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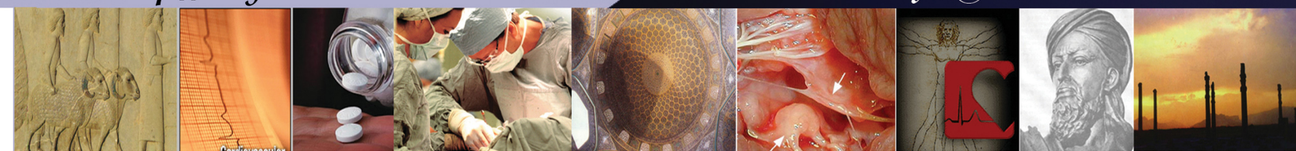

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Trans-snuff box approach as a new access site for coronary angiography and angioplasty versus trans-radial approach in terms of feasibility, safety, and complications

Farshad Roghani-Dehkordi[1], Alireza Riazi[1], Davood Shafie[2], Alireza Khosravi[3], Masoumeh Sadeghi[4], Mehrbod Vakhshoori[2], Soraya Massoudi[5], Mohammad Kermani-Alghoraishi[1]

Original Article

Abstract

BACKGROUND: The feasibility and safety of coronary angiography (CAG) and angioplasty via trans-snuffbox approach (TSA) is still concerned; therefore, in this study, we aimed to assess possible complications occurring after TSA versus trans-radial approach (TRA).

METHODS: This prospective observational study was undertaken from June 2017 till March 2018. Individuals aged at least 18 years who were admitted for CAG through upper extremity were eligible and categorized to TSA (n = 70) and TRA (n = 56) groups. Occurrence of complications including hematoma, pain or paresthesia, pseudoaneurysm formation, arterial obstruction, limb ischemia, and major adverse cardiovascular events (MACE) including death, myocardial infarction (MI), stroke, and emergency vessel revascularization was assessed after the procedure and in two separate visits three and six months afterwards.

RESULTS: The mean age of participants in TSA and TRA groups was 55.1 ± 9.7 and 56.5 ± 9.6 years, respectively (P = 0.415). Men were the dominant group in both approaches [TSA: 44 (62.8%), TRA: 36 (64.3%), P = 0.868]. Success rates in TSA and TRA were 88.6% and 94.6%, respectively (P = 0.230). Radial artery occlusion (RAO) was reported in two (3.2%) and one (1.8%) case in TRA and TSA, respectively (P = 0.653). MACE incidence was not significantly different in TSA compared with TRA group (1.8% vs. 4.8%, respectively, P = 0.389). There was no major procedural complication, neither in TSA nor in TRA category.

CONCLUSION: Our results revealed that TSA could be classified as an alternative modality to other common CAG and angioplasty methods due to its high safety rate and lower complications. Several comprehensive population-based studies are necessary for confirming these findings.

Keywords: Radial Artery; Vascular Access Devices; Coronary Angiography; Coronary Angioplasty

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Introduction

Since the initiation of coronary angiography (CAG) in 1929, this procedure became one of the most common approaches in terms of diagnosis and treatment of coronary artery diseases (CADs).[1] Based on human vascular system, there are several angiographic procedures available including trans-femoral approach (TFA), trans-radial approach (TRA), and trans-ulnar approach (TUA), that each one has its own advantages and disadvantages.[2-6]

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Address for correspondence: Mohammad Kermani-Alghoraishi; Interventional Cardiology Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran; Email: sm.kermani@med.mui.ac.ir

For instance, the most traditional method named TFA was associated with higher rates of complications such as bleeding or patient discomfort versus its advantages of possibility of passing large-bore devices.[6] On the other hand, trans-radial angiography and intervention first reported by Campeau[7] in 1989, and Kiemeneij and Laarman[8] in 1993, respectively, demonstrated to be superior to TFA in terms of multiple strengths including less likelihood of bleeding, decreased hospital stay length, early mobilization, lower costs, and patient satisfaction in a way that it is becoming the first choice for CAG.[4,9,10] A novel CAG method which its safety has been demonstrated in multiple researches is trans-snuffbox approach (TSA). Its anatomical position contained triangular depressed shape on dorsum of hand at levels of carpal bones including scaphoid and trapezium which is also known as radial fossa clearly visible by thumb extension. Extensor pollicis longus (EPL) and extensor pollicis brevis (EPB) bounded medial and lateral borders of this triangle, respectively. This collection consisted of distal radial artery (RA), cephalic vein, and a branch of radial nerve (Figure 1).[11] TSA was previously suggested and introduced by Kaledin et al.[12] in 2014, Babunashvili[13] in 2016, Roghani-Dehkordi[14] in 2016, and Kiemeneij[15] in 2016.

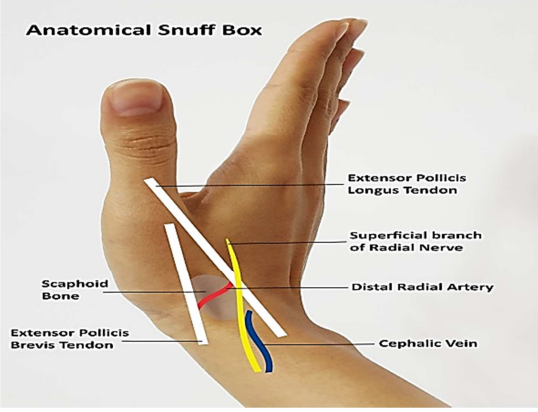

Figure 1. Schematic of anatomical snuffbox

This new technique has some priorities compared with TRA like the followings: early hemostasis due to carpal bones and small distal RA diameter and no requirements for wrist compression, possibility of shifting to TRA in times of TSA failure, less likelihood of compartment syndrome and providing more RA length for candidate patients for coronary artery bypass graft (CABG).[11,16,17] However, this technique is not free from disadvantages and some of them include more needling difficulties, increased chance of nerve irritation, and inability to pass through larger sheaths.[11]

To the best of our knowledge, there are a few studies comparing outcomes of TRA and TSA which revealed controversial results. Due to inconsistencies in results of those aforementioned procedures, in this study, we aimed at evaluating feasibility, safety, and complications of TSA compared to TRA in our centers.

Materials and Methods

This prospective observational study was performed in two distinct referral hospitals (Shahid Chamran Heart Center and Khorshid Hospital) located in Isfahan, Iran. From June 2017 to March 2018, anyone aged more than 18 years who had indications for CAG was eligible to be recruited in our study. Presence of cardiogenic shock with/without hemodynamic instability, suffering from acute coronary syndrome (ACS), being under hemodialysis treatment, past history of CABG, elective candidate for TFA, existence of vascular disorders of upper limb, Raynaud's disease, or hand problems including deformities, osteomyelitis, tenosynovitis, or history of scaphoid fracture were defined as exclusion criteria. Before initiation of procedure, all research aims were explained to each individual and a written consent form was signed by each participant before procedure initiation. A total of 126 cases of CAG were performed through upper extremity; 70 patients underwent TSA and 56 of them were categorized in TRA. This study was approved by the Ethics Committee affiliated to Isfahan University of Medical Sciences (IR.MUI.MED.REC.1397.278).

After preparation of puncture site, 2-2.5 ml of lidocaine 2% was injected subcutaneously in anatomic snuffbox and 3 cm above first proximal wrist crease in TSA and TRA, respectively. Extra intravenous midazolam (1-2 mg) plus 0.4 mg sublingual trinitroglycerin (TNG) was administered in order to decrease arterial spasm and patient stress. A 21-gauge (G) needle was used for puncturing desired artery and 5-6 French sheaths were passed through 0.018-inch guidewires (Figure 2). In cases of failure, 0.014-inch guidewire was utilized alternatively, especially among patients who underwent TSA. Spasmolytic cocktail which consisted of 200-250 micrograms of nitrate and verapamil (2.5 mg) was introduced through the sheaths. Unfractionated heparin [2500-5000 international unit (IU)] was used as an anticoagulant in both groups. After TSA termination, the first 10 to 15 minutes of initial hemostasis was

accomplished by local pressure using contralateral thumb over puncture site and other four fingers below the patients' wrist with addition of appropriate bandage packs for completion of hemostasis in the next 1-2 hours. TR band was used for inducing hemostasis after sheath extraction in individuals who underwent TRA.

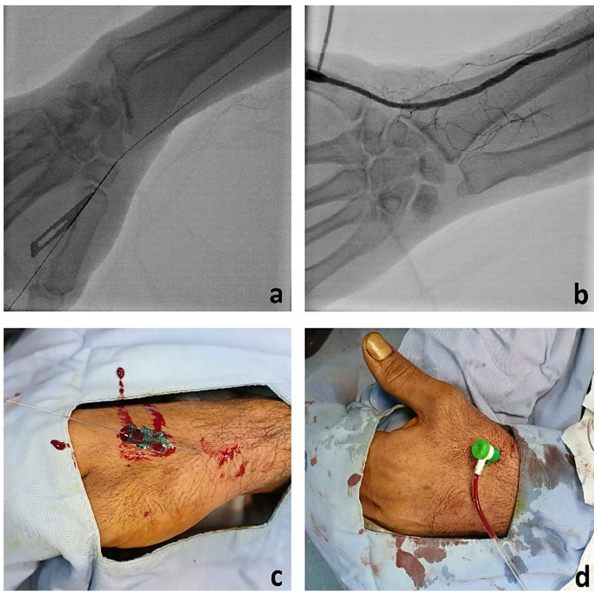

Figure 2. Angiographic depiction of the snuffbox approach (a, b) and cannulation of distal radial artery (RA) in anatomical snuffbox (c, d)

Age, sex, body mass index (BMI), presence/absence of hypertension (HTN), diabetes mellitus (DM), dyslipidemia, smoking status, family history of cardiovascular diseases (CVDs), and past history of myocardial infarction (MI) were recorded in the checklist.

Correct arterial cannulation in each group was defined as successful access. Furthermore, properties of each approach were assessed by measurement of pre-defined variables including access and total procedure time plus fluoroscopy time as well as contrast volume. Complications including hematoma, pain/paresthesia, pseudoaneurysm formation, arterial obstruction, limb ischemia, and major adverse cardiovascular events (MACE) including death, MI, stroke, and emergency vessel revascularization were assessed after procedure and in two distinct clinical visits three and six months apart by the same interventional cardiologist.

Continuous and categorical variables were reported as mean ± standard deviation (SD) and frequencies with percentages, respectively. The basic characteristics and procedural properties were compared between groups. The comparisons were made using the independent t-test (quantitative variables) and chi-square test (qualitative variables). SPSS software (version 15.0, SPSS Inc., Chicago, IL, USA) was used for all analyses and P-values less than 0.05 were considered statistically significant.

Results

The mean age of our study population in TSA and TRA group was 55.1 ± 9.7 and 56.5 ± 9.6 years, respectively (P = 0.415). Men were the dominant group in both approaches [TSA: 44 (62.8%), TRA: 36 (64.3%), P = 0.868]. In 70 patients who underwent TSA, we had a successful access rate of 88.6% (n = 62), while this percentage was 94.6% for TRA (53 out of 56 cases) (P = 0.230). Table 1 provides data of general characteristics of individuals according to distinct arterial approach.

Table 1. Basic characteristics of participants in trans-snuffbox and trans-radial methods

Variables	TSA (n = 70)	TRA (n = 56)	P
Age (year)	55.1 ± 9.7	56.5 ± 9.6	0.415
BMI (kg/m^2)	28.4 ± 3.3	27.2 ± 3.9	0.074
Gender (male)	44 (62.8)	36 (64.3)	0.868
HTN	40 (57.1)	38 (67.8)	0.218
DM	52 (74.2)	45 (80.3)	0.421
Dyslipidemia	22 (31.4)	16 (28.6)	0.728
Current smoker	47 (67.1)	44 (78.6)	0.154
Family history of CVDs	3 (4.3)	6 (10.7)	0.163
History of previous MI	13 (18.6)	10 (17.8)	0.917
CAG success	62 (88.6)	53 (94.6)	0.230
PCI	35 (50.0)	20 (35.7)	0.108

Values are expressed as mean ± standard deviation (SD) for quantitative variables and number (%) for qualitative variables. Chi-square test and independent t-test were used where appropriate.
TSA: Trans-snuffbox approach; TRA: Trans-radial approach; BMI: Body mass index; HTN: Hypertension; DM: Diabetes mellitus; CVD: Cardiovascular disease; MI: Myocardial infarction; CAG: Coronary angiography; PCI: Percutaneous coronary intervention

Table 2. Procedural properties of trans-snuffbox approach (TSA) and trans-radial approach (TRA)

Variables		TSA (n = 62)	TRA (n = 53)	P
Access time (minute)		4.75 ± 0.46	4.65 ± 0.65	0.419
Total procedure time (minute)	CAG	17.50 ± 5.10	16.90 ± 4.80	0.333
	PCI	32.50 ± 8.30	33.40 ± 7.70	0.432
Fluoroscopy time (minute)	CAG	5.50 ± 1.10	5.10 ± 1.80	0.451
	PCI	10.60 ± 1.90	10.10 ± 2.80	0.265
Contrast volume (ml)	CAG	122.00 ± 20.00	127.00 ± 15.00	0.764
	PCI	256.00 ± 22.00	250.00 ± 19.00	0.523

Values are mean ± standard deviation (SD). Independent t-test was used.
TSA: Trans-snuffbox approach; TRA: Trans-radial approach; CAG: Coronary angiography; PCI: Percutaneous coronary intervention

Although participants who underwent TSA had higher BMI means (28.4 ± 3.3 vs. 27.2 ± 3.9), their differences was not statistically significant (P = 0.074). There were no remarkable differences between the groups in term of HTN, DM, dyslipidemia, smoking status, CVDs family history, or previous MI experience. 35 (50.0%) of TSA and 20 (35.7%) of TRA patients underwent percutaneous coronary intervention (PCI) in addition to CAG.

Procedural characteristics of TSA and TRA, as depicted in table 2, were the same and no significant differences were found in terms of access and total procedure duration or fluoroscopy time as well as contrast volume between two groups.

In three- and six-month follow-up duration, there were no adverse complications including hematoma, pain or paresthesia, pseudoaneurysm formation, and limb ischemia in neither TSA nor TRA groups as well as in-hospital period. Radial artery occlusion (RAO) was found in two (3.2%) and one (1.8%) patient in TRA and TSA, respectively (P = 0.653). The only relation found in terms of MACE (death, MI, stroke, and emergency vessel revascularization) was mostly observed in patients who underwent TRA in comparison to TSA [3 (4.8%) vs. 1 (1.8%), respectively] that was non-significant (P = 0.389).

Discussion

The ultimate aim of the current study was evaluating the relation between TSA and TRA in terms of complications. Our findings revealed that there was not any incidence of hematoma, pain or paresthesia, pseudoaneurysm formation, and limb ischemia. MACE occurred less frequently in patients who underwent TSA comparing to TRA group. Since this approach was associated with lower rates of complications, it could be utilized as an alternative modality on their own or in cases of failure of other arterial approaches. Several studies were in agreement with our outcomes. For instance, 100 patients were divided equally into two groups and underwent CAG with either TRA or TSA. Their final outcomes showed that patients with experience of TRA had higher prevalence of hematoma, numbness, and spasm and stayed in hospital for a longer duration (P < 0.001).[18] Likewise, 54 individuals with mean age of 59.3 years were recruited in a study in order to evaluate the feasibility of CAG with left snuffbox artery. In addition to 100% success rate, no cases of RAO, hand paresthesia, or hematoma were reported.[19] Similar findings were also found in 94% (142 out of 151 cases) of successful snuffbox approaches showing no ischemic adverse events with an exception of mild bleeding reported in only one subject.[20] Roghani-Dehkordi et al. performed the aforementioned approach on 235 individuals with a success rate of 94.1%. Their results declared that complications including distal forearm ecchymosis and RAO were observed in 5.1% and 0.9% of patients, respectively, with no reports of hematoma or major cardiovascular events including infection, need for amputation, nerve palsy, or hand dysfunction.[16] With an 88% success rate in CAG or angioplasty with left snuffbox artery performed on 150 individuals with mean age of 65.9 ± 12.8 years with dominant male gender, Kim et al. found that except forearm bruising and edema observed in 4.9% of patients which healed conservatively, no major vascular adverse events occurred.[21]

On the other hand, few studies revealed opposing results especially in terms of procedure process. Aoi et al. performed a retrospective study in order to investigate the association of distal RA approach and conventional approach based on safety and complications. They enrolled 202 individuals with distal RA approach and 206 subjects with traditional TRA as control group. Their outcomes suggested that in spite of more than 90% of successful access rate in each group, distal RA approach was associated with prolonged access time and more prevalence of minor bleeding at puncture site compared to conventional RA access

($P < 0.001$) with no considerable differences in terms of hematoma formation ($P = 0.770$).[22] Moreover, Koutouzis et al. randomly assigned 200 individuals prepared for CAG into two distinct groups of TSA and TRA and evaluated their outcomes according to modality itself and their complications. Their results revealed that although there were no statistically significant differences in terms of their pre-defined complications including RAO, hematoma, or vascular spasm, patients who underwent TSA had less successful access rate, prolonged access time, and higher rates of attempts and skin punctures comparing to traditional approach (30% vs. 2%, $P < 0.001$, 269 ± 251 seconds vs. 140 ± 161 seconds, $P < 0.001$, 6.8 ± 6.2 vs. 3.4 ± 4.5, $P < 0.001$, and 2.4 ± 1.7 vs. 1.6 ± 1.2, $P < 0.001$, respectively).[23]

In addition to implementation of both TSA and TRA by expert physician in this regard, reasonable follow-up duration enabling us to assess occurrence of probable complications in each group by the same interventional cardiologist were some strengths of this study. By the way, quite small sample size which might influence the generalization of our findings would be presumed as a limitation in this research project. Furthermore, we did not gather data about each modality itself which might be effective in concluding feasibility of procedures.

