular risk factors in Iran: The Isfahan Healthy Heart Programme. Ann Acad Med Singapore 2007; 36(3): 175-80.
17. Sarrafzadegan N, Kelishadi R, Baghaei A, Hussein Sadri G, Malekafzali H, Mohammadifard N, et al. Metabolic syndrome: An emerging public health problem in Iranian women: Isfahan Healthy Heart Program. Int J Cardiol 2008; 131(1): 90-6.
18. Bahonar A, Khosravi A, Esmaeelian H, Babak A, Sarrafzadeghan N, Rahmati M, et al. Methods of implementing the operational phases of the health professionals education project-Isfahan Healthy Heart Program (IHHP-HPEP). ARYA Atheroscler 2009; 5(3).
19. Sarrafzadegan N, Kelishadi R, Esmaillzadeh A, Mohammadifard N, Rabiei K, Roohafza H, et al. Do lifestyle interventions work in developing countries? Findings from the Isfahan Healthy Heart Program in the Islamic Republic of Iran. Bull World Health Organ 2009; 87(1): 39-50.
20. Baghaei A, Sarrafzadegan N, Rabiei K, Gharipour M, Tavasoli AA, Shirani S, et al. How effective are strategies for non-communicable disease prevention and control in a high risk population in a developing country? Isfahan Healthy Heart Programme. Arch Med Sci 2010; 6(1): 24-31.
21. Khosravi A, Kiani Mehr G, Kelishadi R, Shirani S, Gharipour M, Tavassoli A, et al. The impact of a 6-year comprehensive community trial on the awareness, treatment and control rates of hypertension in Iran: Experiences from the Isfahan healthy heart program. BMC Cardiovascular Disorders 2010; 10: 61.

22. Sadeghi M, Ramezani J, Sanei H, Rabeiee K, Gharipoor M, Toghianifar N. Adherence to evidence-based therapies and modifiable risk factors in patients with coronary artery disease-the hlcp project. ARYA Atheroscler 2006; 2(3).
23. Gharipour M, Kelishadi R, Toghianifar N, Mackie M, Yazdani M, Noori F. Sex based pharmacological treatment in patients with metabolic syndrome: Findings from the Isfahan healthy heart program. Afr J Pharm Pharmacol 2011; 5(3): 311-6.
24. Najafian J, Toghianifar N, Mohammadifard N, Nouri F. Association between sleep duration and metabolic syndrome in a population-based study: Isfahan Healthy Heart Program. J Res Med Sci 2011; 16(6): 801-6.
25. Sarrafzadegan N, Rabiei K, Alavi M, Abedi H, Zarfeshani S. How can the results of a qualitative process evaluation be applied in management, improvement and modification of a preventive community trial? The IHHP Study. Arch Public Health 2011; 69: 9.
26. Sarrafzadegan N, Talaei M, Sadeghi M, Kelishadi R, Oveisgharan S, Mohammadifard N, et al. The Isfahan cohort study: Rationale, methods and main findings. J Hum Hypertens 2011; 25(9): 545-53.
27. Kelishadi R, Mohammadifard N, Sarrazadegan N, Nouri F, Pashmi R, Bahonar A, et al. The effects of a comprehensive community trial on cardiometabolic risk factors in adolescents: Isfahan Healthy Heart Program. ARYA Atheroscler 2012; 7(4): 184-90.
28. Mohammadifard N, Sarrafzadegan N, Nouri F, Sajjadi F, Alikhasi H, Maghroun M, et al. Using factor analysis to identify dietary patterns in Iranian adults: Isfahan Healthy Heart Program. Int J Public Health 2012; 57(1): 235-41.
29. Ahmadi A, Gharipour M, Nouri F, Sarrafzadegan N. Metabolic syndrome in Iranian youths: A population-based study on junior and high schools students in rural and urban areas. J Diabetes Res 2013; 2013: 738485.
30. Sarrafzadegan N, Gharipour M, Sadeghi M, Nouri F, Asgary S, Zarfeshani S. Differences in the prevalence of metabolic syndrome in boys and girls based on various definitions. ARYA Atheroscler 2013; 9(1): 70-6.
31. Sarrafzadegan N, Kelishadi R, Sadri G, Malekafzali H, Pourmoghaddas M, Heidari K, et al. Outcomes of a comprehensive healthy lifestyle program on cardiometabolic risk factors in a developing country: The Isfahan Healthy Heart Program. Arch Iran Med 2013; 16(1): 4-11.
32. Ahmadi A, Gharipour M, Nouri F, Kelishadi R, Sadeghi M, Sarrafzadegan N. Association between adolescence obesity and metabolic syndrome: Evidence from Isfahan Healthy Heart Program. Indian J Endocrinol Metab 2014; 18(4): 569-73.
33. Najafian J, Mohammadifard N, Naeini FF, Nouri F. Relation between usual daily walking time and metabolic syndrome. Niger Med J 2014; 55(1): 29-33.
34. Roohafza H, Khani A, Sadeghi M, Bahonar A, Sarrafzadegan N. Health volunteers' knowledge of cardiovascular disease prevention and healthy lifestyle following a community trial: Isfahan healthy heart program. J Educ Health Promot 2014; 3: 59.
35. Nouri F, Sarrafzadegan N, Mohammadifard N, Sadeghi M, Mansourian M. Intake of legumes and the risk of cardiovascular disease: Frailty modeling of a prospective cohort study in the Iranian middle-aged and older population. Eur J Clin Nutr 2016; 70(2): 217-21.
36. Mohammadifard N, Sajjadi F, Maghroun M, Alikhasi H, Nilforoushzadeh F, Sarrafzadegan N. Validation of a simplified food frequency questionnaire for the assessment of dietary habits in Iranian adults: Isfahan Healthy Heart Program, Iran. ARYA Atheroscler 2015; 11(2): 139-46.
37. Hosseini E, Lachat UGent C, Mohammadifard N, Sarrafzadegan N, UGent K. Associations of dietary glycemic index and glycemic load with glucose intolerance in Iranian adults. Int J Diabetes Dev Ctries 2014; 34(2): 89-94.
38. Mohammadifard N, Mansourian M, Sajjadi F, Maghroun M, Pourmoghaddas A, Yazdekhasti N, et al. Association of glycaemic index and glycaemic load with metabolic syndrome in an Iranian adult population: Isfahan Healthy Heart Program. Nutr Diet 2017; 74(1): 61-6.
39. Azizi F, Ghanbarian A, Momenan AA, Hadaegh F, Mirmiran P, Hedayati M, et al. Prevention of non-communicable disease in a population in nutrition transition: Tehran Lipid and Glucose Study phase II. Trials 2009; 10: 5.
40. Mohammadifard N, Kelishadi R, Safavi M, Sarrafzadegan N, Sajadi F, Sadri GH, et al. Effect of a community-based intervention on nutritional behaviour in a developing country setting: The Isfahan Healthy Heart Programme. Public Health Nutr 2009; 12(9): 1422-30.
41. Rabiei K, Kelishadi R, Sarrafzadegan N, Abedi HA, Alavi M, Heidari K, et al. Process evaluation of a community-based program for prevention and control of non-communicable disease in a developing country: The Isfahan Healthy Heart Program, Iran. BMC Public Health 2009; 9: 57.
42. Sarrafzadegan N, Azadbakht L, Mohammadifard N, Esmaillzadeh A, Safavi M, Sajadi F, et al. Do lifestyle interventions affect dietary diversity score in the general population? Public Health Nutr 2009; 12(10): 1924-30.

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Cardiac and renal fibrosis and oxidative stress balance in lipopolysaccharide-induced inflammation in male rats

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Original Article

Abstract

BACKGROUND: Subclinical inflammation induced by persistent exposure to lipopolysaccharide (LPS) is found in some clinical conditions such as obesity or diabetes. This study aimed to investigate the effect of recurrent LPS exposure on inflammatory markers, oxidative stress balance and cardiac and renal fibrosis in male rats.

METHODS: Male Wistar rats were divided into control and LPS-treated. LPS (10 mg/kg/week) was injected intraperitoneally. After 4 weeks, left ventricles and kidneys were homogenized and stained with hematoxylin and eosin (H&E) and Masson trichrome for histological examination. Serum levels of nitrite, interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) were measured and total thiol, malondialdehyde (MDA), superoxide dismutase (SOD) and catalase were evaluated in the heart and kidney homogenates.

RESULTS: Serum inflammatory markers were higher in LPS group than control (nitrite: 37.0 ± 2.2 vs. 25.5 ± 1.9 µmol/l; IL-6: 84 ± 3 vs. 98.0 ± 4.4 pg/ml; TNF-α: 75.5 ± 4.9 vs. 85.3 ± 4.7 pg/ml; respectively, $P < 0.050$). Evaluation of total thiol concentration (heart: 10.0 ± 0.9 vs. 22.5 ± 1.2; kidney: 7.0 ± 0.5 vs. 27.8 ± 3.1 nmol/g tissue, respectively), catalase (heart: 0.18 ± 0.03 vs. 0.66 ± 0.04; kidney: 0.17 ± 0.03 vs. 0.73 ± 0.03, U/g tissue, respectively) and SOD (heart: 8.01 ± 0.70 vs. 12.3 ± 0.4; kidney: 7.02 ± 0.60 vs. 12.0 ± 0.2, U/g tissue, respectively) showed lower levels in LPS-treated group compared to control; while MDA concentration in LPS group was higher than control ($P < 0.05$). Histopathological examination in LPS-treated group indicated infiltration of inflammatory cells and more collagen deposition in left ventricle wall and kidney compared to control group.

CONCLUSION: We concluded that in clinical conditions with chronic LPS, cardiac and renal fibrosis occurs even in absence of preceding tissue injury due to imbalances in oxidative stress.

Keywords: Inflammation, Lipopolysaccharide, Oxidative Stress, Heart, Kidney

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Introduction

Inflammation is a part of complex biological and adaptive immune response of body tissues to harmful stimuli such as irritation that plays a central role in metabolism in a variety of organisms.[1] Inflammatory abnormalities are demonstrated in allergic reactions and some pathological conditions. Moreover, in some diseases including cancer, obesity, atherosclerosis, and ischemic heart disease, inflammatory processes are the origin of etiology.[2-5]

Inflammation is classified as acute or chronic. Acute inflammation is a short-term process, appears within a few minutes or hours to remove injurious stimulus.[6] Chronic inflammation can be caused by excessive calorie consumption, elevated blood glucose levels, and oxidative stress. The danger of chronic low grade inflammation is its silent nature with destructive power and characterized by increasing in the systemic concentrations of cytokines such as interleukins (ILs), C-reactive protein (CRP) and tumor necrosis factor-α (TNF-α).[7] Several clinical observations indicated that chronic low-grade inflammation contributes in the pathogenesis of many diseases, including obesity, atherosclerosis and cardiovascular disease (CVD).[8-11]

The murine endotoxin or lipopolysaccharide

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(LPS) model is a known model of inflammation which is important in understanding the inflammatory response. LPS, an endotoxin of gram-negative bacteria, via toll-like receptor 4 (TLR4), induces inflammation in the rodent.[12] Heart can be used as a target tissue for TLR4 receptor.[13] The effect of gram negative bacteria on myocardium has been reported in human and different animal models of inflammation or bacteremia.[14-16] It is indicated that low levels of LPS activate cardiac myocytes and depress cardiac contractility as well as apoptosis by activating the renin angiotensin system. On the other hand, recurrent exposure to subclinical LPS may alter left ventricular structure and function.[17] Therefore, we hypothesized that chronic exposure to low level of LPS may induce cardiac and kidney fibrosis.

In the present study, we used a chronic low grade inflammation model, which is very close to clinical conditions with chronic low-grade inflammation, by recurrent exposure to LPS, and evaluated its effects on serum inflammatory markers and cardiac fibrosis in male rats.

Materials and Methods

Twenty male Wistar rats (8 weeks old; 200-220 g weight) were purchased from animal house of Mashhad University of Medical Sciences, Mashhad, Iran. The animals were kept under standard conditions (temperature 22 ± 2 °C and 12h light/dark cycle) with free access to food and water. Working with the animals was performed in accordance with approved animal protocols and guidelines established by local ethical committee on animal research. The animals were divided into two experimental groups: control and LPS-treated (n = 10 in each group).

LPS was purchased from Sigma (Sigma chemical Co., USA) and dissolved in physiological saline solution. For induction of subclinical inflammation, the rats received LPS (10 mg/kg/week) intraperitoneally for 4 weeks.[18] Control group received 2 ml/kg/week of saline instead of LPS. After 4 weeks, the animals were anaesthetized with diethyl ether and blood samples were taken for further analysis. Then, they were sacrificed, and left ventricle of hearts and right kidneys were dissected, washed with cold saline and kept in 10% formalin solution for histological examination. Right ventricles and left kidneys were stored at -70 °C for tissue marker measurements. Serums were separated by blood centrifugation at 3000 g for 20 min. Then, the samples were stored at −70 °C for further analyses.

Heart and kidney tissues were homogenized in phosphate-buffered saline (PBS) buffer and the supernatant was used for enzyme-linked immunosorbent assay (ELISA). Levels of nitrite in the tissue homogenates and serum were measured using griess reagent method (Promega Co., USA) as previously described.[19-21] Serum IL-6 and TNF-α levels were measured with standard ELISA kits (ebioscience Co., San Diego, CA, USA) according to the manufacturer instructions. The techniques were conducted using automated ELISA Reader (BioTek Instruments, USA). Tissue's total thiol content, malondialdehyde (MDA), and the activity of superoxide dismutase (SOD) and catalase were measured in homogenate solutions.

MDA which is the index of lipid peroxidation was measured as previously described.[22] 2 ml from reagent of thiobarbituric acid (TBA)/trichloroacetic acid (TCA)/ Hydrochloric acid (HCl) and 1 ml of tissue homogenate were mixed. Then, they were centrifuged within 3000×g and the absorbance was measured at 535 nm.

For measurement of total thiol concentration, 1 ml of tris-ethylenediamine tetraacetic acid (EDTA) buffer (pH = 8.6) was added to tissue homogenate and absorbance was read at 412 nm against tris-EDTA (TE) buffer alone. Then, 20 µl of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was added to solution and kept at room temperature for 15 minutes. Then, absorbance was read again. The absorbance of DTNB reagent was also read as a blank.[23]

SOD and catalase activities were measured using a Ransod kit (Randox Laboratory, UK) by a method which was previously described.[24]

The left ventricles of heart and right kidneys were put in 10% buffered formalin for 24-72 hours prior to processing and paraffin following a routine procedure. Then, samples with 5µm thickness were prepared and stained with hematoxylin and eosin (H&E) and Masson's trichrome, and were examined by light microscopy. Fibrosis was quantified in five fields per animal, and expressed as the percentage of fibrous tissue area stained with Masson trichrome. The fibrotic area was quantified using NIH image software (ImageJ).

All data were expressed as means ± standard error (SE). The data was analyzed using the SPSS software (version 20, IBM Corporation, Armonk, NY, USA). Independent sample t-test was used for comparison of data between groups. Differences were considered statistically significant when $P < 0.050$.

Results

Body weight: There was no significant difference in initial body weight between the groups. Over the course of the 4 weeks study, the animals gained body weight, and LPS-treated group gained less body weight compared to the normal group (230.0 ± 5.2 vs. 245.4 ± 3.7 g) (P < 0.050).

Effect of recurrent LPS administration on serum and heart nitrite levels: As shown in figure 1, significant increase in serum nitrite concentrations were observed in LPS group as compared to control (37.0 ± 2.2 vs. 25.5 ± 1.9 µmol/l; respectively, P < 0.050). Nitrite levels in the heart tissue of LPS-treated group showed a higher level than control, although it was not statistically significant (P = 0.090).

Effect of LPS administration on serum IL-6 and TNF- levels: Figure 1 illustrated the effect of LPS administration on serum IL-6 and TNF-α levels. Serum TNF-α and IL-6 concentrations were significantly higher in LPS-treated group compared to control (IL-6: 84 ± 3 vs. 98.0 ± 4.4; TNF-α: 75.5 ± 4.9 vs. 85.3 ± 4.7 pg/ml; respectively, (P < 0.050).

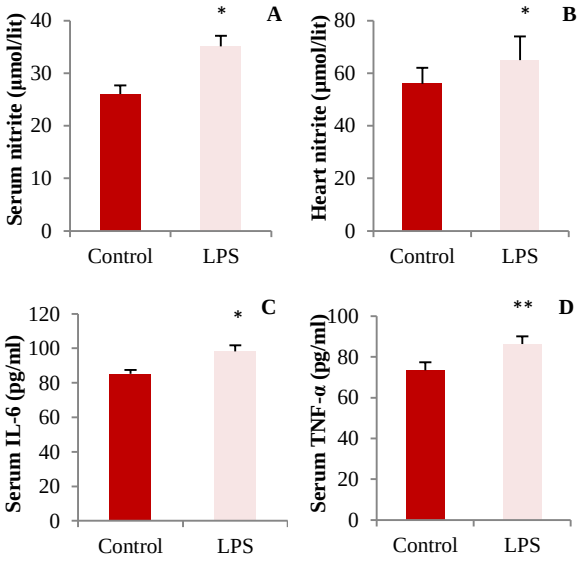

Figure 1. A: Serum nitrite; B: Heart tissue nitrite; C: Interleukine (IL)-6; and D: Serum tumor necrosing factor (TNF)-α concentrations in control and lipopolysaccharide (LPS)-treated groups, (* P < 0.050 and ** P < 0.010 compared to control, n = 10 in each group)

Tissue oxidant and antioxidant factor: As shown in figure 2, MDA contents in heart tissues of LPS-treated group were significantly higher than control (P = 0.010), while, total thiol concentration, catalase and SOD were significantly reduced after LPS treatment (P = 0.010).

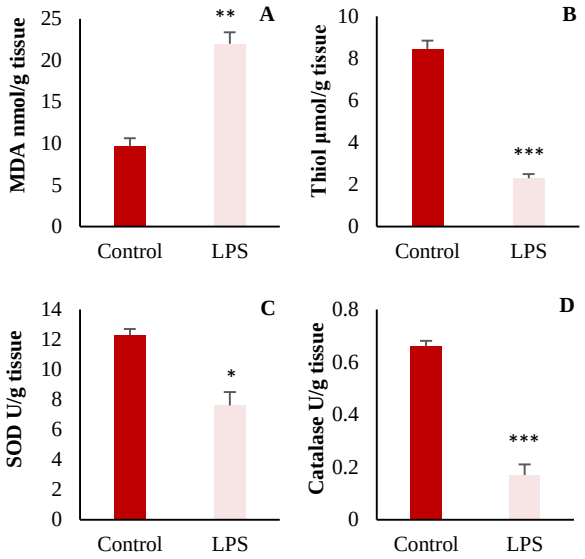

Figure 2. Comparison of A: malondialdehyde (MDA); B: Total thiol concentration; C: Superoxide dismutase (SOD); and D: Catalase in the heart of control and lipopolysaccharide (LPS)-treated groups, (n = 10 in each group, * P < 0.050; ** P < 0.010; *** P < 0.001 compared to control group)

The same results were also observed in oxidant and antioxidant markers in the kidney (Figure 3).

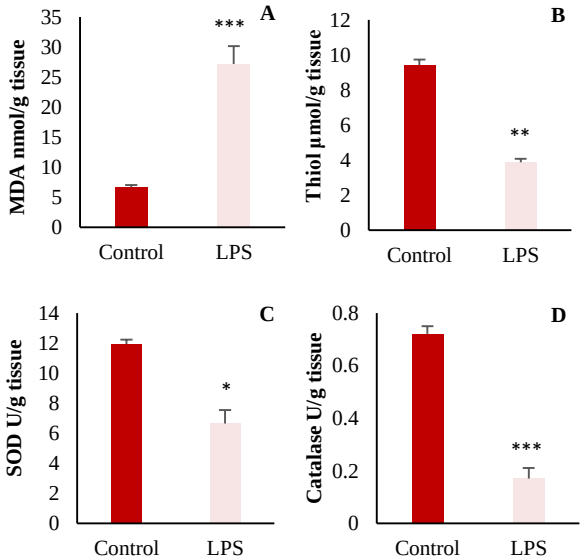

Figure 3. A: Malondialdehyde (MDA); B: Total thiol; C: Superoxide dismutase (SOD); and D: Catalase in the kidney (* P < 0.050; ** P < 0.010; *** P < 0.001 compared to control group)

Histopathological findings: Figures 4-6 illustrate representative examples of photomicrographs of H&E and Masson tichrome stained of the left ventricular tissues and kidneys in control and LPS-treated groups.

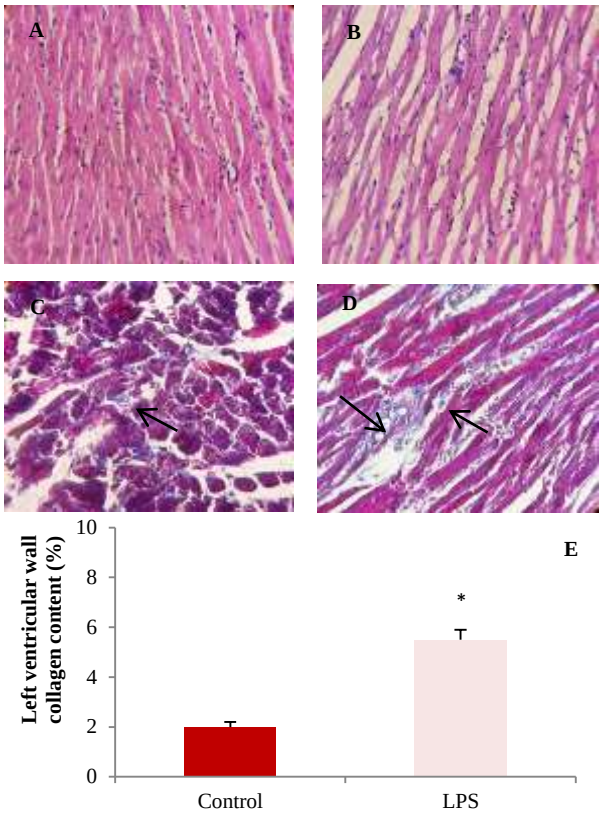

Figure 4. The light micrograph of left ventricles in control and lipopolysaccharide (LPS)-treated groups. A: Control group with normal architecture [×20, hematoxylin and eosin (H&E)]. B: Recurrent LPS treatment [(10 mg/kg/week for 4 weeks (×20, H&E)] showing infiltration of inflammatory cells, edema and disarrangement of fibers. Masson trichrome staining of left ventricular muscles of control (C) and LPS (D) groups show more collagen deposition (blue color, black arrows) in LPS-treated group. Blue color indicates collagen fibers. E: Left ventricular wall fibrosis showed higher collagen content (%) in LPS group compared to control (* P < 0.050 compared to control).

As illustrated in figure 6, the cardiac muscle fibers in control animals have normal histopathological features with single, oval and centrally located nuclei of cardiomyocytes and regularly arranged myofibers. The left ventricle of LPS-treated group showed infiltration of inflammatory cells, disarrangement of fibers, edema, and dilation of blood vessels. Masson's trichrome stained samples showed a significant increase in collagen deposition in left ventricle wall of LPS group compared to control (Figure 5).

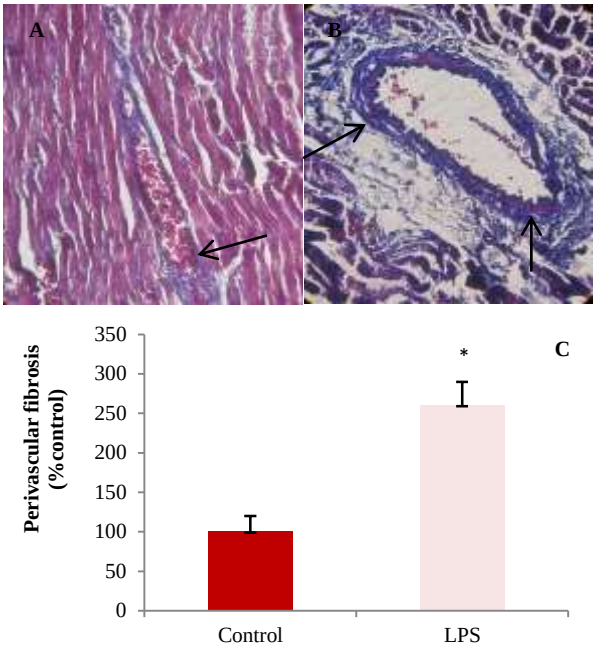

Figure 5. Perivascular fibrosis around left anterior descending coronary artery stained by Masson trichrome. Blue color indicates collagen deposition. A: Control; B: Lipopolysaccharide (LPS); C: Percent of perivascular fibrosis in experimental groups (* P < 0.050 compared to LPS)

Histological evaluation of renal tissue demonstrated a marked increase in inflammatory cells and more fibrotic tissue and collagen content in LPS group than control (Figure 6).

Discussion

Our results showed that chronic low grade inflammation increased heart and kidney factors of oxidative stress, and reduced antioxidative markers. In addition, histopathological examination indicated increased inflammatory cells and collagen deposition in heart and kidney tissues.

In this study, we used subclinical LPS model. The rodent has capacity to tolerate recurrent LPS injection without lethality and this makes them as suitable model for chronic model. Study by Lew et al.,[17] revealed that weekly injection of 10 mg/kg LPS intraperitoneally increased serum LPS levels which is comparable to human;[25,26] and induced a level found in several human conditions.[8]

Previous studies demonstrated that LPS exacerbates hepatic, renal, and cardiac fibrosis in preexisting abnormalities. In the liver in bile duct

ligation model or after hepatic injury, fibrosis was promoted by LPS from gut microbial.[27]

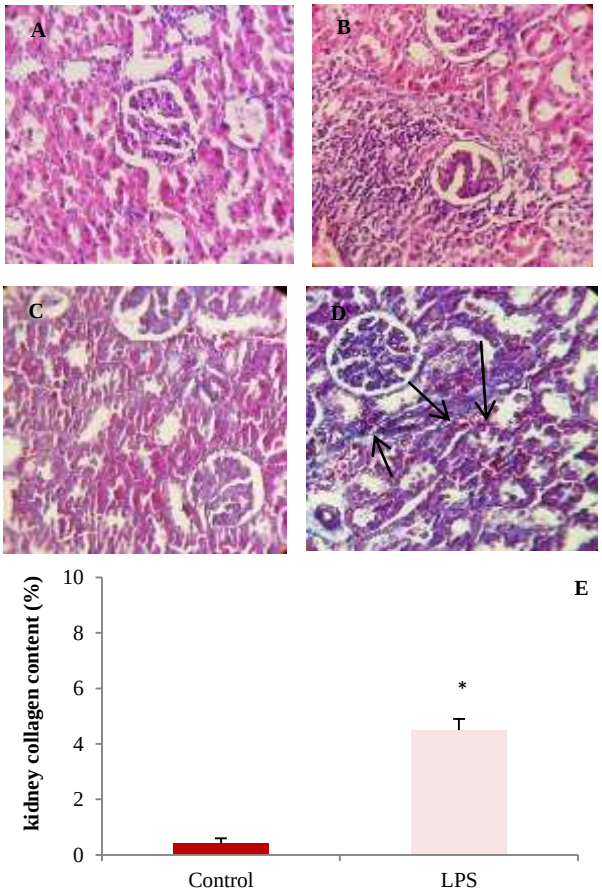

Figure 6. Images A and B are hematoxylin and eosin (H&E)-stained sections demonstrating infiltration of inflammatory cells in kidneys from lipopolysaccharide (LPS)-treated rats. Masson's trichrome stained sections demonstrate collagen deposition (blue color, black arrows) in the kidneys from rats in control and LPS groups (C and D). E: Percent of collagen content in the kidneys in experimental groups (* P < 0.010 compared to control)

Renal fibrosis was also promoted by TLR4 activation in urethral ligation model.[28] Cardiac fibrosis was also exacerbated in myocarditis.[29] In this study, we found that low grade inflammation induced cardiac and renal fibrosis without preexisting heart or kidney injury. Our results were in agreement with that of Lew et al. who used LPS and induced subclinical inflammation in mice.[17] They found that after two and four weeks of LPS administration, collagen fraction area in the left ventricle was higher compared with control group. In addition, they showed that LPS increased cardiac IL-6 and nitric oxide (NO) synthase expression. They also examined if cardiac fibroblasts are target of LPS, and showed that incubation of heart fibroblast with LPS for 48 hours dose-dependently increased nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) messenger ribonucleic acid (mRNA) expression. In our study, although we did not measure inflammatory cytokine expression, however, we found that LPS administration increased serum and cardiac nitrite, serum IL-6 and TNF-α concentrations. IL-6 has a role in fibrosis in different organs by differentiation of fibroblasts to myofibroblasts which produces more collagen.[30] In addition, cardiac and renal fibroblasts increased IL-6 expression after exposure to LPS.

Transforming growth factor-β (TGF-β) and TNF-α are common mediators of tissue fibrosis in pathological conditions.[31] Since TGF-β is not involved in cardiac fibrosis in our model,[17] we did not measure it. Interestingly, we showed that oxidant/antioxidant balance altered in the heart and kidney in subclinical LPS. Higher MDA concentration, lower SOD and catalase activity and total thiol concentration suggest that lipid peroxidation increased and antioxidant enzyme was suppressed after recurrent LPS. It is indicated that LPS promotes the production of inflammatory cytokines that in turn leads to excessive production of free radicals and oxidative stress. It is indicated that higher reactive oxygen species (ROS) is associated with tissue fibrosis in chronic renal failure and myocardial infarction.[32] In the present study, we used LPS for induction of chronic inflammation. It should be considered that in several common diseases in human such as rheumatoid arthritis (RA) and atherosclerosis, the exact mechanism for induction of chronic inflammation is different, and this is the limitation of study. On the other hand, we did not measure LPS level and some serum markers such as TGF-β in serum and thus, we cannot compare it with human plasma level in the same condition.

Conclusion

We concluded that conditions with chronic low grade inflammation which is common in high-fat diet, metabolic syndrome or smoking are associated with cardiac fibrosis as a primary effect which may lead to heart failure (HF) with preserved ejection fraction in chronic condition. Further studies in different models of inflammatory disease especially in chronic condition such as rheumatoid should be done to approve our findings and to find the exact mechanism of inflammation-induced fibrosis.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

References

1. Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1beta generation. Clin Exp Immunol 2007; 147(2): 227-35.
2. Asgharzadeh F, Rouzbahani R, Khazaei M. Chronic low-grade inflammation: Etiology and its effects. J Isfahan Med Sch 2016; 34(379): 408-21.
3. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007; 56(7): 1761-72.
4. Shalapour S, Karin M. Immunity, inflammation, and cancer: An eternal fight between good and evil. J Clin Invest 2015; 125(9): 3347-55.
5. Tahergorabi Z, Khazaei M. The relationship between inflammatory markers, angiogenesis, and obesity. ARYA Atheroscler 2013; 9(4): 247-53.
6. Burgoyne JR, Mongue-Din H, Eaton P, Shah AM. Redox signaling in cardiac physiology and pathology. Circ Res 2012; 111(8): 1091-106.
7. Petersen AM, Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol (1985) 2005; 98(4): 1154-62.
8. Manco M, Putignani L, Bottazzo GF. Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. Endocr Rev 2010; 31(6): 817-44.
9. Shen J, Obin MS, Zhao L. The gut microbiota, obesity and insulin resistance. Mol Aspects Med 2013; 34(1): 39-58.
10. Tahergorabi Z, Khazaei M, Moodi M, Chamani E. From obesity to cancer: A review on proposed mechanisms. Cell Biochem Funct 2016; 34(8): 533-45.
11. Sallam N, Khazaei M, Laher I. Effect of moderate-intensity exercise on plasma C-reactive protein and aortic endothelial function in type 2 diabetic mice. Mediators Inflamm 2010; 2010: 149678.
12. Beutler B, Rietschel ET. Innate immune sensing and its roots: The story of endotoxin. Nat Rev Immunol 2003; 3(2): 169-76.
13. Frantz S, Kobzik L, Kim YD, Fukazawa R, Medzhitov R, Lee RT, et al. Toll4 (TLR4) expression in cardiac myocytes in normal and failing myocardium. J Clin Invest 1999; 104(3): 271-80.
14. Court O, Kumar A, Parrillo JE, Kumar A. Clinical review: Myocardial depression in sepsis and septic shock. Crit Care 2002; 6(6): 500-8.
15. Krishnagopalan S, Kumar A, Parrillo JE, Kumar A. Myocardial dysfunction in the patient with sepsis. Curr Opin Crit Care 2002; 8(5): 376-88.
16. Doi K, Leelahavanichkul A, Yuen PS, Star RA. Animal models of sepsis and sepsis-induced kidney injury. J Clin Invest 2009; 119(10): 2868-78.
17. Lew WY, Bayna E, Molle ED, Dalton ND, Lai NC, Bhargava V, et al. Recurrent exposure to subclinical lipopolysaccharide increases mortality and induces cardiac fibrosis in mice. PLoS One 2013; 8(4): e61057.
18. Lew WY, Bayna E, Dalle Molle E, Contu R, Condorelli G, Tang T. Myocardial fibrosis induced by exposure to subclinical lipopolysaccharide is associated with decreased miR-29c and enhanced NOX2 expression in mice. PLoS One 2014; 9(9): e107556.
19. Elmi S, Sallam NA, Rahman MM, Teng X, Hunter AL, Moien-Afshari F, et al. Sulfaphenazole treatment restores endothelium-dependent vasodilation in diabetic mice. Vascul Pharmacol 2008; 48(1): 1-8.
20. Khazaei M, Fallahzadeh AR, Sharifi MR, Afsharmoghaddam N, Javanmard SH, Salehi E. Effects of diabetes on myocardial capillary density and serum angiogenesis biomarkers in male rats. Clinics (Sao Paulo) 2011; 66(8): 1419-24.
21. Nematollahi S, Nematbakhsh M, Haghjooyjavanmard S, Khazaei M, Salehi M. Inducible nitric oxide synthase modulates angiogenesis in ischemic hindlimb of rat. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2009; 153(2): 125-9.
22. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med 1990; 9(6): 515-40.
23. Hosseinzadeh H, Sadeghnia HR. Safranal, a constituent of Crocus sativus (saffron), attenuated cerebral ischemia induced oxidative damage in rat hippocampus. J Pharm Pharm Sci 2005; 8(3): 394-9.
24. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121-6.
25. Warren HS, Fitting C, Hoff E, Adib-Conquy M, Beasley-Topliffe L, Tesini B, et al. Resilience to bacterial infection: Difference between species could be due to proteins in serum. J Infect Dis 2010; 201(2): 223-32.
26. Copeland S, Warren HS, Lowry SF, Calvano SE, Remick D. Acute inflammatory response to endotoxin in mice and humans. Clin Diagn Lab Immunol 2005; 12(1): 60-7.
27. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, et al. TLR4 enhances TGF-

beta signaling and hepatic fibrosis. Nat Med 2007; 13(11): 1324-32.
28. Pulskens WP, Rampanelli E, Teske GJ, Butter LM, Claessen N, Luirink IK, et al. TLR4 promotes fibrosis but attenuates tubular damage in progressive renal injury. J Am Soc Nephrol 2010; 21(8): 1299-308.
29. Blyszczuk P, Kania G, Dieterle T, Marty RR, Valaperti A, Berthonneche C, et al. Myeloid differentiation factor-88/interleukin-1 signaling controls cardiac fibrosis and heart failure progression in inflammatory dilated cardiomyopathy. Circ Res 2009; 105(9): 912-20.
30. Kong P, Christia P, Frangogiannis NG. The pathogenesis of cardiac fibrosis. Cell Mol Life Sci 2014; 71(4): 549-74.
31. Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. Nat Rev Cardiol 2013; 10(1): 15-26.
32. Looi YH, Grieve DJ, Siva A, Walker SJ, Anilkumar N, Cave AC, et al. Involvement of Nox2 NADPH oxidase in adverse cardiac remodeling after myocardial infarction. Hypertension 2008; 51(2): 319-25.