Conclusion

Our findings suggested that TSA could be categorized as an alternative CAG method compared to other previously-announced methods due to its high safety and few complications. Multiple comprehensive population-based studies are required for proving these outcomes.

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Conflict of Interests

Authors have no conflict of interests.

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Normal and reconstituted high-density lipoprotein protects differentiated monocytes from oxidized low-density lipoprotein-induced apoptosis

Navkiran Kaur[1], Savita Kumari[2], Sujata Ghosh[3]

Original Article

Abstract

BACKGROUND: The progression of atherosclerosis is an ongoing struggle between cell division and cell death. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), a novel receptor for oxidized low-density lipoprotein (ox-LDL), mediates ox-LDL-induced apoptosis of monocytes. The anti-atherogenic function of plasma high-density lipoprotein (HDL) includes the ability to inhibit apoptosis of macrophage, although the exact mechanism and consequences of apoptosis in the development and progression of this disease are still controversial. Thus, in the present study, the effect of normal HDL (nHDL) and reconstituted HDL (rHDL) on ox-LDL-induced cellular responses in differentiated monocytes in view of apoptosis and LOX-1 receptor expression was investigated.

METHODS: The expression of B-cell lymphoma-2 (Bcl-2), B-cell lymphoma-extra large (Bcl-xL), caspase-3, and cytochrome c (cyt c) was assessed and substantiated in 30 hyper-LDL and control subjects. To assess the expression of LOX-1 on differentiated THP-1 cells, western blotting was carried out, followed by statistical analysis in 30 patients and control subjects.

RESULTS: nHDL/rHDL inhibited the ox-LDL-induced apoptosis in the differentiated human monocytic cells, THP-1 cells, and differentiated monocytes of patient and control subjects. Enhanced expression of scavenger receptor, LOX-1, in the differentiated monocytes was also downregulated in presence of nHDL/rHDL. nHDL/rHDL could inhibit the ox-LDL-induced mitochondrial apoptotic pathway and aberrant expression of LOX-1 in patients. Double immunostaining using fluorescein isothiocyanate (FITC)-conjugated ox-LDL and LOX-1 in apoptotic cells indicates its significant role in the differentiated monocytes.

CONCLUSION: It was observed that nHDL/rHDL could promote macrophage survival by preserving mitochondrial integrity from ox-LDL-induced apoptosis.

Keywords: Atherosclerosis; Lipoproteins; Macrophages; Apoptosis

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Introduction

Atherosclerosis involves the deposition of fatty substances inside the artery walls, which causes thickening and hardening of the arteries. Such conditions affect circulation, causing high blood pressure, ultimately leading to angina, heart attack, stroke, and/or sudden cardiac death.[1] The initial phase of atherogenic process involves low-density lipoprotein cholesterol (LDL-C) accumulation, generation of reactive oxygen species (ROS), and oxidative modification of LDL-C. Exposure of macrophages to oxidized LDL (ox-LDL), a major component of atherosclerotic plaques, appears to be a key event in this process, promoting both inflammation and intracellular cholesterol deposition with the formation of lipid-laden foam cells.[2]

Apoptotic macrophages have been found to be concentrated in the areas of plaque rupture. The death of macrophages in atherosclerotic lesions is multifactorial and strongly correlated to the developmental stage of the atherosclerotic plaques.[3]

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Loss of macrophages can also lead to the accumulation of apoptotic bodies, which can result in the activation of inflammatory mediators.[4]

Serum high-density lipoprotein cholesterol (HDL-C) level is an independent predictor of risk for cardiovascular events in both men and women. HDL particles have been reported to exhibit various anti-atherogenic and cardioprotective effects by modulating the function of various cells including the cells of the artery wall.[5] Thus, the biological role of such potentially important molecules in scavenger receptor-mediated ox-LDL-induced apoptosis of differentiated monocytes needs further evaluation in view of cellular modulation.

Materials and Methods

Blood samples (15 ml) were obtained from 30 cases of age- (30-60 years old) and sex-matched hyper-LDL patients with hypertension (HTN) and subjects with normal LDL (nLDL) (control) diagnosed and managed by Department of Internal Medicine, Post Graduate Institute of Medical Education and Research, Chandigarh, India, in 2010. Patients having diabetes mellitus (DM), smoking habit, renal insufficiency, hyper/hypothyroidism, nephritic syndrome, and acute myocardial infarction (MI) within one year (single/combination) were excluded from this study. Peripheral blood mononuclear cells (PBMC) were separated from blood of each patient and control subject by Ficoll-Histopaque density gradient centrifugation.[6] A formal written informed consent for this study was obtained from each patient. The study followed all ethical guidelines and was approved by the institutional ethical committee (No.:5/4/1-8/2004-NCD-II).

Human monocytic leukemic cell line THP-1 [National Centre for Cell Science (NCCS), Pune, India] and total PBMCs (obtained from blood samples of patients/control subjects) were cultured separately in RPMI-1640 (ICN, USA) supplemented with 2 mM glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, and 10% heat-inactivated fetal calf serum at 37 ^{o}C in a humidified atmosphere of 5% carbon dioxide (CO_2)-95% air. Cell viability was assessed by the exclusion of 0.02% trypan blue dye.[7] The monocytes were differentiated in the presence of phorbol-12 myristate-13-acetate (PMA).[8]

Preparation of lipoproteins: Normal HDL (nHDL) (d = 1.125-1.210 g/ml) and nLDL (d = 1.019-1.250 g/ml) were isolated from human plasma obtained from blood bank of Post Graduate Institute of Medical Education and Research.[9] For purification of apolipoprotein A1 (apo A-I), nHDL was delipidified.[10] The protein part of the delipidified HDL was resolubilised in 50 mM glycine/4 mM sodium hydroxide (NaOH) (pH = 8.8)/500 mM sodium chloride (NaCl)/6 M urea and was fractionated by gel filtration chromatography on Sephacryl S-200 column (1.7 × 82 cm).[10] The collected fractions (each 3 ml) were monitored for the presence of protein at 280 nm. The fraction containing apo A-I was further purified by gel filtration chromatography on Sephacryl S-300 column in the fast protein liquid chromatography (FPLC) (Pharmacia, Sweden). The protein was estimated by bicinchoninic acid (BCA) assay[11] and the molecular weight (M_r) of apo A-1 was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).[12,13] The specificity of apo A-I to anti-apo A-I was assessed by enzyme-linked immunosorbent assay (ELISA).[14] The purified apo A-I and phosphatidyl choline (PC) were used to prepare reconstituted HDL (rHDL).[11] The LDL fraction was oxidized[15] and oxidation of LDL was confirmed by the measurement of thiobarbituric acid reactive substances (TBARS).[16] Ox-LDL was conjugated separately to horseradish peroxidase (HRP) and fluorescein (FLUOS) using Peroxidase Labeling Kit and Fluorescein Labeling Kit (Roche Biochemicals, Germany), respectively, as per manufacturer's instructions.

Apoptosis: The optimum dose and time of PMA for differentiation of THP-1 cells were selected in view of deoxyribonucleic acid (DNA) fragmentation analysis using DNA Ladder Kit (Roche, Germany). Briefly, THP-1 cells (10^6/ml media) were cultured in 6 well plates with different doses of PMA (5 nM-100 nM) for 24 hours. In a separate set of experiments, THP-1 cells were cultured with optimum dose of PMA for different time periods (6-24 hours). DNA was isolated as per manufacturer's instructions, analyzed by agarose gel (1.8%) electrophoresis and visualized under ultraviolet (UV) light in the gel documentation system (Bio-Rad Laboratories, USA). Cells cultured without PMA under the same conditions were taken as control.

For selection of optimum dose of ox-LDL, PMA-differentiated THP-1 cells (0.25 × 10^6/500 µl serum free media/well) were cultured in presence of different doses of ox-LDL (25-100 µg/ml) for 24 hours. In another set of experiment, PMA-differentiated THP-1 cells were triggered in presence of optimum dose of ox-LDL for different time intervals (12 hours, 18 hours, and 24 hours). After washing, the cells were fixed in 1% paraformaldehyde solution and fluorescein isothiocyanate (FITC)-deoxyuridine triphosphate (dUTP) staining was performed using Apo-Direct

Kit (Roche, Germany). PMA-differentiated cells cultured under the same conditions without ox-LDL as well as controls supplied with the kit were run in parallel. The cells were analyzed by flow cytometry (FC) using CellQuest software program.

The optimum dose and time of nHDL/rHDL for treatment of PMA-differentiated THP-1 cells (0.25×10^6 cells/500 µl media/well) cultured in absence and in presence of ox-LDL were selected in view of apoptotic index using the Cell Death Detection ELISA Kit (Roche, Germany).

Experimental groups: Based on the selection of optimum dose and time for different triggering agents used in the present study, all the parameters were studied in four different groups. Group a consisted of PMA-differentiated cells (THP-1 cells as well as cells from patients and control subjects), group b consisted of the differentiated cells cultured in presence of ox-LDL, and group c and group d consisted of the differentiated cells preincubated with nHDL and rHDL, respectively, followed by triggering with ox-LDL.

Reverse transcription polymerase chain reaction (RT PCR) analysis: Total ribonucleic acid (RNA) was extracted from all groups of THP-1 cells using the Qiagen RNA/DNA Mini Kit (Qiagen, Germany). The quantitation of RNA/DNA was done spectrophotometrically. The level of expression of various pro- and anti-apoptotic transcripts [B-cell lymphoma-2 (Bcl-2)-associated X-protein (BAX), Bcl-2, Bcl-extra large (Bcl-xL), caspase 3, and cytochrome c (cyt c)] was assessed by RT-PCR using primer pair specific to respective transcripts and Titan One-Step RT-PCR Kit (Sigma Aldrich, United States). The amplified transcripts were analyzed by agarose gel electrophoresis (1.8%). The changes in band intensity in case of each parameter were quantified by scanning densitometry using Scion Image 4.3 software (Bio-Rad, USA). Variations in the complementary DNA (cDNA) concentration were normalized by co-amplification with β-actin in each set. The increase/decrease in the expression of each parameter in the cells of group b→d was calculated by taking the normalized value of the same transcript as in cells of group a.

Western blot analysis: THP-1 cells (10^6/ml) from each group were lysed with lysis buffer 10 mM HEPES (pH = 7.5)/150 mM NaCl/10% glycerol/10 mM sodium orthovanadate ($NaVO_4$)/0.2% Triton X-100/cocktail protease inhibitors (1:10, Roche, Germany). The debris was removed by centrifugation (10000 rpm, 10 minutes). After estimation of the protein content, equal amount (60 µg) of each lysate was subjected to 12% SDS-PAGE. The protein bands were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (0.45 µm, Immobilon-P, Millipore Eschborn, Germany) at 80 V for 2 hours at 4 °C,[17] followed by incubation in blocking buffer 5% skim milk in phosphate buffered saline (SM-PBS) containing 0.1% Tween-20 at 4 °C for overnight and washing with PBST (PBS containing 0.1% Tween-20). Membranes were probed with antibodies to Bcl-2 (N-19, 1:500), BAX (N-20, 1:500), cyt c (C-20, 1:250), caspase-3 (N-19, 1:250), and β-actin (AC-15, 1:3000, taken as internal control) for 2 hours at 37 °C. After extensive washing, the blots were incubated with the respective HRP-conjugated secondary antibody (1:1500) for 1 hour at 37 °C. After washing, the membranes were developed with 3,3'-diaminobenzidine (DAB) tetrahydrochloride/hydrogen peroxide (H_2O_2), except for cyt c. Cyt c was detected with enhanced chemiluminescence (ECL) plus Western blotting detection system (Amersham, UK). Antibodies were procured from Santa Cruz Biotechnology (CA, USA).

Assessment of apoptosis in differentiated monocytes: Total PBMCs of each blood sample were cultured with PMA with percentage of monocytes to be 0.7%-0.8% of total PBMCs. The extent of apoptosis in the monocytes/macrophages obtained from a→d groups of patients and control subjects was assessed by Cell Death Detection ELISA (CDD ELISA).

Expression of BAX and Bcl-2 in differentiated monocytes: To assess the expression of BAX and Bcl-2 in the monocytes/macrophages obtained from a→d groups of patients and control subjects, PBMCs from each group were harvested separately with PBS-ethylenediaminetetraacetic acid (EDTA), washed and fixed in 1% paraformaldehyde (20 minutes, 4 °C). After washing, cells were incubated in permeabilization buffer for 10 minutes and centrifuged (1200 rpm, 5 minutes). Subsequently, cells were treated with primary antibody against BAX (1:20)/Bcl-2 (1:50) for 1 hour, washed, and incubated with fluorochrome-conjugated respective secondary antibody (1:1000) for 30 minutes. After washing, cells resuspended in PBS and monocytes/macrophages were analyzed by FC using CellQuest software program. Result was expressed in view of mean fluorescence intensity (MFI) of the FITC-positive cells in each group. In each set, a negative control was used in order to rule out non-specificity.

Assessment of lectin-like oxidized LDL receptor-1 (LOX–1) expression on differentiated monocytes: To assess the expression of LOX-1 on differentiated THP-1 cells (group a→d), membrane fraction of

the cells from each group was prepared and subjected to Western blotting.[17,18]

Expression of LOX-1 on differentiated monocytes obtained from a→d groups of patients and control subjects was assessed by FC. Total PBMCs isolated from each blood sample were cultured separately in presence of PMA. Cells were harvested and incubated with FITC-conjugated ox-LDL for 1 hour at room temperature. After washing, cells were analyzed by FC. The result was expressed in view of the MFI of the FITC-positive cells in each group. In each set, a negative control was used to rule out irrelevant specificity.

Statistical analysis for normally-distributed parametric tests was performed by paired t-test/independent t-test as per requirement, and further, in patient samples, correlation and regression analysis was done.

Results

nHDL (d = 1.125-1.210 g/ml) and nLDL (d = 1.019-1.250 g/ml) were isolated and purified, purification of apoliporotein A-I, nHDL was delipidified and the protein part of the delipidified HDL was resolubilised followed by gel filtration chromatography on Sephacryl S-200 column (1.7 × 82 cm).

Purification and characterization of apo A-I: Four different types of lipoproteins (nLDL, ox-LDL, nHDL, and rHDL) were used in the present study. The apo A-I was purified from HDL by sequential gel filtration chromatography on Sephacryl S-200 column and Sephacryl S-300 HR16/60 column. The M_r of the purified apo A-I was confirmed in SDS-PAGE, in which it migrated as a single band of 29 kDa. The ELISA titer of anti-apo A-1 was 1:10000 with 15.6 ng of the purified apo A-I. The rHDL was prepared using the purified apo A-I along with PC.

Evaluation of ox-LDL-induced apoptosis in differentiated THP-1 cells: Differentiation of THP-1 cells in the presence of different doses (5-100 nM) of PMA was evaluated in view of DNA fragmentation analysis. A ladder-like appearance was visualized in the DNA isolated from THP-1 cells triggered with 100 nM PMA for 24 hours. In another set of experiment, when THP-1 cells were cultured with 100 nM PMA for different time intervals (6-24 hours), no ladder was detected in DNA isolated from the cells cultured with PMA till 18 hours. Thus, in the subsequent experiments, THP-1 cells were differentiated with 100 nM PMA for 18 hours.