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Right ventricular (RV) echocardiographic parameters in patients with pulmonary thromboembolism (PTE)

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Original Article

Abstract

BACKGROUND: Acute pulmonary thromboembolism (PTE) is a common disease with a high mortality rate, and a variable and nonspecific clinical presentation. To detect the nonspecific signs and symptoms associated with this condition, several right ventricular (RV) echocardiographic parameters have been proposed as practical marker.

METHODS: This cross-sectional study was performed on 93 patients with PTE diagnosed by computed tomography (CT) angiography, and 57 patients with negative PTE based on CT angiography. During the experiment, all patients underwent both transthoracic echocardiography (TTE) and multi-slice CT pulmonary angiography. Transthoracic echocardiography measurements were obtained as patients went through both experimental procedures. These measurements were later compared between the patients with and without PTE.

RESULTS: Tricuspid annulus plain systolic excursion (TAPSE) (1.65 ± 0.09 vs. 2.00 ± 0.08 cm, P < 0.001) and left ventricular (LV) end-diastolic diameter (4.54 ± 0.26 vs. 5.40 ± 0.24 cm, P < 0.001) were significantly lower in patients with PTE as compared to patients without it. Whereas, RV end-diastolic and end-systolic diameters at the papillary muscle levels (3.41 ± 0.09 vs. 3.02 ± 0.12 cm, and 2.48 ± 0.08 vs. 2.16 ± 0.06 cm, respectively, P < 0.001 for both), and tricuspid valve (TV) annulus tissue Doppler imaging (TDI) measurements (6.02 ± 0.10 vs. 5.78 ± 0.14, P < 0.001) were significantly greater in patients with PTE. On the other hand, no significant difference was found between the two groups of patients regarding pulmonary artery pressure (PAP) (P = 0.416), and RV fractional shortening (P = 0.157). Moreover, our results indicated that RV/LV (cut-off point: 0.6898) had high sensitivity (93.5%), specificity (100%), positive predicting value (PPV) (100%), and negative predicting value (NPV) (90.4%) in diagnosing PTE.

CONCLUSION: TTE may be valuable as a substitute diagnostic method for patients with PTE. This technique may also assist in detecting the severity of the illness, by evaluating RV/LV in cut-off point of 0.6898.

Keywords: Pulmonary Thromboembolism, Transthoracic Echocardiography, Computed Tomography Angiography

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Introduction

Acute pulmonary thromboembolism (PTE) is a common disease with a 3-month death rate of up to 17.4%.[1,2] Even if patients are treated with anticoagulation, the death rate in hemodynamically ranges from 8.1% to 15.1%.[2] Furthermore, over 42 million deaths were reported from the United States within a 20-year period, in a previously conducted study.[1] About 1.5% of patients were diagnosed with PTE, and PTE was the presumed cause of death for 200,000 patients.[1] Considering local regions, a study based on three National Healthcare Group hospitals in 2006, estimated the population-based incidence of PTE to be 15 per 100,000.[3] In addition, lower rates of venous thromboembolism were found for Asians, compared to Caucasians and Eurasians.[3]

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Since PTE is associated with variable and nonspecific symptoms, accurate diagnosis is challenging. Yet, computed tomography (CT) angiography is used as a common diagnostic technique for PTE. Furthermore, several right ventricular (RV) echocardiographic parameters have been proposed as sensitive markers, to detect nonspecific signs and symptoms of this illness. Studies showed that acute PTE increases the pressure of the pulmonary arterial system and RV that results in RV dysfunction, and may progress further to right heart failure and circulatory collapse. Thus, these studies suggest that patients with PTE experience RV echocardiographic changes;[3] however, there has not been a specific study to further analyze these changes. In our study, we evaluated RV echocardiographic finding, in patients with PTE, diagnosed by CT angiography.

Materials and Methods

This cross-sectional study was conducted in Department of Cardiology, Alzahra Hospital, Isfahan, Center of Iran, from August to December 2015. The transthoracic echocardiography (TTE) findings of patients who had PTE diagnosed by CT angiography, were compared to patients without PTE.

Inclusion criteria consisted of patient referred to cardiology department of Alzahra hospital with a probability diagnosis of PTE, with age of 18-70 years, and satisfaction to enter the study. Exclusion criteria consisted of low quality images based on patient's condition, patients with chronic lung disease, history of heart failure, or heart attack, history of high pulmonary arterial pressure, including a previous history of pulmonary embolism[4-6] proven by CT angiography, life expectancy of less than 3 months, pregnancy, kidney malfunction (creatinine clearance of 30 ml/minute), unable to complete CT testing (e.g., allergy to intravenous contrast material, unavailability of CT, patient too ill), or hemodynamic instability at presentation (such as cardiogenic shock, systolic blood pressure of more than 90 mmHg,[7] or use of inotropic drug). Patients that did not tolerate the protocol-required TTE, were excluded from this study.

The study flowchart is shown in figure 1. 160 patients with a probability diagnosis of PTE, diagnosed by internist and based on inclusion and exclusion criteria were included. Then, CT angiography was performed for all patients, and PTE was confirmed by showing a complete or partial filling defect in the pulmonary vessels.

Patients with signs and symptoms of pulmonary embolism undergone 64 multi-slice CT pulmonary angiography, after intravenous injection of Wheezy Pack contrast agent.[4] 150 patients had completed data and entered to the study; 93 from PTE group and 57 from individuals without PTE. Pulmonary arterial CT obstruction index (PACTOIR) was calculated in case group by in charge radiologist. Case group were divided into two subtype of moderate to severe PTE (PACTOIR: ≥ 40), and mild to moderate PTE (PACTOIR: < 40).

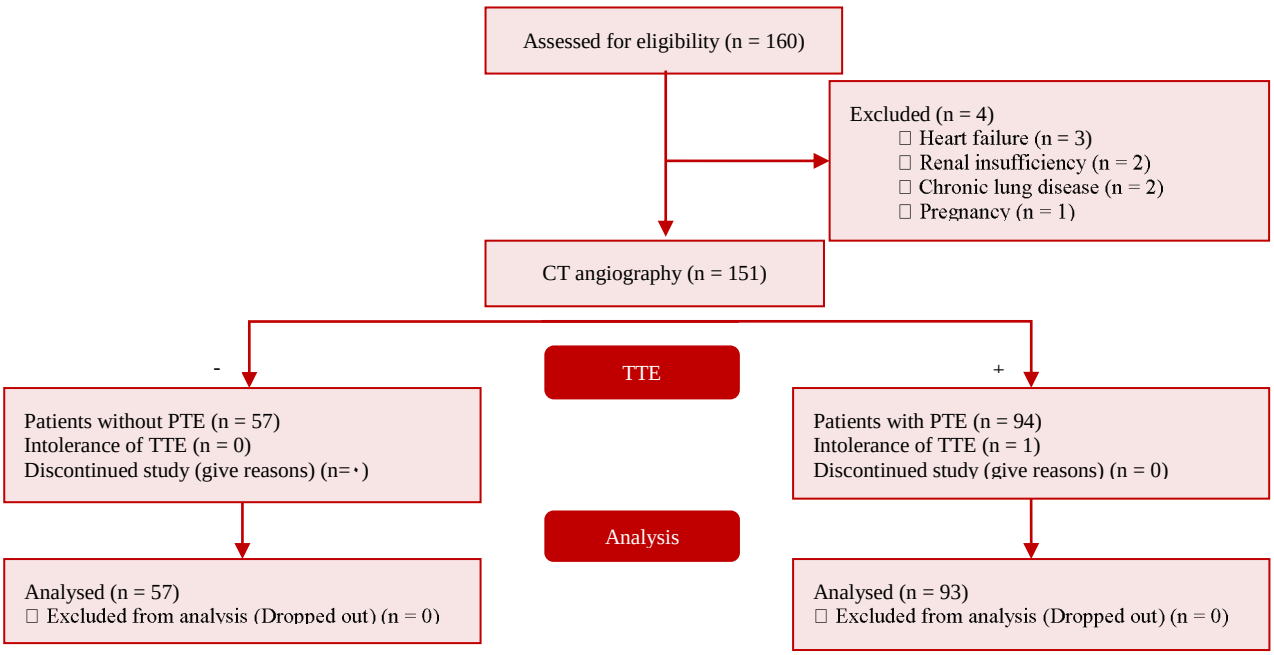

Figure 1. Study flowchart
CT: Computed tomography; TTE: Transthoracic echocardiography; PTE: Pulmonary thromboembolism

The patients underwent TTE (Vivid 3, GE Medical Systems, Horten, Norway) in left lateral decubitus position, with measurements of parameters based on the recommendations of the American Society of Echocardiography, up to 24 hours after acute PTE diagnosis.[5] The left ventricular (LV) and RV distances were measured in the 4-chamber apical view at papillary muscle level, to obtain the RV/LV ratio. The parasternal, apical, and subcostal views were used for the objective calculation of the RV systolic function. The pulmonary artery systolic pressure (PAPs) was resultant from the tricuspid regurgitation (TR), added to right atrial pressure as assessed from the inferior vena cava diameter and collapsibility.

Standard features of echocardiography included parasternal (long/short axis), apical (2/4 chamber), and subxiphoid (long axis) that were recorded in all patients. In addition, LV ejection fraction (LVEF) was measured using Simpson method. The diameter of the right ventricle and left ventricle at the papillary muscle level, and their ratio in systole and diastole were measured in apical view (4 chamber).

RV fractional shortening was calculated by the following formula:

(RV end-diastolic diameter - RV end-systolic diameter) / RV end-diastolic diameter

Myocardial velocity in systolic and diastolic (early/late) in view of the apical (4 chamber) at the junction of the RV free wall and anterior cusp tricuspid valve (TV) was obtained with tissue Doppler imaging (TDI), and transmitral to basal septal myocardial early diastolic velocity ratio (E/Em) was measured.[8]

The common method of evaluating the performance of the right ventricle through the tricuspid valve annulus is M-mode imaging using tricuspid annulus plain systolic excursion (TAPSE).

Data were analyzed for patients with completed data, and reported as mean ± standard deviation (SD) for continuous, and frequency (percent) for categorical variables. To compare qualitative variables between the groups, chi-square test was performed. Kolmogorov-Smirnov test was used for evaluating normal distribution of all quantitative parameters. Moreover, Student's t test was used for variables distributed in a normal way. Besides, Mann-Whitney test was performed for variables that had not normal distribution. Roc-curve was used in order to evaluate the diagnostic accuracy of studied variables in distinguishing pulmonary thromboembolism,[8] and the optimal cut-off values were defined as the point at which the value of "sensitivity + specificity - 1"[9,10] was maximum (Youden index). We used Pearson correlation to find correlation between studied variables. The two tailed p-value of less than 0.050 was considered significant. Statistical analysis of data was performed using SPSS software (version 20, SPSS Inc., Chicago, IL, USA).[11,12]

Results

Demographic features in terms of age (P = 0.446) and gender (P = 0.864) were similar in both groups. Ten patients were dropped out and finally, 150 patients completed the study. In patients with PTE and patients without PTE, 40.9% (n = 38) and 38.6% (n = 22) were men, respectively.

As is shown in table 1, TAPSE was significantly lower in PTE group as compared to patients without PTE (P < 0.001). Moreover, RV end-diastolic and end-systolic diameters in PTE group were significantly greater (P < 0.001).

Table 1. Characteristics and transthoracic echocardiography (TTE) results of the study sample

CT angiography results Variables	PTE (n = 93)	Without PTE (n = 57)	P
Gender: Men [n (%)]	38 (40.9)	22 (38.6)	0.864
Age (year) [Mean ± SD]	55.68 ± 4.19	56.26 ± 4.62	0.446
TAPSE (cm) [Mean ± SD]	1.65 ± 0.09	2.00 ± 0.08	< 0.001
TDI (E/E') [Mean ± SD]	6.02 ± 0.10	5.78 ± 0.14	< 0.001
RVEDD (cm) [Mean ± SD]	3.41 ± 0.09	3.02 ± 0.12	< 0.001
RVESD (cm) [Mean ± SD]	2.48 ± 0.08	2.16 ± 0.06	< 0.001
LVEDD (cm) [Mean ± SD]	4.54 ± 0.26	5.40 ± 0.24	< 0.001
PAP (mmHg) [Mean ± SD]	25.00 ± 83.30	26.00 ± 86.70	0.416
RV Fractional shortening (%) [Mean ± SD]	27.00 ± 2.70	27.64 ± 2.60	0.157
RV/LV [Mean ± SD]	0.75 ± 0.04	0.56 ± 0.03	< 0.001
PACTOIR [Mean ± SD]	47.48 ± 6.53	-	-

CT: Computed tomography; PTE: Pulmonary thromboembolism; SD: Standard deviation; TAPSE: Tricuspid annular plane systolic excursion; TDI: Tissue Doppler imaging; RVEDD: Right ventricular end-diastolic diameter; RVESD: Right ventricular end-systolic diameter; LVEDD: Left ventricular end-diastolic diameter; PAP: Pulmonary artery pressure; RV: Right ventricle; LV: Left ventricle; PACTOIR: Pulmonary artery computed tomography obstruction index ratio

Table 2. Diagnostic accuracy of studied variables in detecting pulmonary thromboembolism (PTE)

Variables	TP	FP	TN	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR^+	LR^-	Overall accuracy (%)
TDI (E/E') (cut-off point: 5.95; area: 0.947)	75	0	57	18	80.6	100	100	76.00	-	0.194	88.00
PAP (cut-off point: 35.50; area: 0.880)	93	7	50	0	100	87.7	93.00	100	8.13	0	95.33
RV fractional shortening (cut-off point: 0.2419; area: 0.391)	83	45	12	10	89.2	21.1	64.84	54.54	1.13	0.510	63.33
RV/LV (cut-off point 0.6889; area: 0.993)	87	0	57	6	93.5	100	100	90.47	-	0.065	96.00

PTE: Pulmonary thromboembolism; TP: True positive; FP: False positive; TN: True negative; FN: False negative; PPV: Positive predictive value; NPV: Negative predictive value; LR^+: Positive likelihood; LR^-: Negative likelihood; TDI: Tissue Doppler imaging; PAP: Pulmonary artery pressure; RV: Right ventricle; LV: Left ventricle

Whereas, LV end-diastolic diameter in patients with PTE was significantly lower than patients without PTE ($P < 0.001$). Furthermore, we found that TV annulus TDI (E/E') in patients without PTE was significantly lower as compared to patients with PTE ($P < 0.001$). On the other hand, PAPs ($P = 0.416$) and RV fractional shortening ($P = 0.157$) were similar in both groups, and no statistically significance were observed.

According to tables 2 and 3, RV/LV (cut-off point: 0.6898) had the highest diagnostic accuracy in distinguishing PTE among the other studied variables with the sensitivity of 93.5%, specificity of 100%, positive predicting value (PPV) of 100%, and negative predictive value (NPV) of 90.47%. In following, PAP (cut-off point: 35.5) had the second place in the term of highest diagnostic accuracy in distinguishing PTE with sensitivity of 100%, specificity of 87.7%, PPV of 93%, and NPV of 100%.

In addition, we found that PACTOIR for patients with PET had a significant positive correlation with RV end-systolic diameter (Pearson correlation: 0.211). None of demographic and TTE results had significant differences in patients with different severity of PTE ($P > 0.050$) (Table 4).