Apoptotic cells were found to be increased significantly ($P \leq 0.001$) with 25 µg/ml, 50 µg/ml, and 100 µg/ml ox-LDL, respectively, at 24 hours as compared to the untreated cells and they were maximum with 100 µg/ml ox-LDL.

A time-dependent significant ($P \leq 0.001$) increase in % apoptotic cells was observed at 12 hours (10%), 18 hours (25%), and 24 hours (29%) as compared to that of respective control cells (5%). Since no significant increase in the number of apoptotic cells was noticed at 24 hours as compared to that at 18 hours, in the subsequent experiments, differentiated THP-1 cells were cultured with 100 µg/ml ox-LDL for 18 hours.

Effect of nHDL and rHDL on ox-LDL-induced apoptosis in THP-1 cells: Effect of various doses (25-100 µg/ml) of nHDL and rHDL on ox-LDL (100 µg/ml, 18 hours)-induced apoptosis in differentiated THP-1 cells was assessed separately by CDD ELISA. A significant ($P < 0.001$) reduction in the extent of apoptosis was noted in presence of nHDL and rHDL (Figure 1A and 1B, $P < 0.001$), which was maximum at 100 µg/ml dose for both molecules. Moreover, ox-LDL-induced apoptosis in the differentiated THP-1 cells was also reduced significantly ($^{**}P < 0.050$, $^{***}P < 0.001$) on 12 hours preincubation with nHDL and rHDL (100 µg/ml) separately (Figure 1C and 1D, $^{**}P < 0.050$, $^{***}P < 0.001$). Since no significant change in the extent of apoptosis was noted at 12 hours of preincubation with nHDL/rHDL as compared to that at 6 hours, in the subsequent experiments, PMA-differentiated cells were preincubated for 6 hours with 100 µg/ml of nHDL/rHDL and then cultured with ox-LDL (100 µg/ml, 18 hours).

HDL suppresses apoptosis in differentiated THP-1 cells: Impact of nHDL/rHDL on ox-LDL-induced apoptotic signal transduction pathways was assessed in differentiated THP-1 cells with respect to the expression level of different regulatory and effector proteins of apoptosis, at both transcript and protein levels of BAX (Figure 2A and 2B), Bcl-2 (Figure 2C and 2D), and Bcl-xL (Figure 2E and 2F). An enhanced expression of BAX was noted in group b as compared to group a, while it reduced appreciably in group c and d when compared to group b. The level of expression of Bcl-2 and Bcl-xL decreased in group b as compared to group a, whereas it was upregulated in group c and d. We examined the effect of nHDL/rHDL on ox-LDL-induced release of cyt c and activation of caspase-3 in differentiated THP-1 cells as shown in figure 3. Cyt c expression was downregulated in group c and d as compared to group b. The level of expression of caspase-3 transcript was upregulated in group b as compared to group a, whereas an appreciable decrease in its level was noted in group c and d as compared to group b.

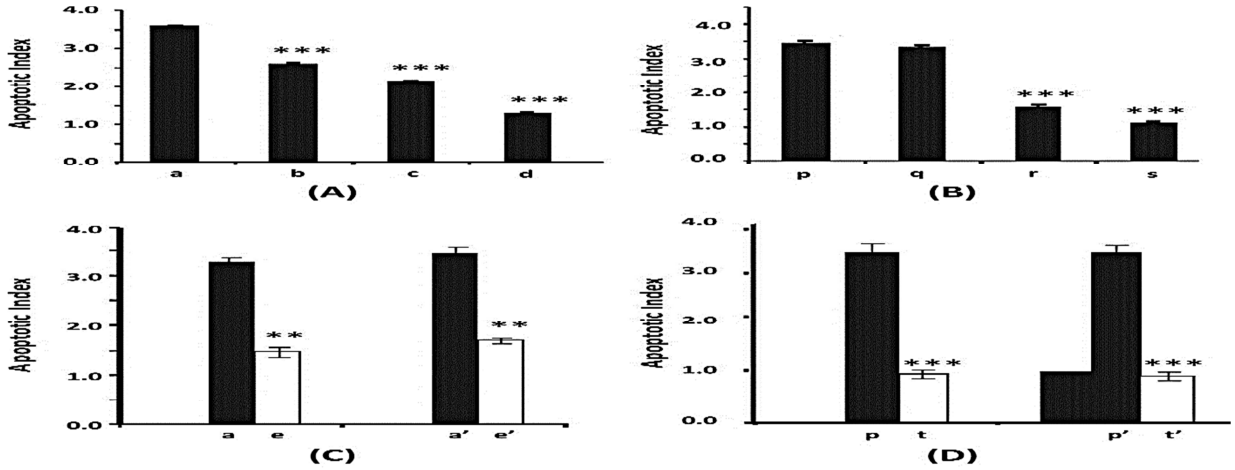

Figure 1. A and B) The bar graph showing the apoptosis in phorbol-12 myristate-13-acetate (PMA)-differentiated THP-1 cells cultured in presence of oxidized low-density lipoprotein (ox-LDL) preincubated without (a and p) and with different doses of 25 µg/ml (b and q), 50 µg/ml (c and r), and 100 µg/ml (d and s) of normal high-density lipoprotein (nHDL) and reconstituted HDL (rHDL), respectively; C and D) The assessment of apoptosis in PMA-differentiated THP-1 cells cultured in presence of ox-LDL without (a and e) and with 100 µg/ml of nHDL (a' and e') for 6 hours and without (p and t) and with (p' and t') 100 µg/ml rHDL, respectively, for 12 hours. Each bar represents the mean ± standard deviation (SD) of three independent experiments performed in duplicates.

Further, in Western immune blot, a prominent band (17 kDa) of activated caspase-3 was found in group b, which was not detectable in group c and reduced in group d.

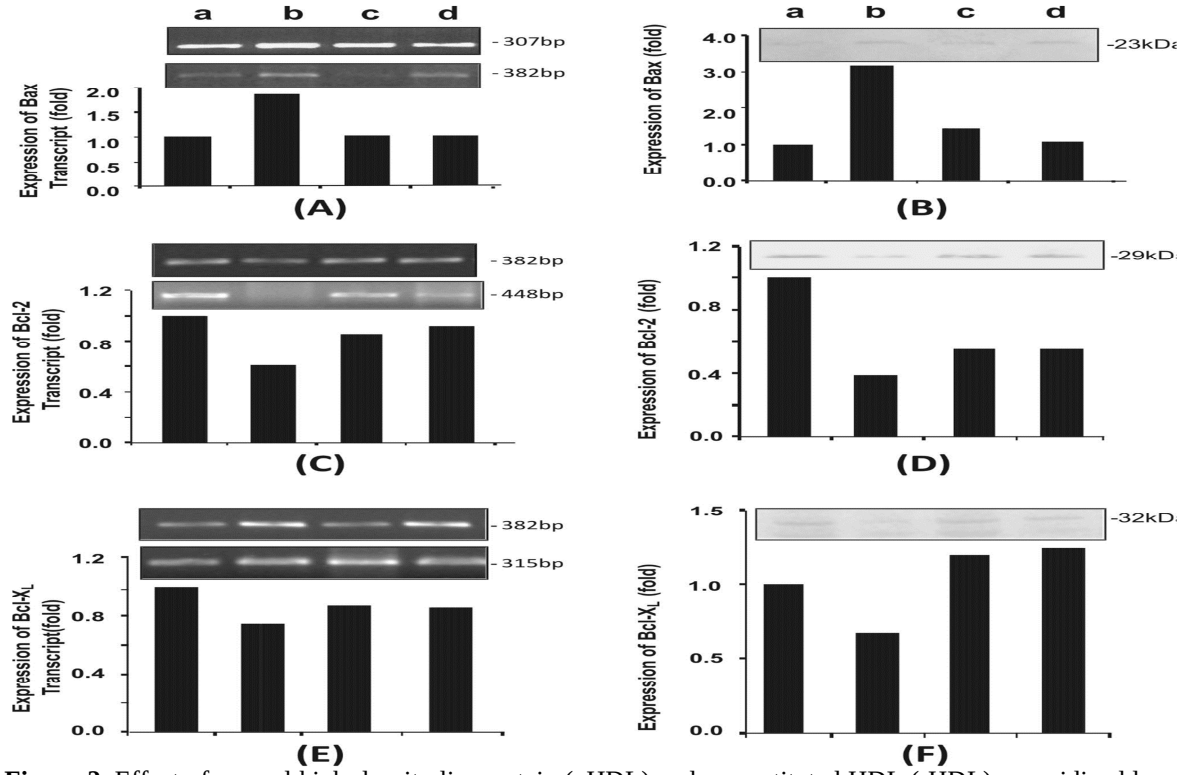

Figure 2. Effect of normal high-density lipoprotein (nHDL) and reconstituted HDL (rHDL) on oxidized low-density lipoprotein (ox-LDL)-induced alteration in expression of B-cell lymphoma-2 (Bcl-2)-associated X-protein (BAX) (A), Bcl-2 (C), and Bcl-extra large (Bcl-xL) (E) in differentiated THP-1 cells detected by reverse transcription polymerase chain reaction (RT-PCR) and Western immunoblotting BAX (B), Bcl-2 (D), Bcl-xL (F), respectively. Bar graphs represent the level of expression of BAX, Bcl-2, and Bcl-xL molecules under different conditions both at messenger ribonucleic acid (mRNA) and protein levels.

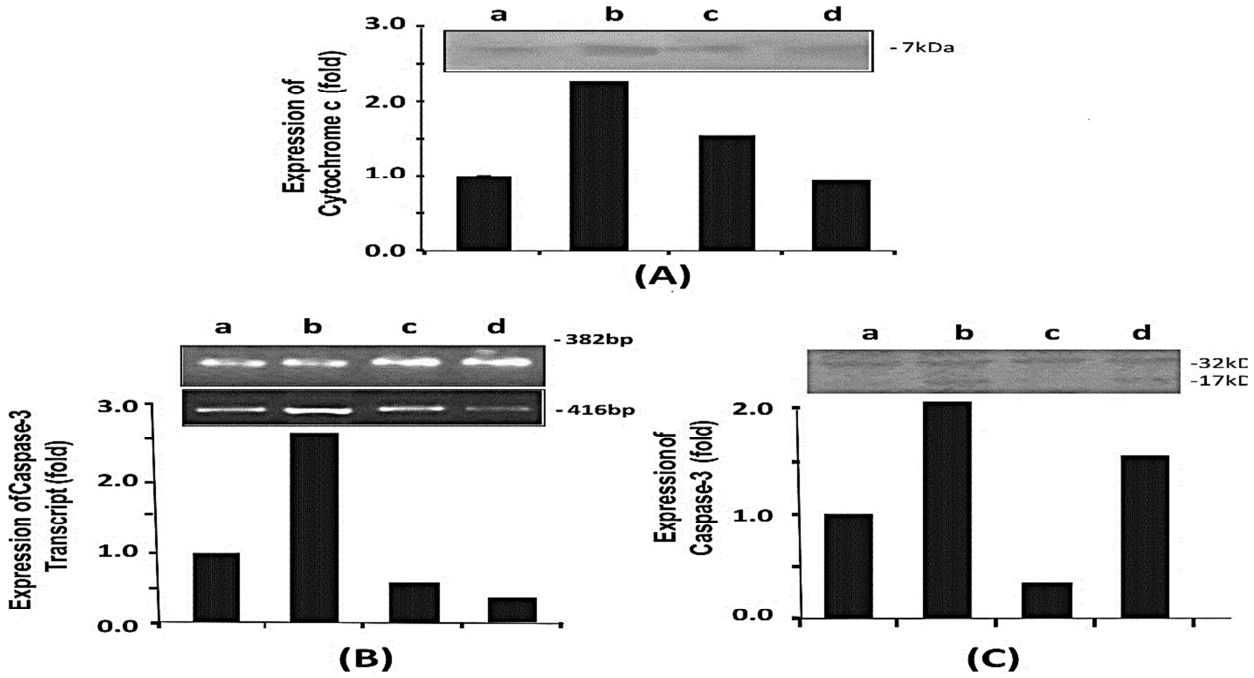

Figure 3. Effect of normal high-density lipoprotein (nHDL) and reconstituted HDL (rHDL) on oxidized low-density lipoprotein (ox-LDL)-induced release of cytochrome c (cyt c) (A) and activation of caspase-3 in differentiated THP-1 cells was determined both at messenger ribonucleic acid (mRNA) (B) and protein level (C). Bar graph represents the level of expression of caspase-3 under different conditions with respect to that of control.

Effect of nHDL/rHDL on ox-LDL-induced apoptosis in differentiated monocytes: We further substantiated our studies on monocytes obtained from patients and control subjects (Figure 4A).

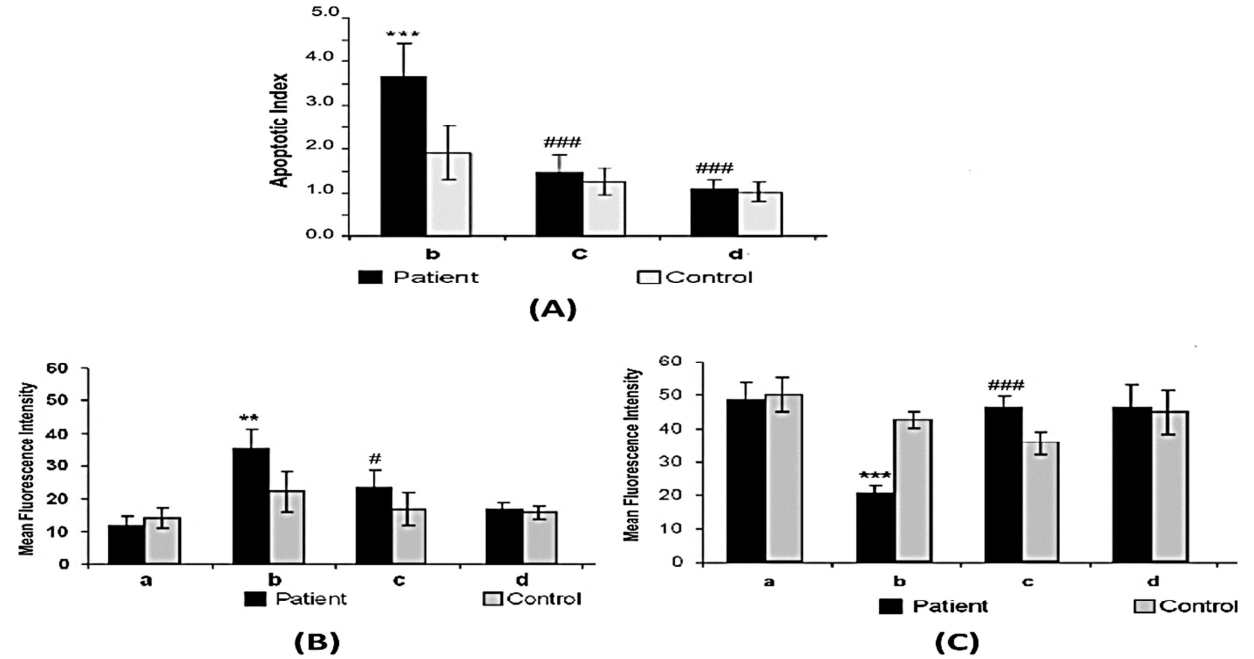

Figure 4. Bar graphs showing the effect of normal high-density lipoprotein (nHDL) and reconstituted HDL (rHDL) on oxidized low-density lipoprotein (ox-LDL)-induced apoptosis (A) and expression of B-cell lymphoma-2 (Bcl-2)-associated X-protein (BAX) (B) and Bcl-2 (C) proteins in differentiated monocytes obtained from patients and control subjects

Apoptotic index and the extent of apoptosis was increased by 4-fold and 1.2-fold in group b in patients and control subjects, respectively. A significant ($P < 0.001$) decrease in the extent of apoptosis was noted in group c and d as compared to that in group b of patients' samples.

Our findings were authenticated by the study on the level of expression of BAX and Bcl-2 in the monocytes obtained from a→d groups of patients and control subjects. In case of patients' samples, BAX was significantly increased in group b ($P < 0.050$) as compared to group a, while its expression was found to be decreased in group c ($P < 0.010$) and d as compared to group b (Figure 4B). Further, levels of Bcl-2 was significantly reduced in group b ($P < 0.001$) as compared to group a, whereas it was increased in group c ($P < 0.001$) as compared to group b (Figure 4C) in patient sample.

Assessment of LOX–1 expression: The level of LOX-1 was variable in different groups (a→d) of THP-1 cells. It was found to be increased in group b as compared to group a, while its expression was decreased in group c and d in comparison to group b (Figure 5A and 5B). Our result was further substantiated by studying the LOX-1 expression in a→d groups of monocytes obtained from hyper-LDL and normal-LDL subjects (Figure 5C). In case of hyper-LDL subjects, the level of LOX-1 was increased significantly in group b ($P < 0.001$) compared to group a. However, a significant decrease in its level of expression was noted in group c ($P < 0.001$) and d ($P < 0.010$) as compared to group b. In case of control subjects, no significant alteration in the level of LOX-1 expression was noted between the groups.

Discussion

The major finding of this study is that the increased expression of pro-apoptotic members (BAX, caspase-3, cyt c) was found to be decreased in the presence of nHDL and rHDL in differentiated monocytes. Ox-LDL was used to induce apoptosis in PMA-differentiated human monocytes. It has been reported that PMA could trigger differentiation or apoptosis, depending on the cell type.[19] This dual effect of PMA may be due to its ability to activate different isoforms of protein kinase C (PKC), the activation of which can either lead to the disordered growth, cell transformation, and inhibition of apoptosis or it can result in cell growth inhibition and induction of apoptosis.[20]

In the present study, it was observed that pre-incubation of differentiated cells with 100 µg/ml of nHDL/rHDL for 6 hours followed by co-incubation with ox-LDL for 18 hours could inhibit apoptosis significantly. Nofer et al.[21] reported that apoptosis of endothelial cells could also be suppressed by HDL and associated lysosphingolipids.

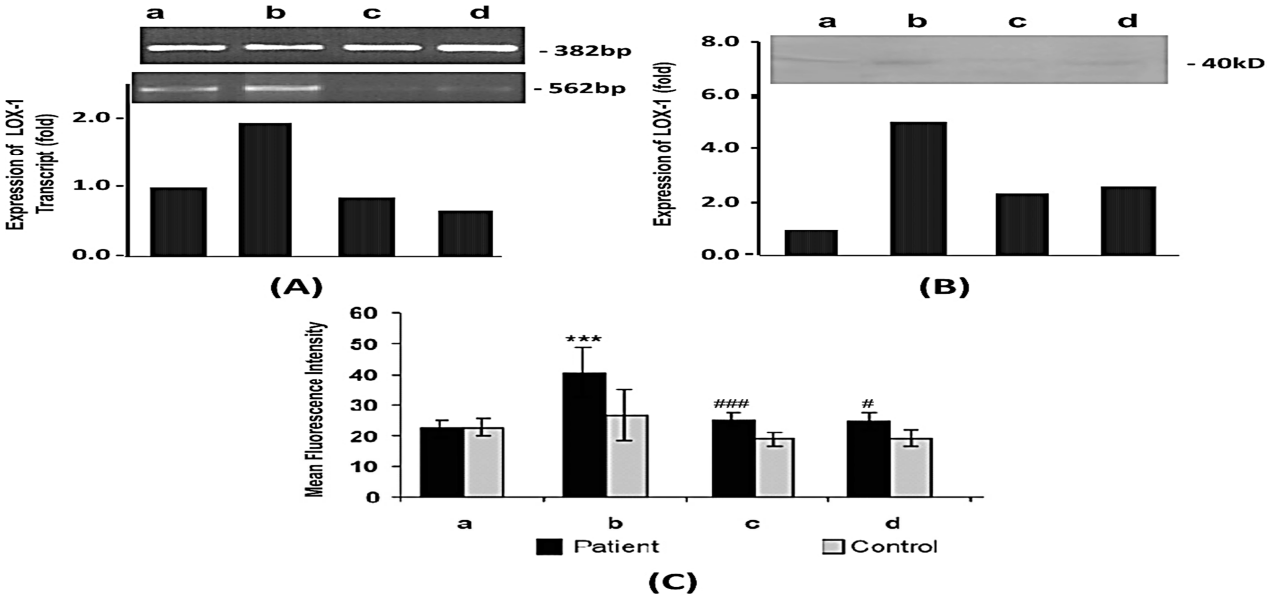

Figure 5. A and B) The expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) at messenger ribonucleic acid (mRNA) and protein level in differentiated monocytes triggered in presence of oxidized low-density lipoprotein (ox-LDL) with and without normal high-density lipoprotein (nHDL) and reconstituted HDL (rHDL), respectively; C) Bar graphs showing the effect of nHDL and rHDL on ox-LDL-induced LOX-1 expression in differentiated monocytes obtained from patients and control subjects in different experimental groups (a→d)

A significantly higher extent of apoptosis and an increased expression of BAX was found in group b of hyper-LDL subjects as compared to that of normal-LDL subjects which was consistent with the reports of Rosa et al., who demonstrated the effect of mitochondrial apoptotic pathway in macrophages during atherosclerosis.[22] It has also been reported that macrophages could express BAX in atherosclerotic plaques and cholesterol withdrawal could reduce the expression of BAX and apoptotic cells in these plaques,[23] which supported our observation in group c and d in patients' samples, in which, decrease in the extent of apoptosis and BAX level was observed.

Mehta et al.[24] reported that LOX-1 mediated ox-LDL-induced apoptosis in vascular endothelial cells by stimulating p38 mitogen-activated protein kinase (MAPK) which was in good agreement with our observations. It is possible that uptake of ox-LDL through LOX-1 could activate mitochondrial apoptotic signal transduction pathways.

It can be suggested that nHDL/rHDL could either prevent the binding of ox-LDL to LOX-1 by preventing the accumulation of oxidized lipids in the ox-LDL through lecithin-cholesterol acyltransferase (LCAT) enzyme, thereby inhibiting ox-LDL-mediated signaling through this receptor or it might degrade the ox-LDL in the system because several apolipoproteins, including apo A-I, have intrinsic antioxidant property.[1]

Conclusion

Apo A-I plays a prominent role and should be measured as a component for assessing cardiovascular risk in humans. Role of LOX-1 in macrophage-cholesterol homeostasis might provide some interesting targets for atheroprotection. The present study showed the protective role of HDL in the disease process as substantiated by inhibition of ox-LDL-mediated apoptosis in differentiated monocytes obtained from patients. Despite a wealth of information on this subject, further research is required to substantiate the findings in more numbers of patient samples, so that identification of appropriate drug targets can be done which will prevent the progression of atherosclerosis.

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Conflict of Interests

Authors have no conflict of interests.

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Endothelial dysfunction in patients with lone atrial fibrillation

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Original Article

Abstract

BACKGROUND: Atrial fibrillation (AF) is the most common tachyarrhythmia in patients with cardiovascular diseases (CVDs) and may have significant complications such as stroke. The present study aims to evaluate endothelial dysfunction in patients with lone atrial fibrillation (LAF) through flow-mediated dilation (FMD) in the brachial artery, as a non-invasive method for evaluating functional and structural markers of endothelial dysfunction.

METHODS: In this case-control study, 43 patients with LAF were selected. 51 age and sex-matched healthy individuals were selected as the control group. The brachial artery diameter of the subjects in both groups was measured through FMD. The obtained data were analyzed by SPSS software.

RESULTS: Patients with LAF and healthy subjects did not have any difference in terms of gender, heart rate (HR), and systolic blood pressure (SBP) ($P > 0.05$ for all). FMD of the patients with AF was significantly lower ($P = 0.04$) than FMD of the healthy controls.

CONCLUSION: Our findings showed that LAF was associated with systemic endothelial dysfunction. AF plays an important and independent role in reducing FMD.

Keywords: Atrial Fibrillation; Arrhythmia; Dysfunction

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Introduction

Atrial fibrillation (AF) is the most frequent tachyarrhythmia which affects more than 5% of people over 65 years. It is a progressive arrhythmia with an increasing rate of prevalence with age. Although AF is not an independent factor for predicting mortality in patients with cardiovascular diseases (CVDs), its complications and morbidity, such as stroke, are significant.[1,2] In 80% of cases, AF is associated with some conditions and CVDs. These underlying causes include ischemic heart failure (IHF), heart failure, valvular heart disease (VHD), hypertension, diabetes mellitus (DM), alcohol consumption, thyroid, and pulmonary diseases. Based on the study population, about 2-10% and in some studies, up to 30% of patients have no specific cause for AF.[3-5] Lone atrial fibrillation (LAF) has a different pathophysiology process and develops in patients younger than 60 years with no evidence of hypertension or structural heart disease.[6]

Several demographic, genetic, and anthropometric factors have been suggested as the risk factors for AF. Although the prevalence of AF increases with age, LAF can occur in younger patients.[7] Indeed, LAF appears due to physiological changes associated with the autonomic system tone, especially the parasympathetic pathway (vagal AF), insulin sensitivity, and imbalance between serum electrolytes at the cell level with a short refractory period.[7]

On the other hand, some studies have shown that endothelium is not just a barrier between intracellular and intravascular components; instead,

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it is an extensive organ with many essential tasks that play a crucial role in tissue oxygenation, vasomotor control, homeostasis, inflammation, and immune response.[8]

Sometimes the endothelium suffers some injury and an abnormal activity called endothelial dysfunction (ED). Indeed, ED is a clinical syndrome described by impaired reactivity of the vasculature.[9]

Given that endothelial dysfunction is involved in the clinical manifestations of many diseases, its evaluation has a potential diagnostic and prognostic role.[10] Besides, it has recently been shown that endothelial dysfunction can be reversible. This could be important because reversibility at an early stage can increase the disease treatment chance, and even changes in endothelial dysfunction in advanced diseases may be effective in reducing progression and complications of them.[8,11-15] In arrhythmia, there is a cause-effect relationship between conditions that impair endothelial function and metabolic activity. The relationship between these two functions and basal mechanisms has not been investigated so far. The present study is intended to investigate endothelial dysfunction in patients with LAF through flow-mediated dilation (FMD) in the brachial artery as a non-invasive technique for evaluating the functional and structural markers of endothelial dysfunction. We aimed to help identify the functional and structural markers of LAF and decrease its complications or exacerbation through the administration of inhibitory drugs in clinical trials.

Materials and Methods

In this case-control study, 43 patients who fulfilled diagnostic criteria of LAF were enrolled in this study through the non-randomized convenience sampling method from patients who visited the Shahid Chamran Hospital in Isfahan, Iran, from January 2017 to August 2017. 51 age-and gender-matched healthy subjects according to medical history, physical examination, and routine laboratory tests without a history of palpitation or AF were also enrolled as a control group. The control group were mainly selected from patients' relatives who fulfilled the study inclusion criteria.

AF was considered lone in patients younger than 60 years of age if there were no known associated CVDs or precipitating factors for AF. Lone AF was confirmed by 12-lead electrocardiogram (ECG).

Subjects with a history of hypertension, DM, coronary artery disease (CAD), heart failure, peripheral artery disease (PAD), stroke, infectious diseases, known liver or kidney disorders, hypercholesterolemia, hypertriglyceridemia, gynecologic disorders (such as polymenorrhea, cystic ovary diseases), morbid obesity [body mass index (BMI) > 35], abuse of substances, anemia (hemoglobin < 12 mg/dl), sinusitis, known lung disorder, recent body trauma or surgery, and migraine were not included in the study for both case and control groups.

The study exclusion criteria were using any drugs, pregnancy, and lactation. Furthermore, all participants had not used hormonal contraceptives or vasoactive drugs in the last three months before the study. All cases were in AF rhythm at the time of the study, but after ventricular rate control (less than 90 beats/minute) and if sinus rhythm was achieved before FMD measurement, they would be excluded. The ventricular rates in all patients with AF were controlled with beta-blockers or calcium channel blockers.

Informed consent was obtained from all participants, and the study protocol was approved by the ethics committee of Isfahan University of Medical Sciences with ID IR.396.3.540. This article was the result of a thesis on residency course with number 396540.

In the beginning, all subjects underwent a complete examination that included physical examination, blood sampling for routine biochemistry measurements, echocardiography, and ECG. Then, forearm FMD was performed under fasting conditions or consumption of low-fat meals before testing.

A high-resolution B-mode ultrasonographic system (ATL Ultrasound, HDI 5000, Bothell, Washington, USA) with a linear transducer mid-frequency of 7.5 MHz was used to determine FMD of the brachial artery. FMD was performed between 8 AM and 9 AM in a room with a controlled temperature by an expert cardiologist who was utterly blind to the study. FMD was not measured during the menstrual phase in female patients.

All subjects were fasting at least 4 hours before the study. They were instructed not to eat, drink caffeinated beverages, or take vitamin C supplements at least 12 hours before the study. After 10 minutes of rest at the supine position, heart rate and blood pressure were measured, and the baseline brachial artery diameter was determined from 3-4 cm above the non-dominant elbow pit. When the 2D long-axis image of the vessel was identified and recorded digitally, the ultrasound probe position was kept constant and remained intact throughout the test. Artery diameters were determined with ultrasonic calipers from the leading edge of the anterior wall to the leading edge of the

posterior wall of the brachial artery at the end of the diastole (the beginning of R wave on ECG). External ECG monitoring during echocardiography and FMD studies ensures the proper timing of images regarding the cardiac cycle. To adapt the heart beat rate to changes with that of patients with AF, the mean artery diameter was obtained from 5 consecutive cardiac cycles. The same method was applied to healthy subjects. After determining the base diameter (Dbase), the sphygmomanometer cuff was placed on the arm and inflated to 300 mmHg for 5 minutes. The cuff was then released, and the second scan was taken every 10 seconds during 120 seconds after the cuff deflation at the end of the diastolic period. The highest 10 second averaged interval throughout the 2-minute post-occlusion collection period represented the peak hyperemic diameter. This measurement determined the endothelium-dependent dilation diameter (Dafter). Changes in the diameter were computed as a percentage relative to the baseline diameter. FMD was calculated using the following formula:

$$FMD = [(Dafter-Dbase)/Dbase] \times 100$$

Two other observers supervised the procedures.

The obtained data were gathered in the checklist and entered SPSS software (version 22, IBM Corporation, Armonk, NY, USA) for analysis. The continuous variables were expressed as mean ± standard deviation (SD). Normal distribution of data was evaluated with Kolmogorov-Smirnov (K-S) test. Differences in continuous variables between the case and control groups were analyzed using the independent-sample t-test in normal distribution variables. If the data distribution was not normal, the Mann-Whitney U test was used. Categorical variables were presented as absolute numbers (%). These variables were analyzed between groups using the chi-square test. P-value of less than 0.05 was considered as the level of significance. All of the statistical analyses were performed by a blind analyzer about the details of the study.

Results

The present study included 43 patients with LAF (24-57 years, 72.1% male), and 51 healthy subjects (23-56 years, 70.6% male). The clinical characteristics of the patients with LAF and healthy subjects are shown in table 1. There were no differences between the two groups in demographic characteristics. Seven patients in the LAF group and six participants from the healthy subjects were someday smokers (P = 0.52).

FMD parameters of the case and control groups have been demonstrated in table 2. As shown in this table, the mean of FMD in the control group (healthy subjects) was significantly higher than that of the patients with LAF (P = 0.04).

Table 1. Demographic and clinical characteristics of the study participants

Clinical characteristics	Patients with LAF (n = 43)	Healthy subjects (n = 51)	P
Age (years)	41.95 ± 8.25	39.11 ± 8.47	0.10*
Resting HR (beats/minutes)	79.48 ± 8.53	77.25 ± 6.82	0.16*
SBP (mmHg)	109.09 ± 11.31	112.78 ± 10.94	0.09*
DBP (mmHg)	68.39 ± 6.70	68.90 ± 6.18	0.65*
Weight (Kg)	73.04 ± 9.98	72.64 ± 8.74	0.78*
Height (cm)	169.90 ± 8.46	169.47 ± 8.01	0.79*
BMI (Kg/m^2)	25.23 ± 2.34	25.28 ± 2.50	0.96*
FBS (mg/dl)	87.79 ± 6.97	86.27 ± 7.00	0.29*
TG (mg/dl)	104.79 ± 23.76	108.43 ± 24.19	0.48$
LDL (mg/dl)	83.83 ± 20.02	88.96 ± 22.77	0.31*
HDL (mg/dl)	53.76 ± 8.71	51.09 ± 9.48	0.11*
Uric acid (mg/dl)	5.32 ± 0.83	5.20 ± 0.78	0.45$
Creatinine (mg/dl)	0.86 ± 0.20	0.89 ± 0.18	0.36$
ALT (U/l)	29.76 ± 6.79	27.84 ± 7.32	0.28*
TSH (mIU/l)	1.41 ± 0.75	1.49 ± 0.66	0.68$
Hemoglobin (g/dl)	14.38 ± 1.02	14.10 ± 1.02	0.16*
Platelet count (per µl)	198.25 ± 33.40	197.60 ± 27.86	0.71*
GFR ($ml/min/1.73m^2$)	121.23 ± 33.11	118.26 ± 28.43	0.70*
Smoker [n (%)]	7 (16.3)	6 (11.8)	0.52**

LAF: Lone atrial fibrillation; HR: Hear rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; FBS: Fasting blood sugar; TG: Triglycerides; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; ALT: Alanine aminotransferase; TSH: thyroid-stimulating hormone; GFR: Glomerular filtration rate

Data are reported as mean ± standard division (SD) or [n (%)].