Table 3. Pearson correlation between quantitative variables in patients with pulmonary thromboembolism (PTE)

		TAPSE	TDI(E/E')	RVEDD	RVESD	LVEDD	PAP	RV fractional shortening	RV/LV	PACTOIR
TAPSE	Correlation	1	-	-	-	-	-	-	-	-
	P	-	-	-	-	-	-	-	-	-
TDI (E/E')	Correlation	0.051	1	-	-	-	-	-	-	-
	P	0.629	-	-	-	-	-	-	-	-
RVEDD	Correlation	0.054	-0.193	1	-	-	-	-	-	-
	P	0.610	0.063	-	-	-	-	-	-	-
RVESD	Correlation	0.039	-0.192	0.229*	1	-	-	-	-	-
	P	0.710	0.065	0.028		-	-	-	-	-
LVEDD	Correlation	-0.082	-0.079	-0.143	-0.065	1	-	-	-	-
	P	0.434	0.454	0.173	0.534		-	-	-	-
PAP	Correlation	-0.002	-0.008	-0.049	-0.097	0.119	1	-	-	-
	P	0.982	0.938	0.643	0.354	0.255	-	-	-	-
RV fractional shortening	Correlation	0.002	0.029	0.523**	-0.709**	-0.047	0.052	1	-	-
	P	0.984	0.779	< 0.001	< 0.001	0.653	0.621	-	-	-
RV/LV	Correlation	0.084	-0.029	0.571**	0.161	-0.892**	-0.124	0.273**	1	-
	P	0.422	0.785	< 0.001	0.123	< 0.001	0.235	0.008	-	-
PACTOIR	Correlation	-0.054	-0.180	0.125	0.211*	0.007	0.091	-0.094	0.064	1
	P	0.610	0.084	0.234	0.042	0.946	0.388	0.371	0.544	-

*: Correlation is significant at the 0.050 level (2-tailed); **: Correlation is significant at the 0.010 level (2-tailed).
TAPSE: Tricuspid annular plane systolic excursion; TDI: Tissue Doppler imaging; RVEDD: Right ventricular end-diastolic diameter; RVESD: Right ventricular end-systolic diameter; LVEDD: Left ventricular end-diastolic diameter; PAP: Pulmonary artery pressure; RV: Right ventricle; LV: Left ventricle; PACTOIR: Pulmonary artery computed tomography obstruction index ratio

Table 4. Characteristics and transthoracic echocardiography (TTE) results in patients with pulmonary thromboembolism (PTE) based on PTE severity

Variables \ PTE severity	Mild to moderate (n = 15)	Moderate to severe (n = 78)	P
Gender: Men [n (%)]	8 (53.3)	30 (38.5)	0.283
Age (year) [Mean ± SD]	55.66 ± 4.01	55.69 ±4.25	0.983
TAPSE (cm) [Mean ± SD]	1.66 ± 0.08	1.65 ± 0.10	0.971
TDI (E/E') [Mean ± SD]	6.07 ± 0.08	6.02 ± 0.11	0.088
RVEDD (cm) [Mean ± SD]	3.40 ± 0.09	3.41 ± 0.09	0.594
RVESD (cm) [Mean ± SD]	2.44 ± 0.14	2.49 ± 0.06	0.191
LVEDD (cm) [Mean ± SD]	4.51 ± 0.07	4.55 ± 0.28	0.578
PAP (mmHg) [Mean ± SD]	36.93 ± 0.45	38.55 ± 4.99	0.215
RV Fractional shortening (%) [Mean ± SD]	27.00 ±4.00	26.00 ± 2.00	0.337
RV/LV [Mean ± SD]	0.75 ± 0.01	0.75 ± 0.04	0.928

PTE: Pulmonary thromboembolism; TAPSE: Tricuspid annular plane systolic excursion; TDI: Tissue Doppler imaging; RVEDD: Right ventricular end-diastolic diameter; RVESD: Right ventricular end-systolic diameter; LVEDD: Left ventricular end-diastolic diameter; PAP: Pulmonary artery pressure; RV: Right ventricle; LV: Left ventricle; SD: Standard deviation

Discussion

Acute PTE is a disease with high prevalence, frequently underdiagnosed, treated poorly, and often associated with complications. Prognosis of PTE is related to the pre-existing cardiovascular disease, the degree of pulmonary hypertension and vascular obstruction, and mainly, to the presence of RV dysfunction.[13] Current studies have been focused on performance of echocardiography to evaluate RV function in patients with PTE, which further helps to identify cases with a higher risk of morbidity and mortality, and in need of a more aggressive treatment.[14]

PTE is associated with various signs and symptoms which may be mild or even absent, mainly in cases involving only the segmental pulmonary branches. Moreover, high or intermediate probability found based on clinical objective assessment, evokes the use of diagnostic techniques. However, a low probability based on objective clinical assessment does not exclude the need for further diagnosis. Further, maintaining a high level of suspicion by physicians is critical for diagnosis of PTE.

Our results showed that TAPSE was significantly lower in PTE group, as compared to patients without PTE (1.65 vs 2.00 cm). Vitarelli et al. study showed that TAPSE in patients with PTE was 1.5 cm, and 2.3 cm in patients without PTE.[15] On the other hand, Gromadzinski et al. did not find any significant differences in TAPSE measurements between patients with and without PTE (1.45 vs 1.63 cm, P = 0.23).[16]

Furthermore, TAPSE plays an important role as predictor of survival, regarding patients with PTE. However, no studies had before assessed interobserver reliability for this parameter. In the study of Kaul et al., TAPSE correlated powerfully with radionuclide angiography, with low interobserver variability.[13] Even though, we did not evaluate variability of TAPSE in detecting PTE, TAPSE measurements were very low in patients with PTE, even lower than patients without PTE.

Moreover, TAPSE is a well-known, reproducible parameter that does not involve complex equipment and image analysis. In addition, this parameter has a proven prognostic value for detection of congestive heart failure. For patients with heart failure, decreased RV systolic function measured by TAPSE was found to be related to increased mortality, independent of other risk factors associated with this condition.[15]

Furthermore, in a previous study, TAPSE was used to detect the severity of RV function defined by plasma B-type natriuretic peptide (BNP). A significant correlation was also found for plasma BNP level and TAPSE ($r = -0.634$, $P < 0.001$).[16] Interestingly, TAPSE measurement for lower reference value of RV function was recently stated as 16 mm. Moreover, TAPSE has been proposed as a prognostic tool on pulmonary arterial hypertension based on guidelines. In addition, TAPSE ≤ 15 is found to be an indicator of poor outcome.[16,17] These studies and our show that patients with PTE had lower TAPSE, and having low amount of TAPSE has significant correlation with poor outcome in patients with PTE; which indicate that TAPSE measurement is a reliable factor in order to prove PTE, and outcome of these patients.

As obtained, RV end-diastolic diameter and RV end-systolic diameter in were significantly greater PTE group (3.41 vs 3.02 cm and 2.48 vs 2.16 cm, respectively), while end-systolic diameter in patients

with PTE was significantly lower than patients without PTE (4.5 vs 5.4 cm). Gromadzinski et al. study showed that end-systolic diameter in patients with PTE was significantly lower than patients without PTE (4.5 vs 5.7 cm), while no significant correlation was found between RV end-diastolic diameter and PTE (3.4 vs 3.0 cm).[16]

According to our findings, PACTOIR had significant positive correlation with RV end-systolic diameter. Moreover, in Varol et al. study, PACTOIR value for patients with RV dysfunction found to be significantly higher than those without RV dysfunction.[18] Lastly, our results indicate that TV annulus TDI (E/E') in patients without PTE was significantly lower, compared to patients with PTE (5.78 vs 6.02). Some studies showed that in patients with PTE, systolic and diastolic tricuspid annular velocities were significantly lower than in healthy controls.[18-22] There was a good reverse correlation between systolic TR velocity and systolic PAP (PAPs), as well as mean arterial PAP (PAPm), too.[23-25]

Conclusion

Our results showed that TTE can be helpful in patients with signs or symptoms of PTE, as an alternative diagnostic method which determined the severity of this illness. We found significant differences in TAPSE, TV annulus TDI, RV end-diastolic diameter, and RV end-systolic and end-systolic diameters between the patients with PTE and without PTE that by defining scores for each of these factors, we could diagnosis PTE by using TTE. Moreover, we found that RV/LV in cut-off point of 0.6898, had the highest sensitivity, specificity, PPV, and NPV value in diagnosing PTE.

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Conflict of Interests

Authors have no conflict of interests.

References

1. Alpert JS, Smith R, Carlson J, Ockene IS, Dexter L, Dalen JE. Mortality in patients treated for pulmonary embolism. JAMA 1976; 236(13): 1477-80.
2. Carson JL, Kelley MA, Duff A, Weg JG, Fulkerson WJ, Palevsky HI, et al. The clinical course of pulmonary embolism. N Engl J Med 1992; 326(19): 1240-5.
3. Molina JA, Jiang ZG, Heng BH, Ong BK. Venous thromboembolism at the National Healthcare Group, Singapore. Ann Acad Med Singapore 2009; 38(6): 470-8.
4. van Beek EJ, Kuijer PM, Buller HR, Brandjes DP, Bossuyt PM, ten Cate JW. The clinical course of patients with suspected pulmonary embolism. Arch Intern Med 1997; 157(22): 2593-8.
5. Goldhaber SZ, Visani L, De Rosa M. Acute pulmonary embolism: Clinical outcomes in the International Cooperative Pulmonary Embolism Registry (ICOPER). Lancet 1999; 353(9162): 1386-9.
6. Kasper W, Konstantinides S, Geibel A, Olschewski M, Heinrich F, Grosser KD, et al. Management strategies and determinants of outcome in acute major pulmonary embolism: Results of a multicenter registry. J Am Coll Cardiol 1997; 30(5): 1165-71.
7. Givi M, Sadeghi M, Garakyaraghi M, Eshghinezhad A, Moeini M, Ghasempour Z. Long-term effect of massage therapy on blood pressure in prehypertensive women. J Educ Health Promot 2018; 7: 54.
8. Rajabi F, Sadeghi M, Karbasian F, Torkan A. Is thromboprophylaxis effective in reducing the pulmonary thromboembolism? ARYA Atheroscler 2012; 8(1): 16-20.
9. Mirdamadi A, Dashtkar S, Kaji M, Pazhang F, Haghpanah B, Gharipour M. Dabigatran versus Enoxaparin in the prevention of venous thromboembolism after total knee arthroplasty: A randomized clinical trial. ARYA Atheroscler 2014; 10(6): 292-7.
10. Azari A, Bigdelu L, Moravvej Z. Surgical embolectomy in the management of massive and sub-massive pulmonary embolism: The results of 30 consecutive ill patients. ARYA Atheroscler 2015; 11(3): 208-13.
11. Ribeiro A, Lindmarker P, Juhlin-Dannfelt A, Johnsson H, Jorfeldt L. Echocardiography Doppler in pulmonary embolism: Right ventricular dysfunction as a predictor of mortality rate. Am Heart J 1997; 134(3): 479-87.
12. Horlander KT, Mannino DM, Leeper KV. Pulmonary embolism mortality in the United States, 1979-1998: An analysis using multiple-cause mortality data. Arch Intern Med 2003; 163(14): 1711-7.
13. Kaul S, Tei C, Hopkins JM, Shah PM. Assessment of right ventricular function using two-dimensional echocardiography. Am Heart J 1984; 107(3): 526-31.

14. Stein PD, Beemath A, Matta F, Weg JG, Yusen RD, Hales CA, et al. Clinical characteristics of patients with acute pulmonary embolism: Data from PIOPED II. Am J Med 2007; 120(10): 871-9.

15. Vitarelli A, Barilla F, Capotosto L, D'Angeli I, Truscelli G, De Maio M, et al. Right ventricular function in acute pulmonary embolism: A combined assessment by three-dimensional and speckle-tracking echocardiography. J Am Soc Echocardiogr 2014; 27(3): 329-38.

16. Gromadzinski L, Targonski R, Pruszczyk P. Assessment of right and left ventricular diastolic functions with tissue Doppler echocardiography in congestive heart failure patients with coexisting acute pulmonary embolism. Adv Clin Exp Med 2014; 23(3): 371-6.

17. Kjaergaard J, Akkan D, Iversen KK, Kober L, Torp-Pedersen C, Hassager C. Right ventricular dysfunction as an independent predictor of short- and long-term mortality in patients with heart failure. Eur J Heart Fail 2007; 9(6-7): 610-6.

18. Varol K, Gumus C, Yucel H, Sezer F, Seker E, Inci MF, et al. Correlation of right ventricular dysfunction on acute pulmonary embolism with pulmonary artery computed tomography obstruction index ratio (PACTOIR) and comparison with echocardiography. Jpn J Radiol 2015; 33(6): 311-6.

19. Lobo JL, Holley A, Tapson V, Moores L, Oribe M, Barron M, et al. Prognostic significance of tricuspid annular displacement in normotensive patients with acute symptomatic pulmonary embolism. J Thromb Haemost 2014; 12(7): 1020-7.

20. Ghio S, Recusani F, Klersy C, Sebastiani R, Laudisa ML, Campana C, et al. Prognostic usefulness of the tricuspid annular plane systolic excursion in patients with congestive heart failure secondary to idiopathic or ischemic dilated cardiomyopathy. Am J Cardiol 2000; 85(7): 837-42.

21. Hsiao SH, Chang SM, Lee CY, Yang SH, Lin SK, Chiou KR. Usefulness of tissue Doppler parameters for identifying pulmonary embolism in patients with signs of pulmonary hypertension. Am J Cardiol 2006; 98(5): 685-90.

22. Park JH, Kim JH, Lee JH, Choi SW, Jeong JO, Seong IW. Evaluation of right ventricular systolic function by the analysis of tricuspid annular motion in patients with acute pulmonary embolism. J Cardiovasc Ultrasound 2012; 20(4): 181-8.

23. Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et al. Guidelines for the echocardiographic assessment of the right heart in adults: A report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. J Am Soc Echocardiogr 2010; 23(7): 685-713.

24. Caiani EG, Toledo E, MacEneaney P, Bardo D, Cerutti S, Lang RM, et al. Automated interpretation of regional left ventricular wall motion from cardiac magnetic resonance images. J Cardiovasc Magn Reson 2006; 8(3): 427-33.

25. Petkov R, Yamakova Y, Petkova E. TDI velocities of tricuspid annulus in patients with acute pulmonary embolism. Eur Respir J 2013; 42: P4101.

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Effect of cardiac rehabilitation on inflammation: A systematic review and meta-analysis of controlled clinical trials

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Meta-analysis

Abstract

BACKGROUND: This systematic review and meta-analysis aimed to assess the effect of cardiac rehabilitation (CR) on serum C-reactive protein (CRP) as an indicator of the inflammatory state and predictor of recurrent cardiovascular events.

METHODS: PubMed, SCOPUS, Cochrane library, and Google Scholar databases were searched up to January 2014 for original articles which investigated the effect of CR on CRP among adult patients with previous cardiovascular events. The random effects model was used to assess the overall effect of CR on the variation in serum CRP levels.

RESULTS: In the present systematic review and meta-analysis, 15 studies were included. The analysis showed that CR might significantly reduce high-sensitivity CRP (hs-CRP) levels [Difference in means (DM) = -1.81 mg/l, 95% confidence interval (CI): -2.65, -0.98; P = 0.004). However, the heterogeneity between studies was significant (Cochran's Q test, P < 0.001, I-squared = 84.9%). To find the source of variation, the studies were categorized based on study design (quality) and duration. The negative effect was higher among studies which followed their participants for 3 weeks or less (DM = -2.75 mg/l, 95% CI: -3.86, -1.64; P < 0.001) compared to studies which investigated the effect of CR for 3-8 weeks (DM = -0.89 mg/l, 95% CI: -1.35, -0.44; P < 0.001) and those which lasted more than 8 weeks (DM = -1.71 mg/l, 95% CI: -2.53, -0.89; P < 0.001). There was no evidence of heterogeneity when the categorization was based on the follow-up period.

CONCLUSION: Both short- and long-term CR have resulted in improvement in serum hs-CRP levels. CR can be perceived as a beneficial tool to reduce inflammatory markers among patients with previous cardiac events.

Keywords: Cardiac Rehabilitation, Inflammation, C-Reactive Protein

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Introduction

Cardiovascular diseases (CVDs) are a group of disorders which effect the heart and blood vessels and are the leading cause of death, worldwide.[1] Based on the World Health Organization (WHO) reports, 17.3 million people (about 30% of global deaths) died due to CVDs in 2008, among which 7.3 and 6.2 million were reported to dye due to coronary heart disease (CHD) and stroke, the two major subclasses of CVDs, respectively. Inflammation is shown to be one of the important factors in the development and clinical course of most CVDs. Inflammation is actively involved in all levels of atherogenesis, from the initial lesions to the end-stage complications. Therefore, atherosclerosis is presently recognized as a low grade inflammatory vascular disease,[2] which maximizes after the cardiac events. Moreover, reducing the inflammatory state after the cardiovascular event might be of great importance. Inflammatory markers, including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and particularly, C-reactive protein (CRP), are considered

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as risk markers of CVDs.[3] The lipid-lowering therapy as a CVD risk-reducing strategy has also been reported to produce a parallel decrease in CRP. It has been shown that CRP concentrations decrease 15–50% with statin therapy.[4-6]

Cardiac rehabilitation (CR) has been defined as "comprehensive long-term services involving medical evaluation, prescribed exercise, cardiac risk-factor modification, health education, counseling, and behavioral interventions" by the United States Department of Health and Human Services (HHS) and the National Heart, Lung, and Blood Institute (NHLBI). CR has been shown to beneficially effect overall health and metabolic factors including inflammatory markers among patients who have experienced CVDs.[7-11] For instance, it has been shown that exercise training, as an important part of CR programs, might be more effective in reducing the inflammatory markers than standard treatments provided early after acute myocardial infarction.[12,13] Several studies have assessed the effect of CR on inflammatory markers including fibrinogen,[14,15] TNF-α,[16,17] and IL-6.[17,18] However, the majority of studies have selected high-sensitivity CRP (hs-CRP) levels as a widely acceptable marker of inflammation to assess the beneficial effects of CR on inflammatory state.[17,19-22] Furthermore, the use of serum CRP has been suggested as a predictor for CVDs and their recurrence.[23] Studies have led to conflicting results regarding the effect of CR on CRP levels. Some researchers have shown the beneficial effect of CR on CRP levels,[9,18,24,25] while others did not find the same results.[20] This is while the majority of published data are from low quality before-after trials,[8,20,24-26] and the parallel studies had not assessed the difference in the CRP level variation between their intervention group and controls.[9,13,17-19,22,27-29] To the best of our knowledge, no study has attempted to summarize published data about the effect of CR on CRP levels. As the clinical trials provide the most qualified data regarding this association, the present study aimed to systematically review the controlled clinical trials investigating the effect of CR on inflammatory markers in patients with CVDs, and if possible quantify their results and search for their possible sources of heterogeneity through a meta-analysis.

Materials and Methods

The online databases of PubMed, ISI Web of Science, Scopus, Science Direct, and Embase were searched for relevant English and non-English publications up to January 2014. Moreover, experts in this field were contacted and reference lists of the published papers were searched. The keywords used in the present search strategy consisted of those selected from the Medical Subject Headings (MeSH) database and other related non-MeSH terms. The non-MeSH terms consisted of 3 groups. The first group were keywords related to cardiac rehabilitation ("cardiac rehabilitation", "cardiovascular rehabilitation", "cardiopulmonary rehabilitation", OR "cardiac exercise", OR "cardiovascular exercise", OR "cardiopulmonary exercise", "rehabilitation", "cardiac rehab*", "pulmonary rehabilitation").

The second group were keywords related to cardiometabolic markers ["intercellular cell adhesion molecule (ICAM)", "vascular cell adhesion molecule (VCAM)", "adhesion molecule", "E-selectin", "fibrinogen", "white blood cell", "serum amyloid A", "cytokines", "erythrocyte sedimentation rate (ESR)", "total cholesterol (TC)", "inflammatory marker", "interleukin", "smoking", "inflammation", "inflammation mediators", "IL" OR "C-reactive protein", "c reactive protein", "CRP", "inflammatory", "inflammation", "tumor necrosis factor", "TNF", "interleukins", "CRP", "TNF-α", "cholesterol", "lipoproteins, high-density lipoprotein (HDL)", "lipoproteins, low-density lipoprotein (LDL)", "triglycerides", "glucose tolerance test (GTT)", "insulin", "blood glucose", "insulin resistance", "cholesterol", "lipoproteins, HDL", "lipoproteins, LDL", "triglycerides", "GTT", "insulin", "blood glucose", "insulin resistance", "LDL", "HDL", "triglyceride", "triacylglycerol (TG)", "TC", "GTT", "fast blood sugar (FBS)", " fasting blood glucose (FBG)", "fasting insulin", "FBS", "FBG", "insulin sensitivity", "blood sugar", "lipid profile", "serum lipid", "plasma lipid", "blood pressure", "hypertension", "blood pressure", and "hypertension"].

The third group consisted of keywords related to clinical trials ("intervention studies", "intervention", "controlled trial", "randomized", "randomized", "random", "randomly", "placebo", "assignment", "clinical trial", and "trial"). Databases were searched using keywords 1 in combination with 2 and 1 in combination with 3 and duplicate studies were removed. No filter or limitation was implemented while searching the mentioned databases.

To be included in the meta-analysis, a published study had to meet the following criteria: be an original article, a controlled clinical trial, and conducted in an adult human population, and having assessed the effect of CR on serum CRP levels.

As the majority of studies had reported CRP levels as their main outcome variable, in the present study, only these articles were included in the systematic review and meta-analysis. Data were

extracted on publication (the first author's last name, year of publication, and country of the studied population), number of individuals in the intervention and control groups, duration of the intervention, age, gender, and mean and standard deviation (SD) of inflammatory factors at baseline and after CR.

The mean and SD of CRP levels at baseline and after the intervention period was used to calculate the mean change in CRP levels and its corresponding SD which was included in the meta-analysis as an effect size (raw difference in means: DM) from each before and after study. The difference in CRP levels variation between intervention and control groups (DM) was also calculated and used as the effect size for controlled clinical trials. Summary mean estimates with their corresponding SDs were derived using the random effects model (method of DerSimonian and Laird),[30] which incorporates between-study variability. Subgroup analyses were performed to check for specific sources of heterogeneity. Sensitivity analysis was used to explore the extent to which inferences might depend on a particular study or group of studies. Publication bias was assessed by visual inspection of funnel plots.[31] In these funnel plots, the difference in mean CRP was displayed against the inverse of the square of the standard error (a measure of the precision of the studies). Funnel plots generally show a peak at the point where the studies with smaller standard errors are found which usually represents the point of the approximate true effect. Formal statistical assessment of funnel plot asymmetry was performed using Egger's regression asymmetry test and adjusted rank correlation test.[32] Reported P-values are from the intercept of the regression analysis, which provides a measure of asymmetry. In addition, Begg's adjusted rank correlation test was used.[32] Statistical analyses were carried out in Stata (version 11.2; StataCorp, College Station, TX, USA). *P* values of less than 0.05 were considered statistically significant.

Results

In the preliminary online search, 7418 studies were retrieved; about 7354 were excluded after reading titles or abstracts because they did not meet the inclusion criteria (Figure 1). Finally, 43 articles were found which had investigated the effect of CR on one of the inflammatory markers; among these, 15 articles had examined the association between CR and hs-CRP or CRP.[8,9,13,17-22,24-29] One of these 15 eligible studies had examined the effect of a very short-term period (1 session) of CR on hs-CRP levels in patients with ischemic heart disease (IHD).[21] As the objective of the present review was to assess the long-term effect of CR on CRP levels, this study was removed from the qualitative and quantitative synthesis. Moreover, a study performed by Hansen et al. was not included in the present review.[9] This study was a long-term follow-up (about 72 weeks) of two groups of patients randomly undergoing a 40-minute and 60-minute CR program for 7 weeks, and there was no intervention in the follow-up period.[9] Therefore, 13 articles were eligible to be included in the systematic review.[8,13,17-20,22,24-29] The characteristics of studies are presented in table 1. Studies were conducted in USA,[18,20,22] Italy,[8,24,26] South Korea,[17,27] Croatia,[13] Serbia,[28] Belgium,[29] Japan,[25] and Iran.[19] Among these studies, 5 were of lower quality because they had a before-after,[8,20,24-26] while others were parallel studies with control groups.[9,13,17-19,22,27-29] However, only in 2 of the parallel studies the patients were randomly assigned to intervention and control groups.[9,13] Although the study conducted by Hansen et al.[29] was a randomized controlled clinical trial, the intervention and control groups underwent 60 and 40 minutes of CR, respectively; therefore, the study was considered a before-after study which assessed the effect of the CR program on all its participants.

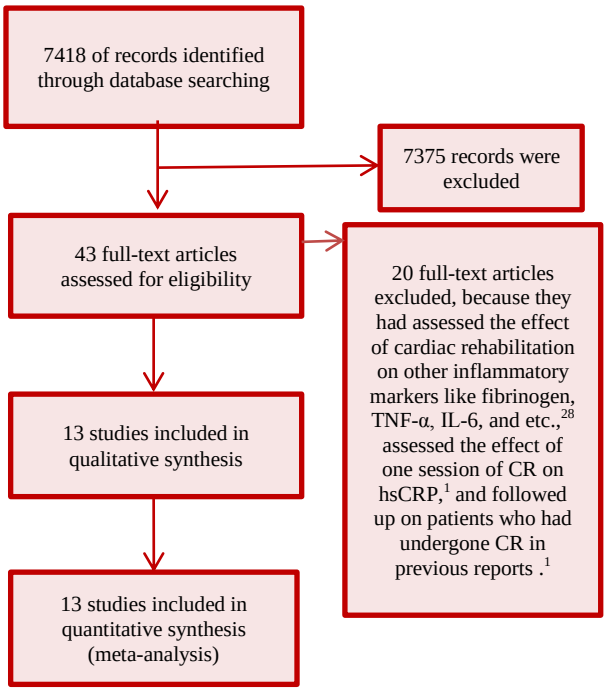

Figure 1. Flow chart for the study selection process
TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6; hs-CRP: High-sensitivity C-reactive protein; CR: cardiac rehabilitation

Table 1. Characteristics of studies evaluated the effect of cardiac rehabilitation (CR) on metabolic syndrome and/or its components

Author/year	Country	Subjects and gender	Mean Age (year)	Design	Intervention	Control	Duration	Outcome variable	Result
Fukuda et al.[25]	Japan	Patients with cardiovascular diseases (F: 6/M: 44)	61	Before-after	Aerobic bicycle exercise 2 or 3 times per week for 3–6 months	-	24 weeks	hs-CRP	hs-CRP levels decreased, but it was not statistically significant.
Ferratini et al.[24]	Italy	Patients after cardiac surgery (F: 68/M: 155)	67	Before-after	Up to 30 minutes of cycling 5 times a week at 70% maximal heart rate	-	3 weeks	hs-CRP	hs-CRP levels significantly decreased after CR program.
Cesari et al.[8]	Italy	Patients with acute coronary syndrome (F:20/M: 92)	58.2	Before-after	3 days/week of endurance training on a cycle-ergometer at 60–70% of VO2 level	-	4 weeks	hs-CRP	hs-CRP levels significantly decreased after CR program.
Aminlari et al.[19]	Iran	Patients with myocardial infarction (56 M/F)	62.7	Parallel (randomization was not mentioned)	Exercise training, education, and behavior modification therapy were performed 3 times per week. The exercise training included arm and leg ergometry and treadmills. Behavioral modifications were smoking cessation, healthy nutrition, hypertension control, and etc.	No intervention	8 weeks	CRP	CRP levels significantly decreased in the intervention group compared to controls.
Kim et al.[27]	South Korea	Patients with acute myocardial infarction (F:32/M: 109)	63.24	Parallel without randomization	Warm-up (10 minutes), exercise (30 minutes), and cool-down (10 minutes) The intensity of exercise was adjusted on a test result basis by calculation of heart rate reserve first, followed by the increased target heart rate from 40% to 85% of the value in phases	General training on exercise or risk factors management. instructed to maintain their own exercise	16 weeks	hs-CRP	The exercise group showed a significantly lower value of hs-CRP than the control group.
Rankovic et al.[28]	Serbia	patients with ischemic heart disease (F: 23/M:29)	60.22	Parallel (randomization was not mentioned)	Continual aerobic exercise for 45 minutes on a treadmill, room bicycle or walking The intensity of physical exercise was limited to the submaximal physical capacity at the level of 70-80% of maximal heart frequency at the stress test taken before cardiovascular rehabilitation. Physical exercise was applied 3 times a week.	Did not have physical training in the last 6 months, except for usual household activities.	3 weeks	hs-CRP	CRP levels decreased significantly in the exercise group compared to controls.

Table 1. Characteristics of studies evaluated the effect of cardiac rehabilitation (CR) on metabolic syndrome and/or its components (continue)

Author/year	Country	Subjects and gender	Mean Age (year)	Design	Intervention	Control	Duration	Outcome variable	Result
Cesari et al.[26]	Italy	Patients after cardiac surgery (F: 35/M: 51)	72.5	Before-after	Aerobic exercise at cycle ergometer and short lasting calisthenic exercises, with the resistance sequentially provided by the weight of single body segments and gentle, passive stretching involving all the main joints The training frequency was 6 times per week for a total of 12 training sessions.	-	15 days	hs-CRP	hs-CRP levels significantly decreased after CR program.
Lavie et al.[20]	USA	Patients with coronary heart disease (F:72/M:73)	65.37	Before-after	3 times per week group exercise and educational sessions, and individual exercise (between 1 and 3 times per week) on non-rehabilitation days	-	12 weeks	hs-CRP	hs-CRP levels significantly decreased only among obese participants but not among lean subjects.
Kim et al.[17]	South Korea	Patients with coronary artery disease (F:11/M: 28)	50.08	Parallel (randomization was not mentioned)	Supervised exercise under prescription based on symptom-limited treadmill exercise test at hospital lasted 6 weeks + a home based and self-managed exercise lasting 8 weeks The exercise: warm-up, 30- to 40-min exercise on a treadmill or bicycle ergometer, and a cool-down	followed up with standard care as outpatients	14 weeks	hs-CRP	hs-CRP significantly decreased only among patients undergoing cardiac rehabilitation compared to controls.
Hansen et al.[29]	Belgium	Patients with coronary artery disease (F: 25/M: 109)	63.15	before-after	60 or 40 minutes of exercise (42% on the treadmill, 33% on the cycle ergometer, and 25% on the arm cranking device)		7 weeks	CRP	hs-CRP levels decreased in both exercise groups.
Balen et al.[13]	Croatia	Patients with acute myocardial infarction (F: 16/M: 44)	60	Randomized controlled clinical trial	45-minute aerobic activity on a cycle-ergometer and 30-minute organized program of supervised walking on a standardized track	standard care	3 weeks	hs-CRP	hs-CRP significantly decreased in both groups, but after the intervention, values were significantly lower among patients with cardiac rehabilitation.

Table 1. Characteristics of studies evaluated the effect of cardiac rehabilitation (CR) on metabolic syndrome and/or its components (continue)

Author/year	Country	Subjects and gender	Mean Age (year)	Design	Intervention	Control	Duration	Outcome variable	Result
Shin et al.[18]	USA	patients with coronary artery disease after percutaneous coronary intervention (F: 11/M: 28)	56.58	Parallel	Cardiac rehabilitation following hospital discharge and 8 weeks of home stay exercise plus statin therapy All subjects were prescribed daily lipid lowering medication consisting of 100 mg aspirin and 75 mg clopidogrel throughout the experimental period.	80 mg daily of fluvastatin	14 weeks	hs-CRP	hs-CRP significantly decreased in both groups but after intervention values were significantly lower among patients with cardiac rehabilitation.
Milani et al.[22]	USA	Patients with coronary heart disease (F:75/M: 202)	66.27	Parallel	Patients received formalized exercise instruction and met 3 times per week for group exercise classes, and were encouraged to exercise on their own (1 to 3 times per week) in between sessions.	Did not have cardiopulmonary exercise tests.	12 weeks	hs-CRP	hs-CRP levels significantly decreased only among patients with cardiac rehabilitation.

F: Female, M: Male; hs-CRP: High-sensitivity C-reactive protein

Results of meta-analysis: Of the 15 eligible article for the systematic review, 2 studies did not report data about baseline and post-intervention serum CRP levels;[19,25] These data could not be obtained even after contacting the authors twice through email, 1 week apart. Therefore, 13 studies were included for the quantification of the effect of CR on serum CRP levels.[8,9,13,17,18,20-22,24,26-29] In total, 992 adult participants with CVDs were included in the current analysis. The analysis showed that CR significantly reduces hs-CRP levels [DM = -1.81 mg/l, 95% confidence interval (CI): -2.65, -0.98; P = 0.004). However, the heterogeneity between studies was significant (Cochran's Q test, P < 0.001, I-squared = 84.9%) (Table 2).

Table 2. Forest plot demonstrating weighted mean differences with 95% confidence interval (CI) for all eligible studies investigating the effects of cardiac rehabilitation on C-reactive protein/high-sensitivity C-reactive protein levels grouped by study designs using random effects model

Study	DM (95% CI)
Before-after studies	
Lavie et al.[20] (obese)	-3.40 (-4.94, -1.86)
Lavie et al.[20] (lean)	-1.00 (-2.60, 0.60)
Hansen et al.[29]	-0.80 (-1.66, 0.06)
Cesari et al.[26]	-0.20 (-6.36,5.96)
Ferratini et al.[24]	-2.90 (-3.15, -2.65)
Cesari et al.[8]	-0.93 (-1.47, -0.39)
Subtotal (I-squared = 92.0%, P < 0.001)	-1.74 (-2.93, 0.55)
Parallel studies with no randomization	
Milani et al.[22]	-2.40 (-15.04, 10.24)
Shin et al.[18]	-1.49 (-3.48, 0.50)
Kim et al.[17]	-1.00 (-2.68, 0.68)
Rankovic et al.[28]	-1.51 (-3.11, 0.09)
Kim et al.[27]	-1.50(-2.85, 0.15)
Subtotal (I-squared = 0.0%, P = 0.991)	-1.39 (-2.19, 0.59)
Randomized clinical trial	
Balen et al.[13]	-4.50 (-6.63, -2.37)
Subtotal (I-squared = 0%, P < 0.001)	-4.50 (-6.63, -2.37)
Overall (I-squared = 84.9%, P < 0.001)	-1.81 (-2.65, -0.98)

DM: Difference in means; CI: Confidence interval

To find the source of variation, the studies were categorized based on their design (quality) and duration. When categorizing studies based on their design, the significant reducing effect of CR was observed in before-after studies (DM = -1.74 mg/l, 95% CI: -2.93, -0.55; P = 0.001), parallel studies (DM = -1.39 mg/l, 95% CI: -2.19, -0.59; P < 0.001), and randomized controlled (DM = -4.5 mg/l, 95% CI: -6.63, -2.37; P < 0.001) clinical trials. The heterogeneity was still significant among before-after studies (Cochran's Q test, P < 0.001, I-squared = 92%), while there was no evidence of heterogeneity between parallel studies (Cochran's Q test, P = 0.990, I-squared = 0.0%). Studies were also categorized based on their follow-up period (Table 3). Although, the reducing effect was statistically significant for all these subgroups, the negative effect was higher among studies which followed their participants for 3 weeks or lower (short follow-up period) (DM = -2.75 mg/l, 95% CI: -3.86, -1.64; P < 0.001) compared to studies which investigated the CR effect for 3-8 weeks (middle follow-up period) (DM = -0.89 mg/l, 95% CI: -1.35, -0.44; P < 0.001) and those which lasted more than 8 weeks (long follow-up period) (DM = -1.71 mg/l, 95% CI: -2.53, -0.89; P < 0.001). Although the I-squared showed a moderate heterogeneity among studies with short follow-up periods (48.2%), Cochran's Q test did not provide a significant evidence of heterogeneity (P = 0.122). The heterogeneity was not significant for studies with middle- (Cochran's Q test, P = 0.80, I-squared = 0.0%) and long-term follow-up periods (Cochran's Q test, P = 0.290, I-squared = 19.4%).