* Results from independent t-test, ** Results from chi-square analysis, $ Results from Mann-Whitney U test

Table 2. Lone atrial fibrillation (FMD) parameters between case and control

Parameter	Patients with LAF (n = 43)	Healthy subjects (n = 51)	P*
BBD (mm)	4.21 ± 0.74	4.06 ± 0.67	0.29
PAD (mm)	4.45 ± 0.75	4.35 ± 0.66	0.57
FMD (%)	5.79 ± 3.85	7.56 ± 4.38	0.04

LAF: Lone atrial fibrillation; BBD: Basal brachial diameter; FMD: Flow Mediated Dilation; PAD: Peripheral arterial diameter; Data are reported as mean ± standard division (SD).
* Results from Mann-Whitney U test

Discussion

The present study showed that endothelial function, which was evaluated with FMD of the brachial artery, was significantly impaired in patients with LAF compared to the healthy subjects. This result suggests that endothelial dysfunction plays a role in the pathogenesis of AF.

This finding is consistent with the results obtained in previous studies. For the first time, Takahashi et al. reported the systemic endothelial dysfunction in patients with AF.[16] This study showed endothelium-dependent dilation in a group of patients with AF using venous occlusion plethysmography. This study drew attention to the non-invasive evaluation of systemic endothelial function in patients with AF and led to numerous trials which showed that FMD could be used to evaluate endothelial function in patients with AF. These trials show that patients with AF always have an impaired FMD, and their endothelial function can improve upon sinus rhythm restoration.[17-20]

However, most of these studies have been conducted on patients with underlying conditions, including hypertension, CAD, and DM, which are known as risk factors for endothelial dysfunction. A number of studies also confirmed that sustained AF is associated with systemic endothelial dysfunction, even in relatively young patients with no CVDs or risk factors.[21,22] It has been shown that AF is an independent contributor to lower FMD, and a prolonged arrhythmia duration may confer the risk for more profound endothelial damage.[21]

It seems that the relation between AF and ED is in a vicious cycle. In this way, not only AF could cause ED, but ED could also provoke AF. Pathophysiologic mechanisms behind systemic endothelial dysfunction in AF could be explained in these three theories as below:

1. In patients with AF, irregular heartbeats produce turbulent blood flow and oscillating shear stress in systemic vessels. This decreases NO production, and thus endothelial NO synthase expression could be changed and finally cause ED.[23] This theory has been supported by findings of reduced plasma nitrite/nitrate levels in AF.[24]

2. AF could induce damage to the endocardium of the left atrium, which could reduce circulating nitroso-compounds serving as endogenous NO donors to systemic vessels and then cause endothelial dysfunction.[25]

3. Activation of the renin-angiotensin system,[26] neurohumoral activation,[27] and oxidative stress could be also implicated in the development of endothelial dysfunction in AF, particularly with longer arrhythmia duration.[28]

On the other hand, ED could cause AF, and this could be explained in three different categories as below:

1. ED results in the down-regulation of NO and the up-regulation of adhesion molecules that promote increased levels of inflammation and oxidative stress. Consequently, the generation of reactive oxygen species and oxidative injury leads to the electrophysiological remodeling observed in AF.[29] Additionally, it has been shown that NO reduces spontaneous electrical activity in cardiomyocytes isolated from the pulmonary vein. Therefore, NO could be as a regulator of AF arrhythmogenesis, and down-regulation of that could provoke AF.[30]

2. Inflammation could result in atrial ectopy in discharging cells near the pulmonary veins.[31] Thus, patients with ED, who are associated with an increased level of inflammation, are at higher risk of developing AF. Moreover, FMD can potentially identify persons with abnormal vascular biological profiles that precede this arrhythmia.[32]

3. Many shared risk factors for AF, such as hypertension, increasing age, DM, and smoking, have been associated with endothelial dysfunction.[33] These conditions can lead to an increase in the level of vascular endothelial dysfunction and predispose individuals to AF.

Conclusion

Our findings proved that LAF was associated with systemic endothelial dysfunction. Actually, AF plays an important and independent role in reducing FMD.

These findings may be important for further studies to identify the clinical relevance and potential therapeutic outcomes, especially in categorizing thromboembolic risks and preventing AF-induced thromboembolism.

Limitations: The current study is one of the first studies to show FMD in relatively young LAF patients (mean age of 41 years), which is highly important and can negatively affect endothelial

function due to aging and the presence of atherosclerosis risk factors. The first limitation of the study was a relatively small sample size. However, both patient and control groups were sufficiently homogenous, and the differences in the main findings between the groups were substantial. The second limitation was the lack of use of other markers of endothelial function in the study to better evaluate ED beside FMD to show the higher strength of ED in patients with LAF. On the other hand, in the present study, physical activity was not assessed, which could affect endothelial function. However, there were no reports on the negative effects of beta-blockers and calcium channel blockers on endothelial function, but they were not controlled in our investigation.

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Conflict of Interests

Authors have no conflict of interests.

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Circadian pattern of symptom onset in patients with ST-segment elevation myocardial infarction in western Iran

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Original Article

Abstract

BACKGROUND: Circadian variation is known as an important factor in acute myocardial infarction (AMI). Moreover, the circadian pattern may help in disease prevention and better medication prescription. Therefore, the aim of our study was to investigate the circadian pattern of symptom onset in patients with ST-segment elevation myocardial infarction (STEMI).

METHODS: This cross-sectional study was conducted on 777 patients admitted to the Imam Ali Cardiovascular Center, Kermanshah, Iran, with a diagnosis of STEMI from March 2018 to February 2019. Data were collected using a checklist developed based on the study's objectives. Differences between subgroups were assessed using one-way analysis of variance (ANOVA) with post-hoc testing and chi-square test (or Fisher's exact test).

RESULTS: Out of the 777 patients, 616 (79.3%) were men. The mean and standard deviation (SD) of age of the patients was 60.93 ± 12.86 years. 380 patients (48.9%) were current smoker, 40.3% were hypertensive, 21.1% had hypercholesterolemia, 18.3% had diabetes mellitus (DM), 25.2% had history of angina, and about 15.0% had history of myocardial infarction (MI). The occurrence of STEMI was most common during hours between 06:01-12:00 (27.7%), followed by 12:01-18:00 (27.3%), 00:00-06:00 (24.3%), and 18:01-24:00 (20.7%), respectively. Gender was significantly associated with circadian pattern of STEMI. Women showed a double peak of symptom onset in 06:01-12:00 and 12:01-18:00.

CONCLUSION: The present study of Iranian patients displayed circadian pattern of STEMI with 2 peaks in the morning and afternoon, and the both peaks were dominated by women.

Keywords: Circadian Rhythm; Myocardial Infarction; Iran

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Introduction

Acute myocardial infarction (AMI) is a leading cause of cardiovascular death and disability across the world.[1] ST-segment elevation myocardial infarction (STEMI), a classic heart attack, is the most deadly sub-class of myocardial infarction (MI), accounting for more than 35% of MI cases. Some physiological factors may trigger MI, and a number of these factors are known to fluctuate with circadian pattern.[2] In turn, circadian patterns affect cardiovascular physiology by varying in multiple biologic functions such as heart rate, blood pressure (BP), cardiac output, endothelial function, and hormone production and release.[3]

Circadian rhythms of MI were first explained by the World Health Organization-Regional Office for Europe (WHO-Europe) in 1976, which reported a peak incidence in the symptom onset between 8:00 am and 10:00 am (on waking and when resuming activity).[4] Furthermore, epidemiological studies have well documented that the onset of MI significantly changes through the day, with a morning peak (06:00-12:00) in the MI symptoms onset and a secondary peak incidence at night-time.[5,6]

The reasons for morning peak in the MI symptoms onset have been partially illuminated and may be due to the morning BP, platelet agreeability,

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releasing asymmetric dimethylarginine (ADMA), coagulation parameters, lipoprotein levels, decreasing the fibrinolytic activity, and sympathetic neurotransmission.[2,7,8] Although, exogenous factors like sleep deprivation, emotional stress, activity levels, geographic location, and etc. may also modulate these variations.[9]

As MI is the leading cause of mortality in the developed and developing countries, primary prevention of MI is a basic healthcare issue worldwide.[1] Also, new therapeutic approaches based on circadian rhythms have led to the better timing of drugs and treatments to optimize outcomes and minimize adverse effects.[10] Accordingly, identifying the circadian patterns of symptom onset in patients with MI may help in the clinical management of patients to prevent the onset of MI.

Despite a morning peak in the MI symptoms onset, most researchers have reported a relationship between MI symptoms onset and circadian rhythm with varying patterns.[11,12] Moreover, differences in the circadian rhythms of MI in different regions of the world and in different ethnic groups have also been reported.[13] Therefore, the aim of our study was to investigate the circadian pattern of symptoms onset in patients with STEMI.

Materials and Methods

This cross-sectional study was conducted in Imam Ali Cardiovascular Center, Kermanshah University of Medical Sciences, Kermanshah, Iran. The mega general hospital, Imam Ali, with 280 active beds is the main cardiovascular center in western Iran, covering about two millions population mostly Kurdish with Caucasian race.

With the aim of one-year evaluation of patients, one-year data of the patients admitted to the center with a diagnosis of STEMI were assessed. Between March 1, 2018, and February 30, 2019, we evaluated all patients (convenience sampling) who were admitted to the center with a diagnosis of STEMI. In fact, we included all patients based on inclusion criteria. One criterion for inclusion in the study was age ≥ 18 years old. Criteria for the diagnosis of STEMI were based on third universal definition of MI defined by the European Society of Cardiology/American College of Cardiology (ACC)/American Heart Association (AHA)/World Heart Federation (WHF) Task Force for the Universal Definition of Myocardial Infarction.[14] The diagnosis of STEMI was accompanied by the following elements: 1) characteristic chest pain or discomfort 2) electrocardiographic (ECG) changes consistent with new ST-segment elevations or left bundle branch block (LBBB), and 3) elevated markers of MI [creatine kinase-myocardial band (CK-MB), troponins, and etc.]. Those with incomplete personal or medical information were excluded. Although, response rate was 100%.

The Research Ethics Committee of Kermanshah University of Medical Sciences approved the study protocol. In addition, individual personal information has been kept confidentially.

Data were collected by a research assistant who was well trained in data gathering, and using a checklist developed based on the study goals. The checklist was evaluated and verified by expert opinions compromising a statistician and two cardiologists. The checklist comprised five following parts: demographic characteristics (e.g., age), clinical histories (e.g., previous MI), medication (e.g., aspirin), cardiac enzyme (e.g., CK-MB), and the time of onset of MI.

We collected the data from both paperwork records and electronic medical records to move bias from abstracting records. Furthermore, the results were tested through checking hospital managerial information.

Data analysis was performed using SPSS statistical software (version 23.0, IBM Corporation, Armonk, NY, USA). Quantitative variables [e.g., body mass index (BMI) or age] were described using mean ± standard deviation (SD) and qualitative/categorical variables were expressed as frequencies and percentages. Differences between subgroups were assessed using one-way analysis of variance (ANOVA) for continuous and normally-distributed variables and chi-square test (or Fisher's exact test) for categorical variables. A probability value (P-value) of less than 0.050 was considered statistically significant.

Results

During 12 months, a total of 777 patients, 616 (79.3%) men and 161 (20.7%) women, met the inclusion criteria for this study. The mean age of the patients was 60.93 ± 12.86 years, ranging from 19 to 95 years. 380 patients (48.9%) were current smoker, 40.3% were hypertensive, 21.1% had hypercholesterolemia, 18.3% had diabetes mellitus (DM), 25.2% had history of angina, and about 15.0% had history of MI. All of the patients' demographic and clinical characteristics are shown in table 1.

We classified the day into the four six-hour intervals from 00:00 to 06:00, 6:01 to 12:00, 12:01 to 18:00, and 18:01 to 24:00.

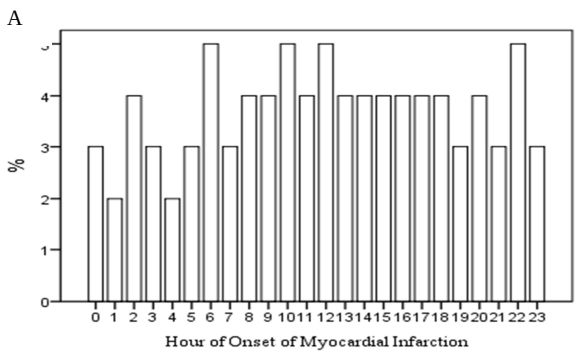
Table 1. The demographic and clinical characteristics of patients (n = 777)

Value	Variable
Age (year)*	60.93 ± 12.86
BMI (kg/m^2)*	25.95 ± 0.87
Sex (male)	616 (79.3)
Prior MI	116 (14.9)
Prior angina	196 (25.2)
Prior CHF	28 (3.6)
Prior stroke	47 (6.0)
Prior AF	16 (2.1)
Prior PCI	67 (8.6)
Prior CABG	31 (4.0)
Current smoker	380 (48.9)
Diabetes	142 (18.3)
HTN	313 (40.3)
Hypercholesterolemia	164 (21.1)
Cancer	7 (0.9)
Aspirin user	215 (27.7)
Clopidogrel user	34 (4.4)
ARB user	86 (11.1)
β-blocker user	165 (21.2)
ACE inhibitors user	149 (19.2)
Statin user	106 (13.6)
CK-MB (U/l)	140.29 ± 116.58
CPK (U/l)	1656.87 ± 1215.35
Troponin (ng/ml)	13.34 ± 63.50
LDH (U/l)	337.69 ± 153.73
EF (%)	37.18 ± 10.65

* Continuous variables are expressed as mean ± standard deviation (SD), others as number (%)

BMI: Body mass index; MI: Myocardial infraction; CHF: Congestive heart failure; AF: Atrial fibrillation; PCI: Percutaneous coronary intervention; CABG: Coronary artery bypass grafting; HTN: Hypertension; ARB: Angiotensin receptor blockers; ACE: Angiotensin converting enzyme; CK-MB: Creatine kinase myocardial band; CPK: Creatine phosphokinase; LDH: Lactic acid dehydrogenase; EF: Ejection fraction

The frequency of symptom onset by a 24-hour period was shown in figure 1A. The occurrence of STEMI was most common during 06:01-12:00 (27.7%), followed by 12:01-18:00 (27.3%), 00:00-06:00 (24.3%), and 18:01-24:00 (20.7%), respectively (Figure 1B).



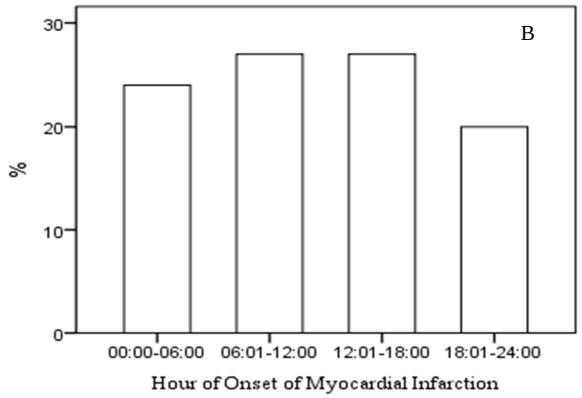


Figure 1. Distribution of symptom onset of ST-segment elevation myocardial infarction (STEMI) by a 24-hour period (A) and by the four six-hour intervals (B)

Sex was significantly associated with circadian pattern of STEMI (P = 0.022) (Table 2). A Bar graph was drawn to show the pattern of symptom onset of STEMI based on gender. Accordingly, women showed a double peak of symptom onset in 06:01-12:00 and 12:01-18:00 (Figure 2).