Table 3. Forest plot demonstrating weighted mean differences with 95% confidence interval (CI) for all eligible studies investigating the effects of cardiac rehabilitation on C-reactive protein/high-sensitivity C-reactive protein levels grouped by study follow-up period using random effects model

Study	DM (95% CI)
Short follow-up period	
Balen et al.[13]	-4.50 (-6.63, -2.37)
Rankovic et al.[28]	-1.51 (-3.11, 0.09)
Cesari et al.[26]	-0.20 (-6.36,5.96)
Ferratini et al.[24]	-2.90 (-3.15, -2.65)
Subtotal (I-squared = 48.2%, P = 0.122)	-2.75 (-3.86, -1.64)
Middle follow-up period	
Hansen et al.[29]	-0.80 (-1.66, 0.06)
Cesari et al.[8]	-0.93 (-1.47, -0.39)
Subtotal (I-squared = 0.0%, P = 0.801)	-0.89 (-1.35, -0.44)
Long follow-up period	
Milani et al.[22]	-2.40 (-15.04, 10.24)
Shin et al.[18]	-1.49 (-3.48, 0.50)
Lavie et al.[20] (obese)	-3.40 (-4.94, -1.86)
Lavie et al.[20] (lean)	-1.00 (-2.60, 0.60)
Kim et al.[17]	-1.00 (-2.68, 0.68)
Kim et al.[27]	-1.50(-2.85, 0.15)
Subtotal (I-squared = 19.4%, P = 0.287)	-1.71 (-2.53, 0.89)
Overall (I-squared = 84.9%, P < 0.001)	-1.81 (-2.65, -0.98)

DM: Difference in means; CI: Confidence interval

In a sensitivity analysis, it was found that the effect of CR on CRP levels was not substantially modified by the result of a certain study. Although a slight asymmetry was seen in Begg's funnel plot, no evidence of publication bias was found using asymmetry tests (Egger's test, P = 0.192, Begg and Mazumdar test, P = 0.170) (Figure 2).

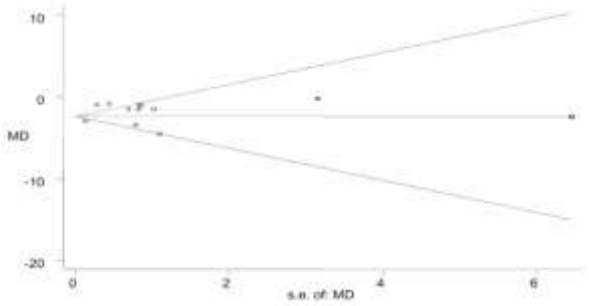

Figure 2. Begg's funnel plot with pseudo 95% confidence interval (CI) of the difference in means versus the standard errors of the difference in means for studies investigating the effect of cardiac rehabilitation on C-reactive protein/high-sensitivity C-reactive protein (b)
MD: Mean difference

Discussion

The systematic review and meta-analysis on clinical trials revealed the significant reducing effect of CR on serum hs-CRP levels. The analysis also showed that CR beneficially affects inflammation both in a short and long period of time. Therefore, CR might reduce the risk of CVDs recurrence not only through its beneficial effects on the lipid profile, but also through its effect on the inflammatory state. To the best of our knowledge, this is the first systematic review and meta-analysis assessing the effects of CR on inflammatory markers.

CRP, as a liver-derived molecule that is increased in inflammatory states, rapidly increases within hours after tissue injuries like CVDs.

Since CVD is at least in part an inflammatory process, CRP has been investigated in the context of arteriosclerosis and subsequent vascular disorders. CRP is recommended as a predictive laboratory marker for CVD risk in patients with high susceptibility to CVDs or their recurrence.[33] CRP has been shown to have some advantages as a marker: (1) It is a stable marker; (2) It does not have clinically significant circadian variation; and (3) It's measurement is easy and reliable.[34] Therefore, decreasing the inflammatory state markers including CRP levels might help patients to decrease the risk of recurrence of CVDs.

Aerobic exercise, as an adjunct to pharmacological therapy in patients with chronic heart failure and CAD, is known to improve peripheral vascular function and skeletal muscle abnormalities. Although exercise causes stress-induced up-regulation of endothelial nitric oxide synthase (eNOS),[35] it is supposed that the modification of inflammatory mediators through exercise reverses peripheral vascular endothelial dysfunction.[16] The present study results are in line with the previous reports suggesting that CR improved the inflammatory markers of patients experiencing CVDs.[8,13,19,22] Therefore, CR must be considered as an important strategy adjacent to conventional therapy used for patients experiencing cardiac events.

Some limitations must be considered while interpreting the present results. The effect of CR on CRP levels was studied because it is an acceptable marker for inflammation and also might be used as a predictor of cardiovascular events. Furthermore, the majority of published studies had selected the same marker to assess the inflammatory state. A limited number of studies were found which had used other inflammatory markers like TNF-α and interleukins.[16-18] Further studies on the effect of CR on other inflammatory markers are highly recommended in order to determine whether CR has the same beneficial effects. In addition, it has been proposed that the beneficial effect of CR might be because of its negative effect on body fat and obesity. Lavie et al. showed that the beneficial effect of CR on CRP levels is only significant in obese patients with CHD.[20] it has also been proposed that circulating hs-CRP and inflammatory cytokines are in relation with obesity among healthy subjects.[36] In the present review, there was only one study which had separately examined the effect of CR on hs-CRP levels among obese and lean participants.[20] Furthermore, the majority of eligible studies did not report the effect of CR on weight or BMI variation; therefore, the association between obesity or body fat and CRP levels variation could not be explored.

In conclusion, the present systematic review provides strong evidence of the beneficial effect of CR on the inflammatory state of patients with CVDs. It is highly recommended that further studies be conducted to explore whether CR affects other inflammatory markers and how dependent the changes in inflammatory markers

as a result of CR are on the magnitude of the change in body composition.

Conclusion

Both short- and long-term CR program have resulted in improvement in serum hs-CRP levels. CR can be perceived as a beneficial tool to reduce inflammatory markers among patients with previous cardiac events. It is highly recommended that further studies be conducted to explore whether CR affects other inflammatory markers and how dependent the changes in inflammatory markers as a result of CR are on the magnitude of the change in body composition.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

References

1. Talaei M, Sarrafzadegan N, Sadeghi M, Oveisgharan S, Marshall T, Thomas GN, et al. Incidence of cardiovascular diseases in an Iranian population: The Isfahan Cohort Study. Arch Iran Med 2013; 16(3): 138-44.
2. Wilensky RL, Hamamdzic D. The molecular basis of vulnerable plaque: Potential therapeutic role for immunomodulation. Curr Opin Cardiol 2007; 22(6): 545-51.
3. Libby P. Inflammation and cardiovascular disease mechanisms. Am J Clin Nutr 2006; 83(2): 456S-60S.
4. van de Ree MA, Huisman MV, Princen HM, Meinders AE, Kluft C. Strong decrease of high sensitivity C-reactive protein with high-dose atorvastatin in patients with type 2 diabetes mellitus. Atherosclerosis 2003; 166(1): 129-35.
5. Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001; 344(26): 1959-65.
6. Ridker PM, Rifai N, Pfeffer MA, Sacks FM, Moye LA, Goldman S, et al. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1998; 98(9): 839-44.
7. Wenger NK, Froelicher ES, Smith LK, Ades PA, Berra K, Blumenthal JA, et al. Cardiac rehabilitation as secondary prevention. Agency for Health Care Policy and Research and National Heart, Lung, and Blood Institute. Clin Pract Guidel Quick Ref Guide Clin 1995; (17): 1-23.
8. Cesari F, Marcucci R, Gori AM, Burgisser C, Francini S, Sofi F, et al. Impact of a cardiac rehabilitation program and inflammatory state on endothelial progenitor cells in acute coronary syndrome patients. Int J Cardiol 2013; 167(5): 1854-9.
9. Hansen D, Dendale P, Raskin A, Schoonis A, Berger J, Vlassak I, et al. Long-term effect of rehabilitation in coronary artery disease patients: Randomized clinical trial of the impact of exercise volume. Clin Rehabil 2010; 24(4): 319-27.
10. Sarrafzadegan N, Rabiei K, Kabir A, Sadeghi M, Khosravi A, Asgari S, et al. Gender differences in risk factors and outcomes after cardiac rehabilitation. Acta Cardiol 2008; 63(6): 763-70.
11. Ghashghaei FE, Sadeghi M, Marandi SM, Ghashghaei SE. Exercise-based cardiac rehabilitation improves hemodynamic responses after coronary artery bypass graft surgery. ARYA Atheroscler 2012; 7(4): 151-6.
12. Sadeghi M, Garakyaraghi M, Khosravi M, Taghavi M, Sarrafzadegan N, Roohafza H. The impacts of cardiac rehabilitation program on echocardiographic parameters in coronary artery disease patients with left ventricular dysfunction. Cardiol Res Pract 2013; 2013: 201713.
13. Balen S, Vukelic-Damijani N, Persic V, Ruzic A, Miletic B, Samardiija M, et al. Anti-inflammatory effects of exercise training in the early period after myocardial infarction. Coll Antropol 2008; 32(1): 285-91.
14. Solov'ev AV, Ermolin GA, Ignashenkova GV, Dikov MM. Fibrinogen and fibrin degradation products in the blood of acute myocardial infarct patients at the hospital rehabilitation stage. Ter Arkh 1987; 59(10): 21-3.
15. Wosornu D, Allardyce W, Ballantyne D, Tansey P. Influence of power and aerobic exercise training on haemostatic factors after coronary artery surgery. Br Heart J 1992; 68(2): 181-6.
16. Conraads VM, Beckers P, Bosmans J, De Clerck LS, Stevens WJ, Vrints CJ, et al. Combined endurance/resistance training reduces plasma TNF-alpha receptor levels in patients with chronic heart failure and coronary artery disease. Eur Heart J 2002; 23(23): 1854-60.
17. Kim YJ, Shin YO, Bae JS, Lee JB, Ham JH, Son YJ, et al. Beneficial effects of cardiac rehabilitation and exercise after percutaneous coronary intervention on hsCRP and inflammatory

cytokines in CAD patients. Pflugers Arch 2008; 455(6): 1081-8.
18. Shin YO, Bae JS, Lee JB, Kim JK, Kim YJ, Kim C, et al. Effect of cardiac rehabilitation and statin treatment on anti-HSP antibody titers in patients with coronary artery disease after percutaneous coronary intervention. Int Heart J 2006; 47(5): 671-82.
19. Aminlari A, Jazayeri SM, Bakhshandeh AR. Association of cardiac rehabilitation with improvement in high sensitive C-reactive protein post-myocardial infarction. Iran Red Crescent Med J 2012; 14(1): 49-50.
20. Lavie CJ, Morshedi-Meibodi A, Milani RV. Impact of cardiac rehabilitation on coronary risk factors, inflammation, and the metabolic syndrome in obese coronary patients. J Cardiometab Syndr 2008; 3(3): 136-40.
21. Madarame H, Kurano M, Fukumura K, Fukuda T, Nakajima T. Haemostatic and inflammatory responses to blood flow-restricted exercise in patients with ischaemic heart disease: A pilot study. Clin Physiol Funct Imaging 2013; 33(1): 11-7.
22. Milani RV, Lavie CJ, Mehra MR. Reduction in C-reactive protein through cardiac rehabilitation and exercise training. J Am Coll Cardiol 2004; 43(6): 1056-61.
23. Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003; 107(3): 363-9.
24. Ferratini M, Ripamonti V, Masson S, Grati P, Racca V, Cuccovillo I, et al. Pentraxin-3 predicts functional recovery and 1-year major adverse cardiovascular events after rehabilitation of cardiac surgery patients. J Cardiopulm Rehabil Prev 2012; 32(1): 17-24.
25. Fukuda T, Kurano M, Iida H, Takano H, Tanaka T, Yamamoto Y, et al. Cardiac rehabilitation decreases plasma pentraxin 3 in patients with cardiovascular diseases. Eur J Prev Cardiol 2012; 19(6): 1393-400.
26. Cesari F, Sofi F, Caporale R, Capalbo A, Marcucci R, Macchi C, et al. Relationship between exercise capacity, endothelial progenitor cells and cytochemokines in patients undergoing cardiac rehabilitation. Thromb Haemost 2009; 101(3): 521-6.
27. Kim C, Kim DY, Moon CJ. Prognostic influences of cardiac rehabilitation in Korean acute myocardial infarction patients. Ann Rehabil Med 2011; 35(3): 375-80.
28. Rankovic G, Milicic B, Savic T, Dindic B, Mancev Z, Pesic G. Effects of physical exercise on inflammatory parameters and risk for repeated acute coronary syndrome in patients with ischemic heart disease. Vojnosanit Pregl 2009; 66(1): 44-8.
29. Hansen D, Dendale P, Berger J, Onkelinx S, Reyckers I, Hermans A, et al. Importance of exercise training session duration in the rehabilitation of coronary artery disease patients. Eur J Cardiovasc Prev Rehabil 2008; 15(4): 453-9.
30. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7(3): 177-88.
31. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315(7109): 629-34.
32. Egger M, Davey-Smith G, Altman D. Systematic reviews in health care: Meta-analysis in context. Hoboken, NJ: John Wiley & Sons; 2001.
33. Pfutzner A, Forst T. High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. Diabetes Technol Ther 2006; 8(1): 28-36.
34. Libby P, Ridker PM. Inflammation and atherosclerosis: Role of C-reactive protein in risk assessment. Am J Med 2004; 116(Suppl 6A): 9S-16S.
35. Gielen S, Adams V, Mobius-Winkler S, Linke A, Erbs S, Yu J, et al. Anti-inflammatory effects of exercise training in the skeletal muscle of patients with chronic heart failure. J Am Coll Cardiol 2003; 42(5): 861-8.
36. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: Associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999; 19(4): 972-8.

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Distal accesses in the hand (two novel techniques) for percutaneous coronary angiography and intervention

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Case Series

Abstract

BACKGROUND: Trans-radial and trans-ulnar accesses have been practiced and recommended as default and alternative techniques for coronary angiography and angioplasty in recent years. In this study, we present new innovative approaches using more distal access points, i.e. trans-snuff box and trans-palmar approaches.

METHODS: We conducted dorsal hand access (trans-snuff box) for angiography and/or angioplasty on 235 patients, and trans-palmar access (superficial palmar branch of ulnar artery) on 175 patients in 3 hospitals in Isfahan City, Iran.

RESULTS: In 221 patients out of 235 ones (94.1%) [men: 76.5%, age: 57.4 ± 10.4 (years); women: 23.5%, age: 62.4 ± 9.5 (years)], our procedure through snuff box (dorsal hand) was successfully performed. In 159 patients out of 175 ones (90.8%) [men: 76.0%, age: 58.1 ± 10.5 (years); women: 24.0%, age: 61.2 ± 9.6 (years)], our procedure through palmar artery was successfully performed. In total, the evaluated patients had mild pain (3.4% for snuff box, and 4.5% for palmar), ecchymosis in distal forearm (5.1% for snuff box, and 2.8% for palmar), with no major complications even one (amputation, infection, thrombosis, need for surgery, hand dysfunction, nerve palsy, and so forth). In addition, percutaneous coronary intervention (PCI) was done in 28.9% and 18.2% of cases via snuff box and palmar approaches, respectively. Meanwhile, hemostasis was very fast and easy with discharge time equivalent to other upper limb accesses.

CONCLUSION: Although our procedures are at their early stages with about a follow-up period of 3-15 months, more researches are recommended to be conducted in forthcoming months and years, and this new innovative approaches could be suggested safe, feasible, and reliable with low complications.

Keywords: Coronary Angiography, Coronary Angioplasty, Trans-Palmar Approach, Trans-Snuff Box Approach, Distal Accesses, Novel Accesses

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Introduction

Angiography and percutaneous coronary intervention (PCI) through arteries of the upper extremities is superior to femoral approach, and is on a rise due to less bleeding, easier practice of hemostasis, more patient convenience, shorter procedure time, lower cost imposed to patients and health system, and shorter period of hospitalization.[1-8] In addition, patients will sooner restore their routine physical activity in case of an upper extremity approach.[4,9]

Currently, the most classic and routine method is trans-radial, suggested and conducted initially by Campeau et al. in 1989, followed shortly thereafter by the first trans-radial coronary stenting by Kiemeneij and Laarman in 1993, and has been done for more than two decades by other researchers and operators.[4,10]

Artery size, anatomical variations, arterial loop, hypoplasia, radial artery occlusion (RAO), previous

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RA harvesting for coronary artery bypass graft (CABG), and so like are the most troublesome issues with trans-radial approach.[2,11,12] In other words, this approach is not always successful with obligatory shift to other routes.[13] Although many investigators have shown that complications of trans-ulnar approach are rather equal to trans-radial approach, and the latter is suggested as an alternative to trans-radial approach,[9,14,15] but trans-ulnar approach has also its own difficulties and limitations; so, more innovative routes with lower complications and higher patient and operator convenience are strongly warranted.

The term "more distal accesses" was introduced for the first time by Roghani et al. as those that are more distal to wrist crease. This new concept was presented technically by Farshad Roghani-Dehkordi at the 4th International Cardiovascular Joint Congress in Isfahan in 2016[16] with attendance of Dr. Ferdinand Kiemeneij, Dr. Tejas Patel, and Dr. Sasko Kedev.