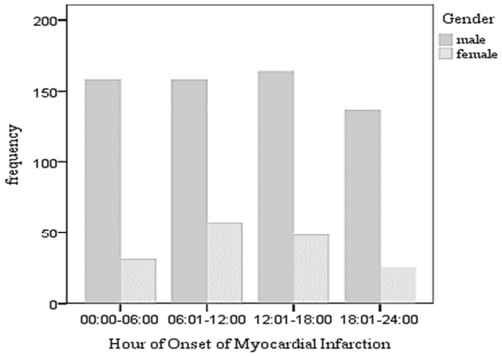


Figure 2. The pattern of symptom onset of ST-segment elevation myocardial infarction (STEMI) stratified by gender

Discussion

This cross-sectional study aimed to evaluate the circadian pattern of symptom onset in patients admitted with a diagnosis of STEMI in Imam Ali Cardiovascular Center at Kermanshah University of Medical Sciences between March 2018 to February 2019. The results of this study illustrated that Iranian patients showed circadian pattern of STEMI with two peaks, the first peak in the morning and the second peak in the afternoon.

Our results concur with the findings of previous studies. Itaya et al. reported two peaks in the onset of STEMI in Japanese population, with the first peak in the morning and the second peak in the evening.[10]

Table 2. Association of various characteristics and circadian patterns of ST-segment elevation myocardial infarction (STEMI) (n = 777)

Characteristic	00:00-06:00 (n = 189)	06:01-12:00 (n = 215)	12:01-18:00 (n = 212)	18:01-24:00 (n = 161)	P
Age ≥ 60 years	91 (50.8)	116 (55.8)	103 (50.5)	68 (44.4)	0.209**
BMI ≥ 25 kg/m^2	108 (57.1)	113 (52.6)	123 (58.0)	100 (62.1)	0.216**
Sex (female)	31 (16.4)	57 (26.5)	48 (22.6)	25 (15.5)	0.022**
Current smoker	97 (51.3)	93 (43.3)	102 (48.1)	88 (54.7)	0.196**
Diabetes	38 (20.1)	45 (20.9)	29 (13.7)	30 (18.6)	0.328**
HTN	79 (41.8)	88 (40.9)	79 (37.7)	67 (41.6)	0.888**
Hypercholesterolemia	41 (21.7)	45 (20.9)	50 (23.6)	28 (17.4)	0.477**
Prior CHF	7 (3.7)	9 (4.2)	6 (2.8)	6 (3.7)	0.522***
Prior MI	29 (15.3)	33 (15.3)	30 (14.2)	24 (14.9)	0.393**
Prior angina	44 (23.3)	62 (58.8)	53 (25.0)	37 (23.0)	0.512**
Prior stroke	14 (7.4)	16 (6.0)	8 (3.8)	12 (7.5)	0.376**
Prior AF	3 (1.6)	4 (1.9)	7 (3.3)	2 (1.2)	0.492***
Aspirin user	39 (20.6)	68 (31.6)	62 (29.2)	46 (28.6)	0.082**
Clopidogrel user	2 (1.1)	13 (6.0)	11 (5.2)	8 (5.0)	0.076**
β-blocker user	29 (15.3)	54 (25.1)	46 (21.7)	36 (22.4)	0.111**
ARB user	16 (8.5)	28 (13.0)	25 (11.8)	17 (10.6)	0.514**
ACE inhibitors user	40 (21.2)	42 (19.5)	34 (16.0)	33 (20.5)	0.567**
Statin user	20 (10.6)	29 (13.5)	37 (17.5)	20 (10.4)	0.449**
CK-MB (U/l)	145.30 ± 131.12	138.75 ± 108.22	142.09 ± 115.12	134.26 ± 112.66	0.844*
CPK (U/l)	1648.98 ± 1234.90	1671.29 ± 1194.06	1674.94 ± 1216.70	1621.67 ± 1232.21	0.978*
Troponin (ng/ml)	15.86 ± 79.41	13.24 ± 49.12	14.94 ± 82.03	8.48 ± 7.30	0.730*
LDH (U/l)	340.00 ± 158.62	239.75 ± 90.01	325.50 ± 79.04	427.00 ± 228.64	0.359*

Continuous variables are expressed as mean ± standard deviation (SD), others as number (%)
* Analysis of variance (ANOVA); ** Chi-square test; *** Fisher's exact test
BMI: Body mass index; HTN: Hypertension; CHF: Congestive heart failure; MI: Myocardial infraction; AF: Atrial fibrillation; ARB: Angiotensin receptor blockers; ACE: Angiotensin converting enzyme; CK-MB: Creatine kinase myocardial band; CPK: Creatine phosphokinase; LDH: Lactic acid dehydrogenase

Rallidis et al. reported that the onset of STEMI was higher during 06:01-12:00 and 12:01-18:00;[15] this observation is consistent with the present findings. In agreement with our study, Gallerani et al.[16] and Park et al.[17] showed that the risk of experiencing MI was increased during the morning. Moreover, Sari et al. reported that incidence of MI between 12:01 and 18:00 was higher than other three 6-hour periods, demonstrating afternoon peak.[13]

Nevertheless, some researchers have reported contradictory results. A report from China demonstrated a peak of symptom onset between 01:00 and 07:00.[18] In contrast, Sumiyoshi et al. reported that onset of AMI was more common in the evening than in the morning.[19] Conversely, a peak from 00:01 to 06:00 for MI onset in the Chinese patients has been reported by Chan et al. in 2012.[12]

For a long time, studies have demonstrated that the onset of MI mostly occurs in the morning hours.[5,20,21] In addition to the morning peak, a secondary peak of MI onset in the evening hours has been reported in some earlier studies.[22,23] This peak was absent in our study as well as in two studies conducted by Leiza et al.[24] and Holmes et al.[25] Actually, although the circadian pattern with a secondary peak of MI onset between 12:01 and 18:00 in the present study is discordant with the western populations, it is similar with the Bulgarian populations.[26] Furthermore, our data are partly similar with the report from Argentine and Uruguay.[27]

The mechanism of a morning peak in AMI onset is well known. In the morning, sympathetic activity and platelets aggregation increase while fibrinolytic activity decreases.[23,28] These changes lead to altered hemostasis and an unbalance between myocardial oxygen store and demand, possibly resulting in thrombotic events in patients. It has long been speculated that the BP increases in morning due to the basal vascular tone changes related to α-sympathetic vasoconstrictor activity.[29] Other changes include renin activity, increased heart rate, and plasma concentrations of cortisol, adrenaline, epinephrine, norepinephrine, and angiotensin II.[30,31] Cortisol may further enhance sensitivity of the coronary vessels to the vasoconstrictor effects of catecholamines, and also, increased plasma levels of epinephrine and norepinephrine may increase vascular resistance in the morning.[32,33] Moreover, hemostatic changes, external triggers, and variations in gene expression

may make a shear stress resulting in atherosclerotic plaque disruption and thrombosis in the morning.[34]

We found that women had two dominant peaks of symptom onset in the morning and afternoon. Itaya et al. reported that men had a dominant peak of symptom onset in the morning (06:01-12:00) and women had two peaks of symptom onset in morning and evening, respectively.[10] Kinjo et al. from Japan reported that women aged 65 years or more showed a morning peak.[35] Gilpin et al. observed a unique pattern of occurrence in female patients with two peaks in the morning and evening,[36] while Leiza et al.[24] and Hansen et al.[37] observed no effect of sex.

For a long time, a sex-based difference has been reported in the field of cardiovascular diseases. In fact, women present some physiological differences compared to men. The effect of sex hormones on cardiac events is obviously reported.[38] This report may help to illustrate why women show a different circadian pattern compared to men in the onset of STEMI.

Conclusion

The present study of Iranian patients displayed circadian pattern of STEMI with 2 peaks in the morning and afternoon, and the both peaks were dominated by women. The results of the current study make clear that circadian pattern of AMI onset with morning and evening peaks may not be applicable worldwide; rather, we can mention 'population pattern' which needs to be illuminated for every population. The factors that are known to affect circadian pattern of MI onset may not be equally effective in all populations.

Limitations: This study had the well-known limitations of a cross-sectional design and our data were derived from a single center. Hence, our participants may not be the representative of the whole STEMI population. However, findings are almost consistent with those in the literature.

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Conflict of Interests

Authors have no conflict of interests.

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Evaluation of cardiac function in children after percutaneous closure of atrial septal defect using speckle tracking echocardiography

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Original Article

Abstract

BACKGROUND: Atrial septal defect (ASD) is among the most common congenital anomalies that its neglect may cause severe right ventricular (RV)-associated cardiac dysfunction. Percutaneous closure of ASD is an efficient technique used commonly worldwide. Varieties of techniques have been used to assess postoperative changes of cardiac function. The current study has aimed to assess outcomes of percutaneous ASD closure using two-dimensional speckle tracking echocardiography (2D-STE).

METHODS: This non-experimental research was conducted on 22 patients who volunteered for percutaneous ASD closure between 2016 and 2018. Cases were assessed three times including prior to percutaneous ASD closure, after 24 hours, and a month after procedure. Cases outcomes were assessed and compared during the time.

RESULTS: Strain rate in RV middle septal wall was significantly different ($P < 0.050$) between before and one month after the procedure. Comparison of indices post procedure and one month later showed better RV strain pattern but they did not have a significant difference ($P > 0.050$).

CONCLUSION: Based on this research, STE is a quick simple method of assessing cardiac chambers and function in details. It seems that this method can replace other traditional echocardiographic methods for cardiac function tests; thus, further studies with larger groups and longer follow-up duration are strongly recommended.

Keywords: Atrial Septal Defect; Vascular Closure Devices; Echocardiography

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Introduction

Atrial septal defect (ASD) is one of the most common congenital anomalies which accounts for 10%-15% of all congenital heart diseases (CHDs) and uncorrected remnants of ASD account for up to 30% of adult heart anomalies.[1] Most of ASD-affected children are asymptomatic while in untreated cases, it may turn to ventricular tachyarrhythmia, right ventricular (RV) dysfunction, and pulmonary hypertension (PHTN).[2]

Percutaneous closure of ASD has been raised by in 1974 for the first time. Performance of this procedure before the age of 25 years accompanies acceptable RV morphology, exercise physiology, and positive effects on remodeling function and dimension;[3] thus, it poses normal life expectancy.[4] Percutaneous approaches of ASD closure have been considerably promoted through the years and assessment of post procedure outcomes plays an important role in detecting short- and long-term quality and efficacy of ASD closure;[1,5] however, information in this regard is poorly explained.[6]

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Echocardiographic methods have been notably developed in recent decades and provided high quality of assessing RV and left ventricular (LV) function. Furthermore, new techniques have opened new windows of quantifying global and regional ventricular functions. Two-dimensional speckle tracking echocardiography (2D-STE) is a relatively new echocardiography technique for myocardial deformation evaluation.[7-9] The percentage of myocardial functioning is achieved using STE. This index is presented as strain rate measuring regional myocardial deformation per specific unit of time. Then global strain pattern is recorded using average of segmental values.[10] Information about use of 2D-STE for assessing outcomes of percutaneous ASD closure on four chambers function is limited in children. One study on adult patients has shown that strain rate of RV worsened after 24 hours of device closure.[5] This study has aimed to assess RV and LV functioning following percutaneous closure of ASD in children.

Although percutaneous closure of ASD has been well-established worldwide to preserve children's cardiac function, especially RV, studies assessing post-procedural outcomes are limited. Most of them have evaluated general function of heart post-procedurally but not in detailed indices. Most of the previous studies assessed RV ejection fraction (EF) as main index of cardiac function.[5,11] Echocardiography strain imaging known as deformation imaging is a quantifying technique of imaging that practically assesses myocardial function. These techniques have been previously utilized for assessment of ischemic diseases, cardiomyopathy, and even cardiac function following use of cardiotoxic chemotherapy remedies.[12,13] 2D-STE is one of the deformation techniques which is a simple quick means of assessing physiological and pathophysiological cardiac conditions.[14]

Materials and Methods

This non-experimental study was conducted on 26 patients who were candidates of percutaneous ASD closure referred to Imam Hossein and Shahid Chamran Hospitals affiliated to Isfahan University of Medical Sciences, Isfahan, Iran, between 2016 and 2018. Inclusion criteria were: a) age less than 18 years old, b) presenting indications of ASD closure, and c) willingness of patients' parents for participation in the study. Indications of ASD closure include one of these factors: a) significant shunt known as pulmonary flow index (Qp) compared to systemic flow index (Qs) (Qp/Qs) ≥ 1.5, b) significant shunt with diastolic rumble during auscultation of the tricuspid valve, c) signs of RV hypertrophy and RV enlargement in electrocardiogram (ECG), and d) signs of cardiomegaly or increased vascular markings in chest X-ray.[15] None of the patients had any symptom or exercise intolerance. Patients who did not refer for follow-up assessments after percutaneous ASD closure and those who had concurrent cardiac complex anomalies that need operation such as ventricular septal defect (VSD), pulmonary stenosis, and patent ductus arteriosus (PDA) were excluded.

Following proposal approval by Ethics Committee of Isfahan University of Medical Sciences (code: IR.MUI.REC.1396.3.721), all study processes were presented for patients' parents and they were requested to sign written consent form of their child participation in the study. Then patients were accurately examined by a pediatric cardiologist and their data such as age, gender, time of ASD diagnosis, and consumed medical remedies were recorded in a checklist. Then patients underwent STE by the pediatric cardiologist. In order to minimize inter-observer bias, all of STE steps were performed by a pediatric cardiologist fellow.

STE was performed measuring RV and LV function through assessment of basal lateral, middle lateral, apical lateral, apical septal, middle septal, basal septal, and global using strain and strain rate method. In order to perform echocardiography, patients were in supine or left lateral decubitus comfortable position using Echo 7 machine (Samsung, Korea), equipped with a 2-7 MHz probes and frame rate was 88 frames per second (fps). Controls were examined once while cases underwent echocardiography in a day prior to ASD closure and then 24 hours after closure. The last assessment was performed after one month of procedure. In order to save data, images of each patient and movie clips were stored on the echocardiography device. We used standard four-chamber view to obtain longitudinal strain and strain rate for RV and LV. Strain software thereafter was utilized to analyze saved data. This software is able to track endocardial borders derived from 2D longitudinal circumferential views. The endocardial border was drawn by the operator and then followed automatically frame by frame. The position of speckles was detected by operator based on the point's spatial coherence (Figure 1).

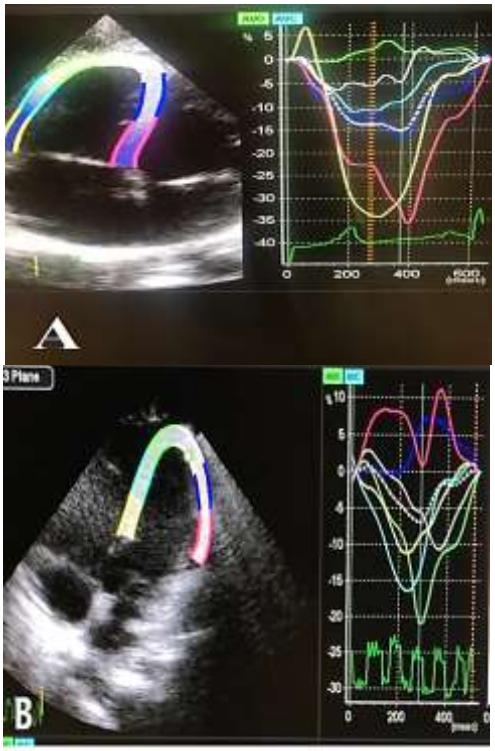

Figure 1. Speckle tracking in right ventricle (RV) (A) and left ventricle (LV) (B)

Then patients underwent percutaneous ASD closure. Echocardiography was performed within 24 hours and once again one month after the procedure. Data mentioned above were reassessed and recorded in the checklist.

The qualitative variables were expressed as the number and percentages. The quantitative variables were expressed as mean and standard deviation (SD). Kolmogorov-Smirnov test (K-S test) was used to check whether the variables had a normal distribution or not. Repeated measures analysis of variance (ANOVA) with post hoc was used for evaluation of the effect of procedure during time. SPSS software (version 22.0, IBM Corporation, Armonk, NY, USA) for further analysis was used for analyzing data. P-value less than 0.05 was considered as significant.

Results

Four cases were excluded from the study as they refused to participate in follow-up visits. Thus, study analysis was performed on 22 remained cases.

Mean ± SD of cases' age was 63.54 ± 60.00 (8-192) months and 11 were male (50%) and 11 were female (50%).

Comparison of indices post procedure showed better RV strain pattern and strain rate immediately after procedure and at one-month follow-up; however, RV strain rate changed to better state one month later as compared with findings obtained in a day after ASD closure (Table 1). In middle septal before the procedure and one month after the procedure, significant differences were seen (P = 0.032) although in other parts significant differences were not seen.

Discussion

In the current study, comparison of indices postoperatively showed better RV function indices after the procedure. Following the ASD closure, volume load on both ventricles changes, and this unloading of RV is the absolute benefit of ASD closure that can improve its function and prevent long-term further complications.[16]

Table 1. Comparison of right ventricular (RV) function indices before, one day, and one month after procedure

Variable		Before procedure (1)	The day after procedure (2)	One month after procedure (3)	P			P*
					1 and 2	1 and 3	2 and 3	
Strain	Global	-16.94 ± 9.36	-18.69 ± 4.81	-19.18 ± 3.54	0.460	0.430	0.700	0.298
	Basal septal	-34.25 ± 11.83	-29.64 ± 16.54	-33.57 ± 9.09	0.780	0.750	0.410	0.332
	Middle septal	-21.71 ± 11.02	-21.74 ± 9.22	-22.41 ± 11.12	0.980	0.840	0.680	0.942
	Apical septal	-11.00 ± 10.19	-11.71 ± 9.85	-9.57 ± 10.92	0.940	0.320	0.370	0.572
	Basal lateral	-20.55 ± 5.59	-20.58 ± 5.28	-21.70 ± 7.02	0.950	0.800	0.600	0.734
	Middle lateral	-15.67 ± 5.54	-17.31 ± 4.10	-18.58 ± 3.52	0.330	0.013	0.340	0.051
	Apical lateral	-11.40 ± 5.74	-9.85 ± 7.69	-10.52 ± 8.31	0.440	0.440	0.900	0.614
Strain rate	Global	-1.41 ± 0.34	-1.54 ± 0.24	-1.61 ± 0.30	0.230	0.230	0.540	0.234
	Basal septal	-2.13 ± 0.31	-1.87 ± 3.78	-2.07 ± 3.61	0.100	0.120	0.630	0.142
	Middle septal	-1.69 ± 0.94	-1.70 ± 0.58[1]	-1.71 ± 0.69[1]	0.130	0.032	0.340	0.045**
	Apical septal	-1.23 ± 0.02	-1.25 ± 0.71	-1.13 ± 0.34	0.240	0.470	0.850	0.464
	Basal lateral	-1.68 ± 0.58	-1.70 ± 0.69	-1.73 ± 0.76	0.740	0.660	0.620	0.878
	Middle lateral	-1.31 ± 0.53	-1.48 ± 0.68	-1.53 ± 0.51	0.670	0.800	0.690	0.709
	Apical lateral	-1.19 ± 0.60	-0.87 ± 0.28	-1.18 ± 0.91	0.530	0.600	0.600	0.464

Data are presented as mean ± standard deviation (SD)

* Repeated measures analysis of variance (ANOVA) with Bonferroni post hoc was used, ** P < 0.050 sets as significant

In our study, this improvement was seen especially in the middle septal segment of RV (the part which was more under the loading pressure due to specific RV geometry). Although our results indicate clinical improvement in RV function which has begun from middle lateral segment, it is not significant statistically.

Our results are similar to the study conducted by Agha et al.[17] which demonstrated that remodeling of both RV and LV was reversible after percutaneous ASD closure in pediatric group; however, their study was performed in older children.

Xu et al.[18] evaluated RV myocardial strains by STE after percutaneous ASD closure in children. They reported that RV strains were significantly higher in children before device closure. At 1 day after closure, all these measures decreased accordingly. This discrepancy between their findings and ours can be due to the differences in ages and ASD dimensions before procedure though they concluded that transcatheter device closure of ASDs improved RV strain indices, so its function recovered to normal over 3 months.

Bussadori et al.[5] performed a similar study on older population following ASD closure. They presented significant improvement of RV functions as the fluid volume decreased but immediately it got worse after closure. They presented this improvement in six-month follow-up as well which may be attributed to the chronic long-term effects of ASD on cardiac function (the mean age of patients was in the third decade). However, benefit of ASD closure on LV function was not statistically significant. Ding et al.,[11] Akula et al.,[19] and Pascotto et al.[20] performed other studies on outcomes of percutaneous ASD closure using echocardiography for function assessments. They presented similar outcomes as RV volumes diminished and EF improved while LV functions improved following intervention but not significantly.

The other study conducted by Vitarelli et al.[21] evaluated the efficacy of three-dimensional (3D) and 2D speckle tracking on assessing outcomes of ASD closure and its ability in prediction of further paroxysmal atrial fibrillation (AF) progression. Their study findings were consistent with our findings regarding RV function improvement. Furthermore, they presented that speckle tracking could successfully predict paroxysmal AF progression among patients with ASD. Ozturk et al.[3] performed another study assessing efficacy of speckle tracking in adults for evaluation of ASD closure outcomes. They presented that all RV-related aspects including right atrium (RA) volume and RV end-diastolic diameter (RVEDD) improved significantly following percutaneous ASD closure. Indices related to left side of heart showed significant decrease in left atrial (LA) diameter postoperatively while LV end-diastolic diameter (LVEDD) remained unchanged. Furthermore, RV longitudinal strain increased significantly following ASD closure.

Compared to other studies, most of them on adults, our study was conducted on children. Considering the better compliance of pediatric hearts, it seems that the improvement of cardiac function in children is faster than adults. The difference in the results of the measurements found in different parts of the RV is due to the fact that we have had more non-symmetric RV hypertrophy and a lower pulmonary pressure in some patients. More improvements in other indices may appear in subsequent pursuits and long-term follow-up. Due to volume overload effect on the RV in comparison to LV, atrial septal correction had further effects in the right side of heart and improved its function more.

Conclusion

Based on this research, our findings indicate improvement of RV function post intervention. As STE is a quick simple method of assessing cardiac chambers and function in details, it seems that this method can replace other traditional echocardiographic methods for cardiac function tests.

Limitations: According to our knowledge, very few studies are currently available using this method in children. Small number of sample population and short-term follow-up are the most significant limitations of our study. Further multicenter studies with larger sample population and long-term follow-up are recommended.

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Conflict of Interests

Authors have no conflict of interests.

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Rationale, design, and preliminary results of the Iran-premature coronary artery disease study (I-PAD): A multi-center case-control study of different Iranian ethnicities

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Original Article

Abstract

BACKGROUND: Premature coronary artery disease (CAD) is still prevalent worldwide and may differ in various ethnicities. Due to the presence of different ethnicities in Iran, the Iran-premature coronary artery disease (I-PAD) study aimed to determine the frequency of premature CAD and related risk factors based on each ethnicity.

METHODS: In this multi-center case-control study, 4000 patients with premature CAD from ten different ethnicities who lived in different cities of Iran and underwent coronary angiography were enrolled (women aged ≤ 70 and men ≤ 60 years). Patients with CAD defined as obstruction equal or above 75% in at least a single coronary artery or left main ≥ 50% were included in the case group, while patients with normal coronary arteries were included in the control group. Lifestyle behaviors, cardiometabolic risk factors, anthropometric measurements, and other variables were collected. Serum, whole blood, buffy coat, plasma, urine, stool, and saliva samples were stored.

RESULTS: The number of patients enrolled until April 2020 was 2071. The mean age of patients was 53.51 ± 7.52 and 934 (45.09%) of patients were women. To date, about 39.6% of the patients were normal. Also, about 26.0% were with one-vessel disease (1VD), 15.0% with two-vessel disease (2VD), and 15.2% with three-vessel disease (3VD). More than 30000 patients' biosamples from across the country have been stored.

CONCLUSION: Knowing the frequency of premature CAD according to different ethnicities with major differences in their lifestyle behaviors and risk factors can assist health decision-makers. In addition, I-PAD biosamples will be an invaluable source.

Keywords: Coronary Artery Disease; Ethnic Groups; Risk Factors; Biological Specimen Banks; Iran

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Introduction

Cardiovascular disease (CVD) is one of the most important causes of disability that accounts for up to 30% of mortality worldwide. The prevalence of CVD in Iran is also high, while some studies report it more than 30%.[1-3]

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Premature coronary artery disease (CAD) in Iran is highly frequent which justifies the reason for further studies and better understanding of its risk factors and determinants.[2-5]

Behavioral and cardiometabolic risk factors like hypertension (HTN), diabetes, dyslipidemia, smoking, unhealthy diet, and low physical activity are important risk factors that have been reported in numerous studies previously. However, studies have shown that some of these factors, such as smoking, family history, unhealthy nutrition, and blood lipids play a stronger role in premature CAD.[5-8]

The prevalence of CAD differs in various populations which may be connected to their ethnicities.[9-12] Iran is one of the countries with multiple ethnicities, including Fars (Persian), Azari (Azeri, Turk, or Tork), Kurd (Kord), Arab, Lor (Lur), Gilak, Balouch (Balouchi or Balooch), Turkman (Torkman or Turkmen), Qashqaei (Qashghai), and Bakhtiari. These ten ethnicities consist more than 95 percent of Iran's population. Fars is considered to be the largest ethnic group in Iran, estimated to comprise between 50% and 70% of the population, followed by Azari, as the second largest ethnic group (15% to 24%), Kurd from 7% to 10%, Gilak from 3% to 6%, Arab from 2% to 4%, Lor from 3% to 5%, and Turkman, Bakhtiari, Qashghaei, and Balouch each with 2%.[13]

Iran-premature coronary artery disease (I-PAD) study aims to investigate the frequency of premature CAD and its risk factors according to different ethnicities in Iran, so the results can be a gateway to some preventive and treatment strategies. This report addresses the rationale, methodology, and some earlier results of I-PAD study.

Materials and Methods

I-PAD is a multi-centric case-control study which is ongoing on Iranian patients with different ethnicities. Patients are men and women who underwent coronary angiography. We recruited our patients from hospitals with catheterization laboratories in different cities. Patients are selected from across the country based on the distribution of different ethnicities (Fars, Azari, Kurd, Arab, Lor, Gilak, Balouch, Turkman, Qashqaei, and Bakhtiari). Each ethnicity was considered positive if the patient and his/her parents had the same ethnicity. We included questions on ethnicity of the patients and their parents after obtaining their consent to participate in the study. If a patient indicated other ethnicity of even one of his/her parents, we excluded him from the study. Sampling will continue through convenience sampling until our study sample reaches 4000 individuals, depending on the percentage of different ethnicities in the population.

Inclusion criteria consist of patients who underwent coronary angiography, age ≤ 70 or ≤ 60 years for women and men, respectively, being related to one of the ethnicities considered in our study, and being familiar with their parent's ethnicity. Having an occlusion of at least single coronary artery equal and above 75% or left main coronary of equal or more than 50% and normal coronary artery were our criteria for case and control groups, respectively. Previous history of documented coronary artery disease such as coronary artery bypass surgery, balloon angioplasty, or percutaneous coronary intervention (PCI) was considered as exclusion criterion.

We calculated our sample size in each group with an error of the first type of 0.05, power of 0.8, and considering an odds ratio (OR) = 1.30. Our sample is about four-thousand patients. Individuals are recruited using convenience sampling in reference hospitals in more than twelve cities in Iran. The total sample was divided proportionally according to the distribution of each ethnic group. Calculated sample for each ethnicity is as follows: 2000 Fars, 510 Azari, 400 Kurd, 250 Gilak, 140 Arab, 140 Lor, 140 Turkman, 140 Bakhtiari, 140 Qashghaei, and 140 Balouch patients.

Initially, we designated focal points who were the heads or one of the authorities of cardiac catheterization centers in each city with one major ethnicity. In addition, we asked them to establish their team who could recruit patients according to our inclusion criteria and complete our questionnaires. We organized necessary training sessions for the executive team on how to interview patients and complete questionnaires. Furthermore, we developed a study practical manual and sent to all teams following our training sessions. We provided all necessary information on I-PAD study to patients who met our inclusion criteria. Then we obtained written consent forms from the patients.

Interviewers in hospitals completed our

questionnaires that included questions on demographics like age, sex, ethnicity, religion, education, income, marriage status, and lifestyle behaviors such as any type of smoking, alcohol or drug use, and nutrition via Food Frequency Questionnaire (FFQ), physical activity by International Physical activity Questionnaire (IPAQ), anxiety level through Hospital Anxiety and Depression Scale (HADS), sleep disorders using the Pittsburgh Sleep Quality Index (PSQI), personal and family history of illnesses and used medications. Questionnaires' reliability and validity were assessed if they were not done before. Individuals removed shoes and heavy cloths prior to anthropometric measurements, then height, waist, hip, neck, and thigh circumferences were measured in all patients, according to standard protocols.

Biobank: Blood, urine, stool, and saliva samples were taken from each participant. The patient should have been fasting for at least 12 hours. Blood samples were taken to measure triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and fasting blood sugar (FBS). Also, after the necessary procedures, in the shortest possible time, serum, buffy coat, plasma, whole blood, saliva, urine, and feces were taken of each patient and stored in specific cryoboxes then in a -80 °C freezer. Isfahan central core laboratory (Isfahan, Iran) executive team was responsible for receiving and storing the biosamples sent from different cities properly.

Quality assurance and quality control: To ensure the quality of the project, several steps were taken, some of which are briefly described here. Initially, the design of questionnaires and data collection forms used in this study was approved and finalized by an expert team in each field. In each questionnaire, detailed, clear, and purpose-based questions were considered. The team included cardiologists, a nutritionist, a psychiatrist, an epidemiologist, a biobank expert, and a statistician.

Then, a comprehensive questionnaire and laboratory protocol were designed, and training sessions for questioners and project executives in different cities were held in Isfahan center to make the implementation of the project more uniform.

Random checking of the data entry in the database was done by a separate expert to check the accuracy of the entered data. Pilot design also helped researchers to identify existing defects, and greatly improved the quality of the study. A unique identity document (ID) for each patient was used on the questionnaire and all biosamples, and it was used throughout the whole study process.

The quality control unit of the Isfahan Cardiovascular Research Institute which is a World Health Organization (WHO) collaborating center was responsible for periodic monitoring and inspections to ensure the best possible performance. The committee members conducted field visits to ensure the highest study performance and high-quality data collection and management.

Performing multiple interim analysis, once every two weeks at the beginning of the project and then, once a month while executing the project, receiving the necessary feedback, and making changes to each of the steps as necessary are among other measures that have been taken.

This study was reviewed and approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.REC.1396.2.055). A written informed consent was obtained from all patients and the Declaration of Helsinki was considered. Patients were provided with a complete description of the study and all their questions were answered. To keep the confidentiality, the IDs assigned to each patient were specific codes that did not correspond to the patient's specification, such as his or her national code. All people involved in this study who somehow had access to patient information were given the necessary training in this regard. Access to patient data was limited as much as possible. The outcome of data was not reported individually and patient information was gradually analyzed and disseminated.

The analysis was done in two descriptive and analytical sections. The prevalence of risk factors in the two groups of patients with premature CAD and the other is reported by ethnicity. Quantitative variables in different groups are reported as mean ± standard deviation (SD). Univariate comparisons between case and control groups was performed according to the type of risk factor by comparison of means (independent t-test or Mann-Whitney) based on statistical assumptions or chi-square test based on qualitative variables. Effect size determination based on different risk factors was extracted by reporting ORs in logistic regression modeling. Conditional and unconditional logistic regression modeling were performed considering the confounders' elimination strategy. The receiver operating characteristic (ROC) curve was used to evaluate the difference between the case and control groups, based on risk factors. All analyses were performed at 5% error level, using Stata software (version 14, Stata Corporation, College Station, TX, USA).

Results

The number of patients enrolled in the study by February 2020 was 2071. The participants were patients with ethnicities such as Fars, Azari, Kurd, Lor, Bakhtiari, Qashqaei, Balouch, Arab, and Gilak. 1137 (54.91%) of the patients were men and 934 (45.09%) of the patients were women (Table 1).

Our enrollment is completed for Bakhtiari and Fars ethnicity; however, around 67% of originally calculated Kurd, 27% of Lor, 84% of Qashghaei, 7% of Arab, and 2% of Gilak, Balouch, and Turk patients were completed to date. The cities of Isfahan, Birjand, Yazd, Shahrekord, Rasht, and Orumiyeh are recruiting patients in a way to complete the sample according to ethnicities. Each city focused on one ethnicity or several specific ethnicities (Figure 1).