We hereby present two novel accesses as follows:

1. Trans-snuff box approach that was also suggested and introduced by Kaledin et al. in 2014,[17] Babunashvili in 2016,[18] Roghani-Dehkordi in 2016,[16] Kiemeneij in 2016,[3] and Latsios et al. in 2018.[19]

2. Trans-palmar approach that was suggested and presented for the first time worldwide by Roghani-Dehkordi at the 4th Iranian Cardiovascular Joint Congress in 2016 (Isfahan, Iran), and at the 2nd Congress of Clinical Cases in Complex Cardiovascular Therapeutics in April 2017 (Shiraz, Iran).

Materials and Methods

In this multi-center and cross-sectional study, we conducted dorsal hand access (snuff box) for angiography and/or angioplasty in 235 patients during 1 Oct. 2016 till 1 Oct. 2017, and palmar approach on 175 patients during 15 Oct. 2016 till 15 Oct. 2018 in Shahid Chamran, Khorshid, and Shahid Saddoughi hospitals (Isfahan, Iran).

Exclusion criteria for trans-snuff box and trans-palmar approaches were patients with Raynaud's disease, upper limb vascular disorders, patients with carpal tunnel syndrome (only for palmar method), patients with neural disorders of radial nerve innervation area (for trans-snuff box) and sensory/motor disorders in territory of median and ulnar nerves (for trans-palmar approach), patients with chronic tenosynovitis, osteomyelitis, patients with marked deformities of hand, patients with recent fracture of scaphoid bone (for snuff box approach) and fractures of bones of medial aspect of the wrist (for trans-palmar approach), patients with ulnar tunnel (Guyon's canal) syndrome (for trans-palmar approach), and patients with hypothenar hammer syndrome (for trans-palmar approach).

Before the procedure, a written and informed consent was taken by first operator.

Local anesthetic for trans-snuff box approach was lidocaine 2% [2-5 ml, subcutaneous (SC)] filling snuff cavity. Anatomically, snuff box artery is deep palmar branch of radial artery (Figure 1).

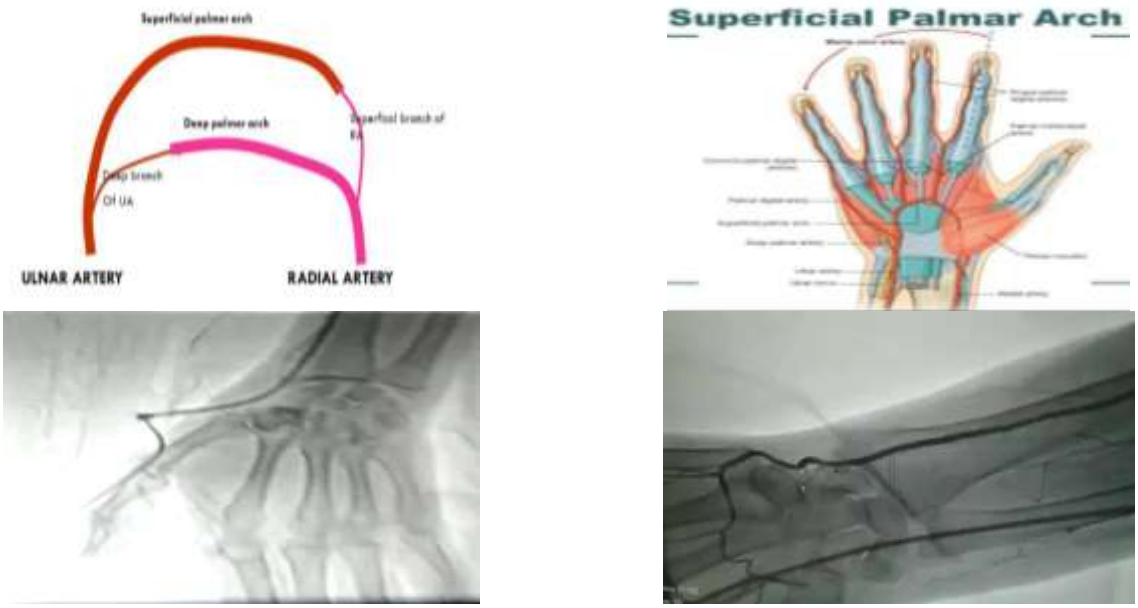

Figure 1. Snuff box artery is deep palmar branch of radial artery and palmar artery is superficial palmar branch of ulnar artery. The figure shows their schematic organization and their radiography.

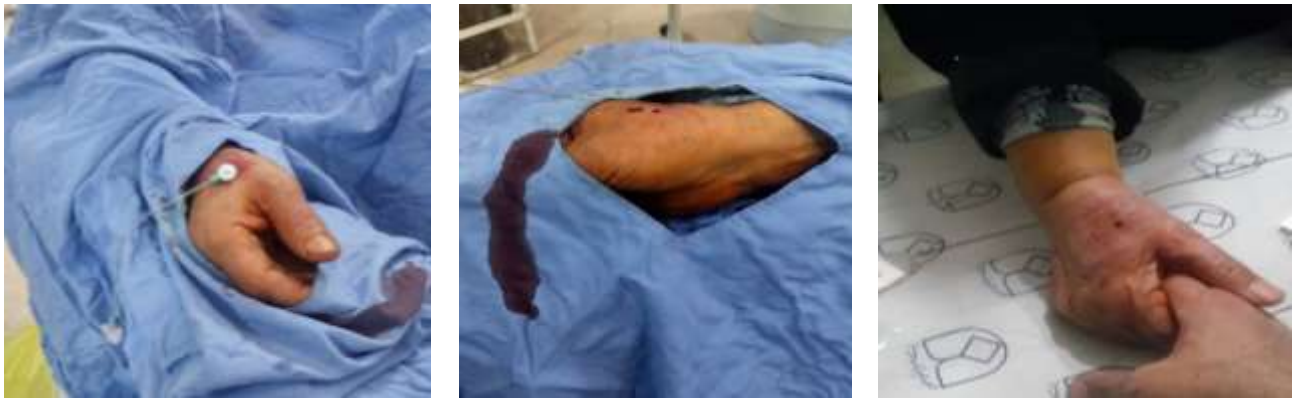

Figure 2. The first successful coronary angiography preformed on deep palmar branch of radial artery in a 65-year-old man in 1 October 2016.

The presence of an appropriate pulse in the anatomical snuff box was verified by manual palpation. We used intravenous (IV) midazolam (1-2 mg) and sublingual trinitroglycerin (TNG) (0.4 mg) in most patients (to minimize patient stress and arterial spasm).

The forearm was positioned on a soft bedding while applying ulnar deviation and semi-flexion position to the wrist (for easy palpation and puncture of snuff artery). Then, the artery was punctured with a 21G needle at an angle of 35-45 degrees. The needle was directed toward the place of strongest pulse. After arterial puncture, we passed 0.018 guide wire gently while holding semi abduction and extension position to the patient wrist (in order to minimize pressure effect of abductor pollicis longus and extensor pollicis brevis tendons). If any resistance was observed, we performed direct dye injection via the needle under fluoroscopic guide to verify the problem. Alternatively, we used 0.014 coronary guide wire in many patients in whom 0.018 inch wire had cross failure. In order to prevent damage to the tip of the introducer and sheath, which might damage the artery, a small skin incision was made, followed by introducing the 4, 5, or 6 F sheath, as needed. In the first 30 cases, we performed sheathogram in order to verify arterial anatomy. Additionally, we also used spasmolytic cocktail containing TNG (250 µg) and verapamil (2.5 mg) intra-arterially. Furthermore, we applied unfractionated heparin (2500-5000 IU) as anticoagulant. For arterial hemostasis, we immediately removed the sheath with local compression using contralateral thumb over puncture site, while other four fingers under patient wrist for 10-15 minutes (for initial hemostasis) and application of pressure using bandage packs for completion of hemostasis (1-2 hours) (Figure 2). Local anesthetic for trans-palmar approach was subcutaneous lidocaine 2% (5 -10 ml) 1 inch proximal to pisiform bone and lateral to the tendon of flexor carpi ulnaris 15 minutes before the procedure. In some patients additional, lidocaine needed which was infiltrated subcutaneously distal to pisiform bone. Anatomically, palmar artery is superficial palmar branch of ulnar artery (Figure 1).

In trans-palmar approach, about 50% of cases were eligible for the operation according to clinical criteria like good palpable pulse. In about 70-80% out of this population, puncture was successful regarding guide wire and sheath insertion. Globally, in about one-third of all patients the procedure would be successful. Criteria of successful procedure were older age, high body mass index (BMI) (obese individuals), athletes, workers, and a history of previous radial occlusion and weak radial pulse in elderly. Technically, palmar artery is highly spastic, and for this reason, it was essential to sedate all the patients before the procedure. For this purpose, we used midazolam (1-2 mg) and sublingual TNG (0.4 mg). Additionally, we used spasmolytic cocktail intra-sheath containing TNG (250 µg) and verapamil (2.5 mg). Besides, we used heparin (2500-5000 IU) as anticoagulant. For this approach, patient's arm was positioned at 45 degrees and the hand was held in mildly extended position (about 20-30 degrees) with avoidance of hyperextension to prevent arterial stretching and collapse. The puncture site (medial aspect of palmar surface 1 cm distal and lateral to pisiform bone and about 1 inch distal to wrist crease) was gently examined for optimal anesthesia with insulin needle and additional lidocaine infiltrated locally (if needed).

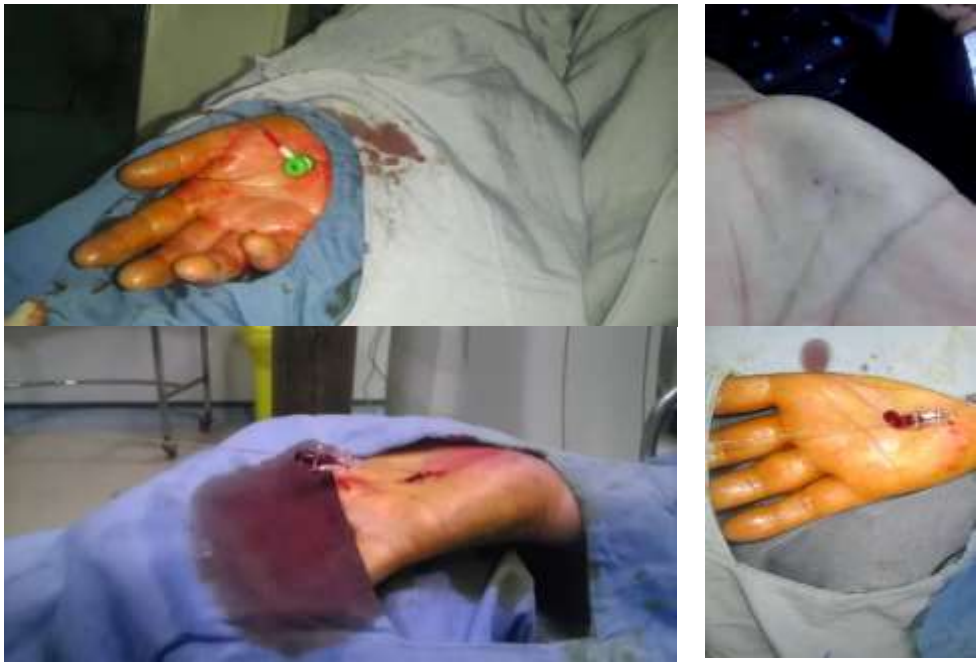

Figure 3. The first successful coronary angiography preformed on right superficial palmar branch of ulnar artery in a 64-year-old man in 15 October 2016.

Superficial palmar artery was punctured by 21G needle, and 0.018 guide wire was passed under fluoroscopy guide (skin incision was crucial before sheath insertion). Then, 4, 5, or 6 F sheath was inserted.

Arterial hemostasis for trans-palmar approach was wrist hyperextension (up to 90 degrees) for 15 minutes followed by local compression thereafter. In a few cases, especially those with PCI and 6f sheaths, we used trans-radial band in ulnar side of distal forearm (Figure 3).

Results

In 221 patients out of 235 ones (94.1%) (men: 76.5%, age: 57.4 ± 10.4 years; women: 23.5%, age: 62.4 ± 9.5 years), our procedure through snuff box artery was successfully conducted. In total, the evaluated patients had ecchymosis in distal forearm (5.1%), asymptomatic snuff artery occlusion (3 cases) that was verified in one case by ipsilateral trans-palmar angiography, no hematoma even one, and no major complications (amputation, infection, thrombosis, need for surgery, hand dysfunction, nerve palsy, and so forth). In 2 patients, we observed asymptomatic RAO during 1-month follow-up period.

In 159 patients out of 175 ones (90.8%) (men: 76%, age: 58.1 ± 10.5 years; women: 24%, age: 61.2 ± 9.6 years), our procedure through palmar artery was successfully performed. Complications for trans-palmar approach included hand ecchymosis (2 cases), hematoma of proximal forearm (5 cases), and hematoma of distal arm (2 cases) that were self-limited in nature with no requirement for further therapy, and had no direct association with puncture at more distal sites. In addition, transient paresis and hyposthesia was observed in 7 cases in terminal branches of ulnar nerve that appeared in 4th and 5th fingers, and completely recovered within 1-2 weeks on follow-up. We had also no event of motor paralysis, cellulitis, and persistent pain. None of the patients with hematoma had major complications (ulnar occlusion, amputation, infection, thrombosis, need for surgery, hand dysfunction, nerve palsy, and so forth). No ulnar (palmar) artery occlusion even one observed.

In addition, PCI was done in 18.2% and 28.9% of cases via palmar and snuff box approaches, respectively. Meanwhile, hemostasis was very fast and convenient for all patients with discharge time equivalent to or even shorter than other upper limb accesses (15-30 minutes in angiography group, and 1-2 hours in angioplasty group). There was no difference in hemostasis time between snuffbox and palmar group.

Discussion

In this novel and innovative research study, we presented new accesses in the upper limb at more distal points in contrast to conventional trans-radial

and trans-ulnar approaches. We also had a follow-up on patients for 3-15 months to check possible complications of these newly presented techniques. Our experience showed the safety and feasibility of more distal accesses in the hand at anatomical snuff box and/or palmar zone. We could enumerate several benefits for these approaches. Firstly, the patient could leave the hospital after several hours with no requirement of hospitalization. Secondly, these approaches were associated with lower risk of upstream artery occlusion at the site of puncture. Thirdly, we did not observe any soft tissue injury at puncture site, damage to the vessel wall by sheath insertion, and possible trauma due to hemostatic procedures. In addition, since we did puncture a smaller and more superficial artery, less time was certainly required for establishment of hemostasis. Of additional advantage, since no extra pressure is exerted on the local veins, no congestion of the hand region is expected. Furthermore, our approaches may be strongly recommended for those cases that show signs of radial and ulnar spasm at more proximal sites. Additionally, if we had a failure in performing these more distal approaches, upstream sites are still preserved for percutaneous coronary intervention. Finally, in the case of proximal arterial occlusions, distal accesses could be used for retrograde angiography and intervention.

However, new techniques have their own limitations, and more time is certainly needed for their performance on a large-scale population with a precisely-designed follow up to check possible benefits, limitations, and complications in long term.

The patients' inconvenience and complications of trans-palmar approach were significantly lower than classic trans-ulnar approach, but the rate of patient eligibility was lower. The failure rate of trans-palmar approach was higher than classic trans-ulnar approach. In the case of access failure, we crossed over to another hand accesses. The main cause of trans-palmar access failure was vascular tortuosity, siphon at distal part of ulnar artery, and spasm, which was negotiated by 0.014 coronary guide wire in many cases during study.

One challenging and important issue with our more distal approaches is that distal arteries are smaller and thinner and for this reason, their puncture needs much more experience and learning curve. Perhaps, our methods may be difficult to be performed by newly trained operators. In contrast to our findings, McNamara et al. reported ischemia of the index finger and thumb secondary to thrombosis of the radial artery in the anatomical snuff box. These researchers ascribed the complications to local inflammation and/or systemic disease.[20] However, there is no reason that our approaches endanger and compromise blood flow to the fingers due to existence of collateral blood vessels.

Regarding postoperative course, recovery and hemostasis was very successful with great comfort and high satisfaction of the patients. Since number of studied cases was rather low, and duration of follow-up was not long, such study with a longer follow-up and on a higher number of cases is strongly warranted. Although our procedures are at their early stages with about a follow-up period of 3-15 months, more researches are recommended to be conducted in forthcoming months and this new innovative approaches could be suggested as safe and reliable with low complications to be used for coronary angiography and/or angioplasty. However, these new approaches require thoughtful decision by operators.

Conclusion

As a conclusion, although these new innovative approaches could be suggested safe, feasible, and reliable to be used for coronary angiography and/or angioplasty with low complications, but they are at their early stages with about a follow-up period of 3-15 months; so, more researches based on large clinical trials are recommended to be conducted in forthcoming months and years.

Acknowledgments

Hereby, we wish to acknowledge Dr. Ferdinand Kiemeneij at Department of Cardiology (Tergooi Blaricum, Blaricum, the Netherlands) for his great scientific support during this study.

Conflict of Interests

Authors have no conflict of interests.

References

1. Liu J, Fu XH, Xue L, Wu WL, Gu XS, Li SQ. A comparative study of transulnar and transradial artery access for percutaneous coronary intervention in patients with acute coronary syndrome. J Interv Cardiol 2014; 27(5): 525-30.
2. Sallam M, Al-Riyami A, Misbah M, Al-Sukaiti R, Al-Alawi A, Al-Wahaibi A. Procedural and clinical utility of transulnar approach for coronary procedures following failure of radial route: Single centre experience. J Saudi Heart Assoc 2014; 26(3): 138-44.
3. Kiemeneij F. Left distal transradial access in the

anatomical snuffbox for coronary angiography (ldTRA) and interventions (ldTRI). EuroIntervention 2017; 13(7): 851-7.
4. Roghani F, Shirani B, Hashemifard O. The effect of low dose versus standard dose of arterial heparin on vascular complications following transradial coronary angiography: Randomized controlled clinical trial. ARYA Atheroscler 2016; 12(1): 10-7.
5. Costa F, van Leeuwen MA, Daemen J, Diletti R, Kauer F, van Geuns RJ, et al. The rotterdam radial access research: Ultrasound-based radial artery evaluation for diagnostic and therapeutic coronary procedures. Circ Cardiovasc Interv 2016; 9(2): e003129.
6. Kumar AJ, Jones LE, Kollmeyer KR, Feldtman RW, Ferrara CA, Moe MN, et al. Radial artery access for peripheral endovascular procedures. J Vasc Surg 2017; 66(3): 820-5.
7. Maniotis C, Koutouzis M, Andreou C, Lazaris E, Tsiafoutis I, Zografos T, et al. Transradial approach for cardiac catheterization in patients with negative Allen's test. J Invasive Cardiol 2015; 27(9): 416-20.
8. Rao SV, Kedev S. Approaching the post-femoral era for coronary angiography and intervention. JACC Cardiovasc Interv 2015; 8(4): 524-6.
9. Roghani-Dehkordi F, Hadizadeh M, Hadizadeh F. Percutaneous trans-ulnar artery approach for coronary angiography and angioplasty; A case series study. ARYA Atheroscler 2015; 11(5): 305-9.
10. Roghani F, Tajik MN, Khosravi A. Compare complication of classic versus patent hemostasis in transradial coronary angiography. Adv Biomed Res 2017; 6: 159.
11. Valsecchi O, Vassileva A, Musumeci G, Rossini R, Tespili M, Guagliumi G, et al. Failure of transradial approach during coronary interventions: Anatomic considerations. Catheter Cardiovasc Interv 2006; 67(6): 870-8.
12. Dehghani P, Mohammad A, Bajaj R, Hong T, Suen CM, Sharieff W, et al. Mechanism and predictors of failed transradial approach for percutaneous coronary interventions. JACC Cardiovasc Interv 2009; 2(11): 1057-64.
13. Biondi-Zoccai G, Sciahbasi A, Bodi V, Fernandez-Portales J, Kanei Y, Romagnoli E, et al. Right versus left radial artery access for coronary procedures: an international collaborative systematic review and meta-analysis including 5 randomized trials and 3210 patients. Int J Cardiol 2013; 166(3): 621-6.
14. Dahal K, Rijal J, Lee J, Korr KS, Azrin M. Transulnar versus transradial access for coronary angiography or percutaneous coronary intervention: A meta-analysis of randomized controlled trials. Catheter Cardiovasc Interv 2016; 87(5): 857-65.
15. Gokhroo R, Kishor K, Ranwa B, Bisht D, Gupta S, Padmanabhan D, et al. Ulnar artery interventions non-inferior to radial approach: Ajmer ulnar artery (AJULAR) intervention working group study results. J Invasive Cardiol 2016; 28(1): 1-8.
16. Roghani-Dehkordi F. Merits of more distal accesses in the hand for coronary angiography and intervention. Proceedings of the 4th International Cardiovascular Joint Congress in Isfahan; 2016 Nov. 24-25; Isfahan, Iran.
17. Kaledin A, Kochanov I, Podmetin P, Ardeev VN. Distal radial artery in endovascular interventions [Online]. [cited 2017]; Available from: URL: https://www.researchgate.net/publication/319162208
18. Babunashvili A. Novel snuff-box technique for trans-radial approach: Let's go distal. Proceedings of the Aim-Radial 2016; 2016 Sep. 23-22; Budapest, Hungary.
19. Latsios G, Toutouzas K, Synetos A, Vogiatzi G, Papanikolaou A, Tsiamis E, et al. Left distal radial artery for cardiac catheterization: Insights from our first experience. Hellenic J Cardiol 2018.
20. McNamara MG, Butler TE, Sanders WE, Pederson WC. Ischaemia of the index finger and thumb secondary to thrombosis of the radial artery in the anatomical snuffbox. J Hand Surg Br 1998; 23(1): 28-32.

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Trifascicular block as primary presentation of the cardiac amyloidosis; A rare case report

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Case Report

Abstract

BACKGROUND: Amyloidosis is a severe systemic disorder produces by the accumulation of inappropriately amyloid deposition in tissues. Cardiac involvement, as a main type of amyloidosis, has a major impact on prognosis. We describe a biopsy-proven cardiac amyloidosis in an old man with unexpected presentation.

CASE REPORT: A 70-year-old man, with a complaint of severe weakness, lightheadedness, and lower limb paresthesia, was admitted to the emergency department. Electrocardiography revealed right bundle branch block and Trifascicular block. Echocardiography study showed a moderately increased thickness of left ventricular wall with concentric pattern as well. Laboratory investigations including serum and urine electrophoresis, and serum free light chain examination as immunofixation assay revealed that κ chains predominated over λ chains in a ratio of 3:2. Our patient with final diagnosis of amyloid light-chain (AL) amyloidosis underwent chemotherapy with melphalan combined with high-dose dexamethasone, CPHPC and monoclonal antibodies for 2 weeks.

CONCLUSION: It shows that rapid diagnosis of AL amyloidosis can enhance the prognosis. Applying an optimal strategy for the treatment leads to effective therapy, too.

Keywords: Amyloidosis, Bundle Branch Block, Echocardiography

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Introduction

Cardiac amyloidosis is determined as dramatically extracellular infiltration of amyloid in the heart.[1]

The predominant organ affected by depositions of amyloids is the heart; however, in some types of this disease, isolated heart involvement can occur. Anatomical distributions of amyloid deposition in the heart include atria, ventricles, and perivascular space as well as valves and conduction system.[2]

Amyloidosis in senile patients affects some organs such as liver, gastrointestinal tract, bone marrow, upper gastrointestinal tract, and endocrine glands; but, the dramatically clinical appearance of cardiac amyloidosis in this patients are very rare.[3]

Hereby, we present an old man with cardiac amyloidosis presented only with Trifascicular block, with early diagnosis and successful management.

Case Report

A 70-year-old man patient weighing 55 kg was admitted to emergency department with a complaint of severe weakness, lightheadedness, and lower limb paresthesia. Before this admission, he also presented worsening of symptoms in the last two weeks. He had no family history of cardiovascular disease, sudden cardiac death, or syncope.

On admission, hemodynamic parameters were in acceptable condition. He had blood pressure of 79/99 mmHg, heart rate of 57 beats/minute, and respiratory rate of 20 breath/minute, and he was also afebrile. Oxygen saturation, at rest, with finger pulse oximeter in index finger was 95%.

Primary physical examination was also done and we found the S4 sound in the apex, in cardiac auscultation. He was in class 2 of the New York Heart Association (NYHA) Functional Classification. Laboratory findings showed a mild hypochromic

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microcytic anemia [hemoglobin = 10.9 g/dl, hematocrit = 30.3%, mean corpuscular volume (MCV) = 73.09 fl, and mean corpuscular hemoglobin (MCH) = 21.67 pg]. He had degrees of hypothyroidism with thyroid stimulating hormone (TSH) of 20 mU/l and serum thyroxine (T4) of 2.4 µg/dl.

Electrocardiography (ECG) showed right bundle branch block (RBBB) with right axis deviation and complete atrioventricular block that diagnosed as a Trifascicular block (Figure 1).

Figure 1. 12-leads electrocardiogram (ECG) of the patient after admission
There is right axis deviation, left bundle branch block, and complete atrioventricular block revealed as Trifascicular block. Secondary ST-T change is also present.

The patient referred to echocardiography unit and transthoracic echocardiography showed a moderate increase in thickness of left ventricular (LV) wall as a concentric pattern and LV diastolic dysfunction (grade 2). The ejection fraction of LV (LVEF) was 55%. Echocardiography revealed normal right ventricle size and function as well [tricuspid annular plane systolic excursion (TAPSE) = 1.9 cm] (Figure 2). Cardiac biomarkers revealed that troponin I was negative with elevated N-terminal prohormone of brain natriuretic peptide (NT-ProBNP) equal to 288 pg/ml.

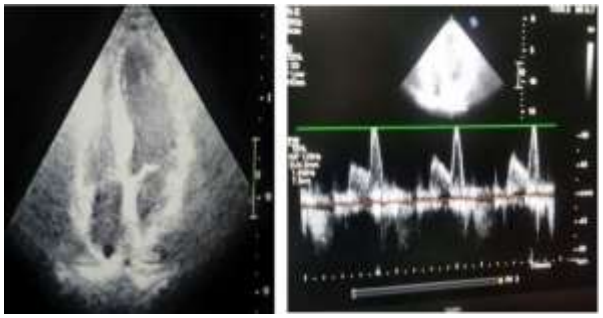

Figure 2. Apical four-chamber view echocardiography
Left ventricular (LV) thickening free wall is seen. No evidences of both dilated atria and interatrial septum are seen. Any evidences of thickening of mitral and tricuspid valve cusps are not seen as well.

Regard to severe weakness and paresthesia, he referred to neurology department for electrodiagnostic study. The electrodiagnostic evaluation revealed absent sensory nerve action potential (SNAP) with low amplitude compound muscle action potentials (CMAPs) of upper and lower limbs. These findings were compatible with chronic sensory-motor polyneuropathy with axonal features, and evidence of ongoing axonal loss.

According to the sustained anemic situation and severe weakness, the patient underwent bone marrow aspiration. Microscopic evaluation of bone marrow aspiration revealed elevated cellularity with myeloid hyperplasia to erythroid hypoplasia ratio of 3:1, and complete maturation. Plasma cells were 22% of all nucleated cells. This finding was compatible with plasma cell myeloma.

Laboratory investigations including serum and urine electrophoresis and serum free light chain examination as immunofixation assay were also done. The results showed that kappa (κ) chains predominated over lambda (λ) chains in a ratio of 3:2. The hepatic test showed elevated alkaline phosphatase value equal to 215 U/l.

Ultimately, by considering all clinical aspects and paraclinical investigations mentioned above, final diagnosis for the patient was amyloid light-chain (AL) cardiac amyloidosis.

Immediately, the targeted therapy was established. Regarding clinical and paraclinical parameters, the patient received melphalan combined with high-dose dexamethasone. Concurrently, immunotherapeutic strategy with CPHPC and monoclonal antibodies were also prescribed. The patient closely monitored for 2 weeks and during this treatment period, he showed hematologic and organ response to the therapy. After this period, kappa to lambda ratio in urine immunofixation electrophoresis was normal, and serum level had a negative result. Heart response included no change in normal left ventricular ejection fraction (55 %), enhance the NYHA class of 2 to 1 without using any medications such as diuretic, and no wall thickness enhancement. NT-ProBNP was in normal range (100 pg/ml). The hepatic test revealed that alkaline phosphatase value had a dramatic decrease in 101 U/l (more than 50%).

After completion of treatment, the patient was discharged and advised to follow his situation and disease by referring to heart clinic every 2 weeks until 6 months, and referring electrophysiologist to consult the indications of an implantable cardiac defibrillator.

Discussion

Cardiac amyloidosis might possibly be related to the inclusion of different organs. The presence of different type features of the clinical and paraclinical evidence shall almost enhance the hesitancy for this disorder. These findings prompt a stepwise indicative process to confirm cardiac amyloidosis.[3] Early diagnosis is a key requisite for successful treatment of AL amyloidosis allowing insulation of the organ damage, and safety treatment to avoid toxicity.[4] The target of common therapies is to put down the pathological plasma cells, and to remove misfolded free light chains. The treatment with this approach can lead to a relapse of amyloid deposits with yield organ enhancement and long-term survival.[5]

Clinical detections in patients with cardiac amyloidosis may had an extensive range of features involving many organs. In AL amyloidosis, there is a massive amount of possible extracardiac findings such as macroglossia, periorbital purpura, and petechial lesions of eyelids due to vascular fragility, neuropathic conditions, and severe fatigue. Hepatic and renal involvements are usual in AL amyloidosis;[6] but nephropathy is a very scarce presentation. Nervous system involvement results in a progressive sensorimotor neuropathy.[3] In our patient, clinical features on admission were only cardiac conductive disturbances that presented with complete atrioventricular block. Another investigation revealed other manifestations such as neuropathy that presented with severe weakness. This manifestation first seemed to be completely independent, and a massive challenge for the diagnosis.

The most prevalent echocardiographic presentation in cardiac amyloidosis is involvement of the LV wall, merely in the absence of hypertension.[7,8] In our patient, we found moderate concentric LV wall thickening of about 2 cm, without any hypertensive manifestation. This is often referred to incorrectly as "hypertrophy" because the pathological process is infiltration, not myocyte hypertrophy.[7]

The composition of increased LV mass in the absence of high ECG voltages may be due to infiltrative diseases, of which amyloidosis is the most ordinary. In our patient, the ECG was not accompanied by low-voltage and pseudo-infarct pattern; this can be sound in 75% of AL cardiac amyloidosis cases.[7] Moreover, our patient had a conductive abnormality with complete AV block. Conduction abnormality in AL cardiac amyloidosis is an often unusual presentation and this event may be dependent on the risk of sudden death.[9] So, these patients should be closely monitored.

Blood tests represent a crucial part of the assessment in suspected amyloidosis. Nowadays, a serum immunoglobulin free light chain (FLC) assay with quantitative analysis of kappa to lambda ratio has become the most useful laboratory method for establishing the diagnosis, prognostic, and follow-up of AL amyloidosis.[10] In this case, we observed a kappa to lambda ratio of 3:1, which revealed definite diagnostic laboratory test of AL amyloidosis.

Cardiac biomarkers represents by negative troponin I with elevated NT-proBNP in patients with amyloid heart disease.[11] In our patient, NT-proBNP levels were increased asymmetrically to the intensity of symptoms of congestive heart failure (CHF) (NYHA class 2). This may be related to the fact that increase the NT-proBNP levels is not only a result of heart failure, but also reflects hormone production by myocytes that are compressed by extracellular amyloid deposits.[3]

Chemotherapy is withstood poorly in many patients with AL amyloidosis,[7] but our patient had a good outcome with protocol therapy and eventually, he discharged with an acceptable situation after 2 weeks of admission. This may be due to considering the indication criteria for other treatment such as high dose melphalan and autologous stem cell transplant (HDM/SCT), and shift the protocol to melphalan combined with a high dose of dexamethasone. This restricted criterion includes age lower than 65 years old, involvement of two or more organs, NT-proBNP and troponin I levels lower than 35 ng/l and 0.1 ug/l, respectively, LV ejection fraction more than 45%, creatinine clearance more than 50 ml/minute, and systolic blood pressure of 90 mmHg.[12] In our case, all of the mentioned parameters were not seen. So, optimal target therapy in this patient was done.

Despite significant developments in chemotherapy for AL amyloidosis, the prognosis of patients with advanced cardiac involvement remains poor. So, early diagnosis of AL amyloidosis is critical, and this approach leads to effective therapy.

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Conflict of Interests

Authors have no conflict of interests.

References

1. Bunker D, Gorevic P. AA amyloidosis: Mount Sinai experience, 1997-2012. Mount Sinai Journal of Medicine: A Journal of Translational and Personalized Medicine 2012; 79(6): 749-56.
2. Dubrey SW, Hawkins PN, Falk RH. Amyloid diseases of the heart: Assessment, diagnosis, and referral. Heart 2011; 97(1): 75-84.
3. Falk RH. Diagnosis and management of the cardiac amyloidoses. Circulation 2005; 112(13): 2047-60.
4. Fikrle M, Palecek T, Kuchynka P, Nemecek E, Bauerova L, Straub J, et al. Cardiac amyloidosis: A comprehensive review. Cor et Vasa 2013; 55(1): e60-e75.
5. Koyama J, Falk RH. Prognostic significance of strain Doppler imaging in light-chain amyloidosis. JACC Cardiovasc Imaging 2010; 3(4): 333-42.
6. Palladini G, Lavatelli F, Russo P, Perlini S, Perfetti V, Bosoni T, et al. Circulating amyloidogenic free light chains and serum N-terminal natriuretic peptide type B decrease simultaneously in association with improvement of survival in AL. Blood 2006; 107(10): 3854-8.
7. Merlini G, Seldin DC, Gertz MA. Amyloidosis: Pathogenesis and new therapeutic options. J Clin Oncol 2011; 29(14): 1924-33.
8. Mohty D, Damy T, Cosnay P, Echahidi N, Casset-Senon D, Virot P, et al. Cardiac amyloidosis: Updates in diagnosis and management. Arch Cardiovasc Dis 2013; 106(10): 528-40.
9. Nordlinger M, Magnani B, Skinner M, Falk RH. Is elevated plasma B-natriuretic peptide in amyloidosis simply a function of the presence of heart failure? Am J Cardiol 2005; 96(7): 982-4.
10. Palladini G, Merlini G. Current treatment of AL amyloidosis. Haematologica 2009; 94(8): 1044-8.
11. Reyners AK, Hazenberg BP, Reitsma WD, Smit AJ. Heart rate variability as a predictor of mortality in patients with AA and AL amyloidosis. Eur Heart J 2002; 23(2): 157-61.
12. Selvanayagam JB, Hawkins PN, Paul B, Myerson SG, Neubauer S. Evaluation and management of the cardiac amyloidosis. J Am Coll Cardiol 2007; 50(22): 2101-10.

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