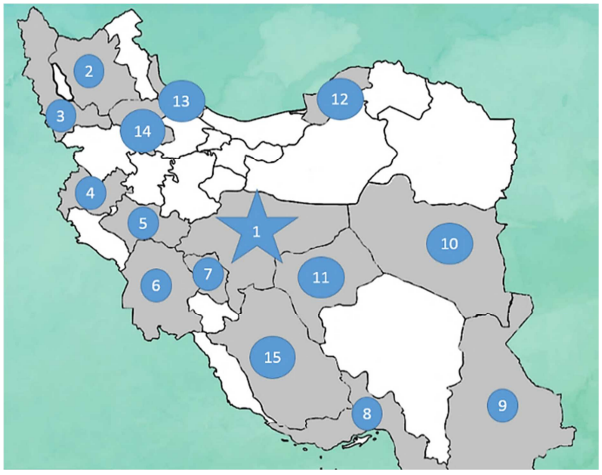

Figure 1. Cities included: Isfahan (1), Tabriz and Maragheh (2), Orumiyeh (3), Kermanshah (4), Khorramabad (5), Ahvaz (6), Shahrekord (7), Bandar Abbas (8), Zahedan and Zabol (9), Birjand (10), Yazd (11), Gorgan (12), Rasht (13), Zanjan (14), Shiraz (15)

To date, about 39.6% of the patients were normal. Also, about 26.0% were with one-vessel disease (1VD), 15.0% with two-vessel disease (2VD), and 15.2% with three-vessel disease (3VD).

Discussion

Currently, premature CAD is one of the most important and prevalent diseases in the world with a growing trend. At the same time, it is one of the diseases that can be reduced by preventive strategies.[1-3,5] The instructions and guidelines available for dealing with heart disease risk factors and their treatment are the same across our country, and even in most countries around the world, as this is one of the biggest problems in the field of disease prevention and treatment. Because patients of different races and ethnicities may have different risk factors or the relative risk of each risk factor may differ in various populations, that can be due to behavioral, environmental, and genetic differences, as well as different gene-environment interactions. In fact, a risk factor may be more important in one ethnicity and less crucial in another.[9-11,13]

There are different ethnicities living in Iran with different lifestyles;[13] yet, no comprehensive study has been conducted to investigate the different cardiovascular risk factors among these ethnicities. The present study (I-PAD) is one of the largest studies in our country and the region on ethnic differences in terms of extent and sample size. This study investigates the risk factors of premature CAD among people of different ethnicities in more than ten cities of Iran. Although there have been limited studies in the past, none has comprehensively covered most Iranian ethnicities or ethnicities were not assured as in our study.[9,10]

Table 1. Frequency distribution of different ethnicities by age and sex

Variables / Ethnicities	Total (n)	Age (year)	Sex		Report of angiography	
		Mean ± SD	Men [n (%)]	Women [n (%)]	Normal [n (%)]	Premature CAD [n (%)]
Fars	1441	53.66 ± 7.33	818 (56.80)	623 (43.20)	559 (40.65)	816 (59.35)
Azari	8	51.00 ± 9.68	6 (75.00)	2 (25.00)	3 (37.50)	5 (62.50)
Kurd	271	53.89 ± 7.93	120 (44.30)	151 (55.70)	114 (42.54)	154 (57.46)
Lor	39	49.26 ± 10.40	23 (59.00)	16 (41.00)	20 (54.05)	17 (45.95)
Bakhtiari	178	52.66 ± 7.66	99 (55.60)	79 (44.40)	64 (38.32)	103 (61.68)
Qashqaei	118	53.77 ± 7.30	62 (52.50)	56 (47.50)	52 (45.61)	62 (54.39)
Gilak	3	53.00 ± 6.08	1 (33.30)	2 (66.70)	1 (33.33)	2 (66.67)
Arab	10	52.70 ± 6.21	7 (30.00)	3 (70.00)	3 (30.00)	7 (70.00)
Turkman	0	-	0 (0)	0 (0)	0 (0)	0 (0)
Balouch	3	56.33 ± 4.04	1 (33.30)	2 (66.70)	1 (33.33)	2 (66.67)
Total	2071	53.51 ± 7.52	1137 (54.91)	934 (45.09)	817 (41.16)	1168 (58.84)

CAD: Coronary artery disease; SD: Standard deviation

One such study by Jahangiry et al. was a cross-sectional study of 3506 participants, aged 30-70 years in Naqadeh (northwestern Iran, including two ethnicities, Kurd and Azari). Patients were evaluated for cardiometabolic risk factors associated with metabolic syndrome. Sixty percent of the study population were Azari. The researchers recommended that the identification of different components of the metabolic syndrome based on two studied ethnicities would be an appropriate step in determining interventional strategies, considering different ethnicities in Iran.[10] Our study, however, examines more ethnicities and risk factors in a national sample representing ten ethnicities in Iran that comprise more than 95% of the total population.

In this study, the ethnicity of parents is considered in addition to individual ethnicity. In fact, in many studies, the boundary between the use of the word "ethnicity" and the word "race" is blurred, and sometimes the two words are used interchangeably.[14,15] In studies of different races, it is much easier to separate races than to study different ethnicities. In addition, in most studies on ethnicity, participants report their own ethnicity and not that of their parents, which in many cases may cause problems in the final analysis.[9-11,16,17] For example, a person who was born of an Azari mother and a Fars father may, for any reason, identify himself as a Fars or an Azari. Even a person born to parents of the same ethnicity, for whatever reason, presents herself/himself as an ethnicity that is the predominant ethnicity of the city where she/he lives. However, in the present study, one of the inclusion criteria was considered to be same parental ethnicity, in order to minimize such errors in data analysis. In addition, the paternal and maternal grandparents' ethnicity is also questioned although it is not included in the inclusion criteria, but it can be considered in later analyses.

Also, considering the dominance of Fars ethnicity in the country (between 50 and 70 percent of the population),[13] Fars people from four different cities (Isfahan, Birjand, Yazd, Shahrekord) were included in this study to investigate possible differences and gain a better understanding of gene-environment interaction. These cities are located in different parts of Iran with probably different risk factors and different environments.

Establishment of biobank is also one of the most important steps in this study. Serum samples, buffy coat, plasma, whole blood, saliva, urine, and feces were taken from each patient. This biobank is able to hold samples for years to be used in future studies like epigenetics. In addition, one of the advantages of this study was feasibility study in Isfahan. Thus, about 5% of all samples were initially studied in Isfahan. All glitches were carefully examined and resolved at each stage of the project.

This study has some limitations like any other study. There are, however, other ethnicities in Iran but their community is not large enough to be considered. In addition, they have no catheterization laboratory in their living areas and should travel to larger cities which made the process of diagnosis of their ethnicity more difficult.

Due to existing limitations, it was not possible to examine other ethnicities such as Taleshi, Georgian, Kurmanji, etc. However, the percentage of these ethnicities in the country is very low (less than 5%), and there may not be enough individual available to meet our criteria.

Conclusion

I-PAD is one of the largest studies of ethnicity in Iran and the Middle East area. Studying the prevalence of premature CAD and its risk factors among different ethnicities of the country can give a much better perspective to health care providers, as well as national health decision-makers. In addition, the biobank established in this project could be one of the most important sources for genetic and epigenetic studies in Iran in the coming years.

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Conflict of Interests

Authors have no conflict of interests.

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A substitution mutation in LRP8 gene is significantly associated with susceptibility to familial myocardial infarction

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Short Communication

Abstract

BACKGROUND: Myocardial infarction (MI) is a multifactorial disease caused by the suspension of blood circulation in a part of the myocardium. Understanding the genetic basis of MI can provide insight regarding the pathogenesis of the disease. The aim of this study was to investigate the association between pathogenic mutations and early-onset MI in five families with familial MI and without common MI risk factor.

METHODS: Patients with MI younger than 50 years with family history of MI and without common diagnostic criteria (obesity, diabetes, familial hypercholesterolemia, opium/alcohol use) were evaluated for pathogenic mutations by whole exome sequencing (WES) and mutation was confirmed by polymerase chain reaction (PCR)-Sanger sequencing.

RESULTS: The c.2855G > A missense mutation with homozygous autosomal recessive inheritance was identified in low-density lipoprotein receptor-related protein 8 (LRP8) gene in all patients of a family.

CONCLUSION: The c.2855G > A (R952Q) mutation in LRP8 gene in homozygous state could be considered as a possible etiology of early-onset familial MI.

Keywords: Myocardial Infarction; Low Density Lipoprotein Receptor-Related Protein 8; Whole Exome Sequencing

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Introduction

Myocardial infarction (MI) is one of the underlying causes of morbidity and mortality and it causes more than 30% of all deaths worldwide.[1] According to the recent report of the heart and cardiovascular center of Iran Health Ministry, about 300 patients die each day due to cardiovascular disease (CVD).[2] High incidence of CVD in Iranian population shows the importance of investigating the cause of MI in this population. MI is a complex multifactorial disease, which involves both environmental and genetic factors and their interactions.[3,4] The coronary artery disease (CAD) risk factors include (but not limited to) diabetes, smoking, hypertension (HTN), hyperlipidemia, age, and gender.[5] Lifestyle risk factors have an important role in the incidence of CAD and MI. However, the role of genetic factors cannot be ignored in etiology of the CAD and MI pathogenesis.[6] The heritability of CAD and MI was estimated approximately 50 to 60 percent by the long-recognized familial clustering of CAD which suggests that genetics plays a critical role in the CAD and MI development.[7] Genetic evaluation for finding pathogenic mutations in patients with early-onset CAD and MI can be useful.[4] Early-onset MI in a first-degree relative which is younger than 55 years in men and younger than 65 years in women could

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be defined as an independent risk factor for CAD.[7] One-quarter of early-onset MIs are unrecognized MI (UMI) and recognition is critical to minimize further cardiovascular complications.[8] Whole exome sequencing (WES) is a valuable screening tool for clinical diagnosis of familial CAD, particularly for cases without common diagnostic criteria and sudden cardiac death.[9] We aimed to identify the pathogenic mutations responsible for early-onset MI in five families with familial MI and without common MI risk factor.

Materials and Methods

Forty patients with early-onset MI and premature CAD were evaluated in the Angiography Center of Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran, and five families with at least five definite diagnosis of patients with MI were selected and referred to the third universal definition of MI.[10] Patients with definite MI and premature CAD on the basis of coronary angiography were referred for genetic counseling in Amin Genetic Counseling Center, Marvdasht, Iran. Both environmental and hereditary factors can cause CAD and MI. Therefore, we selected the familial form of CAD and MI disease with at least five patients in a pedigree to increase the chances of identifying genetic factors, and excluded patients and families with environmental risk factors of CVD (such as diabetes, familial hypercholesterolemia, and opium consumption) from the study. In this study, five families with these characteristics were investigated. Information about family history of CAD and MI, diabetes (2 hours postprandial glucose ≥ 200 mg/dl, fasting blood glucose ≥ 126 mg/dl, or use of insulin or hypoglycemic agents), dyslipidemia [triglycerides (TGs) > 150 mg/dl, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dl, or high low-density lipoprotein cholesterol (LDL-C) based on Adult Treatment Panel III (ATP III)],[11] HTN (use of antihypertensive drugs or positive past history of HTN), smoking or opium consumption, age, and gender were collected from probands and their family members. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood of patients and their family members using a High Pure PCR Template Preparation Kit ("Roche Life Science", Germany) (this research was approved by the local institutional review board). All DNA samples' concentration was 30 µg and A260/280 ratio was ~ 1.8. After quantitative and qualitative assessment using standard techniques, DNA was subjected to WES. WES was carried out for five probands. Paired-end sequencing with 101-base reads was performed on Illumina's HiSeq2000 platform (Illumina, San Diego, CA, USA). In a proband, WES result showed 1048 variants. After data and variants analysis, a missense mutation (c.2855G > A) was identified in exon 19 of lipoprotein receptor-related protein 8 (LRP8) gene in homozygous state. For suspected mutation (LRP8 gene c.2855G > A variant), polymerase chain reaction (PCR) primers (Table 1) were designed manually and using Primer-BLAST and Primer3 to amplify the mutation containing fragment. PCR amplification was carried out in a total volume of 25 µl containing 12.5 µl PCR Master Mix (Promega, Madison, USA), 30 ng of genomic DNA, 0.5 µM of each primer, and 5.5 µl double-distilled water (DDW). Sanger sequencing was performed in proband and other patients in pedigree.

Results

In this study, we collected information about probands and their family members and excluded families with cardiovascular risk factors from the study as explained above. WES was carried out for five probands. The c.2855G > A (p.R952Q) (rs5174) missense mutation was identified in exon 19 of LRP8 gene, chr1:2,315,167 in homozygous state in proband of a family by WES (Table 2). Pedigree analysis of the family was consistent with autosomal recessive inheritance of CAD (Figure 1). Proband (V.1) was a 39-year-old man with early-onset MI at the age of 35. Family history showed that his father (IV.8), grandfathers (III.2, III.9), uncle and aunt (IV.9, IV.11), and his male cousin (V.6) were diagnosed with early-onset MI and premature CAD. Although next generation sequencing (NGS) is a high throughput sequencing method, it has not been approved as a clinical diagnostic test; therefore, we designed PCR primers and amplified the fragment containing the c.2855G > A mutation point. Sanger sequencing was done which confirmed the sequencing data.

Table 1. Sequence of forward and reverse polymerase chain reaction (PCR) primers

Gene	Primer	Primer length (bp)	Tm	GC%
LRP8	Forward: TTTGCCAAAGCTAACCCACTG	21	59	47
	Reverse: CCTCATGGGTAGTGCAACCA	20	59	55

LRP8: Low-density lipoprotein receptor-related protein 8

Table 2. The characteristics of c.2855G > A (p.R952Q) mutation in low-density lipoprotein receptor-related protein 8 (LRP8) gene

Gene and transcript	Variant	Location	Zygosity	Inheritance	Associated disease	OMIM	CADD score	Polyphen
LRP8 NM_004631	c.2855G>A p.R952Q	1p32	HOM	AR	Type 1 MI	602600	34	Probably damaging

OMIM: Online Mendelian Inheritance in Man; CADD: Combined annotation dependent depletion; LRP8: Low-density lipoprotein receptor-related protein 8; NM: NCBI reference sequence (locus); HOM: Homozygous; AR: Autosomal recessive; MI: Myocardial infarction

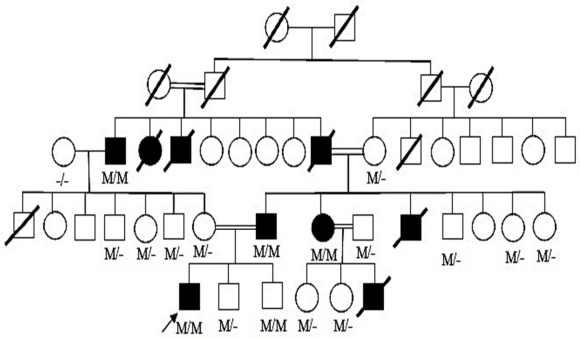

Figure 1. Pedigree of a family demonstrating autosomal recessive inheritance of early-onset myocardial infarction (MI); individuals with early-onset MI are indicated by solid squares (men) or solid circles (women). Unaffected individuals are indicated by open symbols. Deceased individuals are indicated by a slash (/). The proband is indicated by an arrow. Genetic status: M/M indicates the presence of mutation (homozygous); M/– indicates heterozygous status, and –/– indicates the absence of the mutation.

Mutation was confirmed in homozygous state in proband by PCR-Sanger sequencing and segregation analysis revealed mutation in heterozygous and homozygous states in pedigree (Figure 2). There are no data about first and second generations. Proband's youngest brother had the c.2855G > A mutation in LRP8 gene in homozygous state without CAD that may be due to his younger age.

Discussion

According to the latest statistics of World Health Organization (WHO), twelve million people die each year due to CAD worldwide.[12] In Iranian population, CAD is the most common cause of death,[12] and MI is the most severe type of CAD, which is ranked as the leading cause of death worldwide.[13] Recent update of American Heart Association in 2017 showed that 12.2% of patients aged ≥ 20 years had a parent or sibling with angina or heart attack before age of 50 years.[14] As mentioned above, early-onset MI in a first-degree relative could be considered as an independent risk factor for CAD,[7] and WES is a valuable screening tool to evaluate patients with suspected inherited CAD.[9] According to the NGS-based study on early-onset CAD that was carried out on approximately 5000 cases with early-onset CAD in order to find genes of significant associations with CAD in 2015, 2% of studied patients with early-onset CAD harbored at least a rare variant on LDL receptor (LDLR).[15]

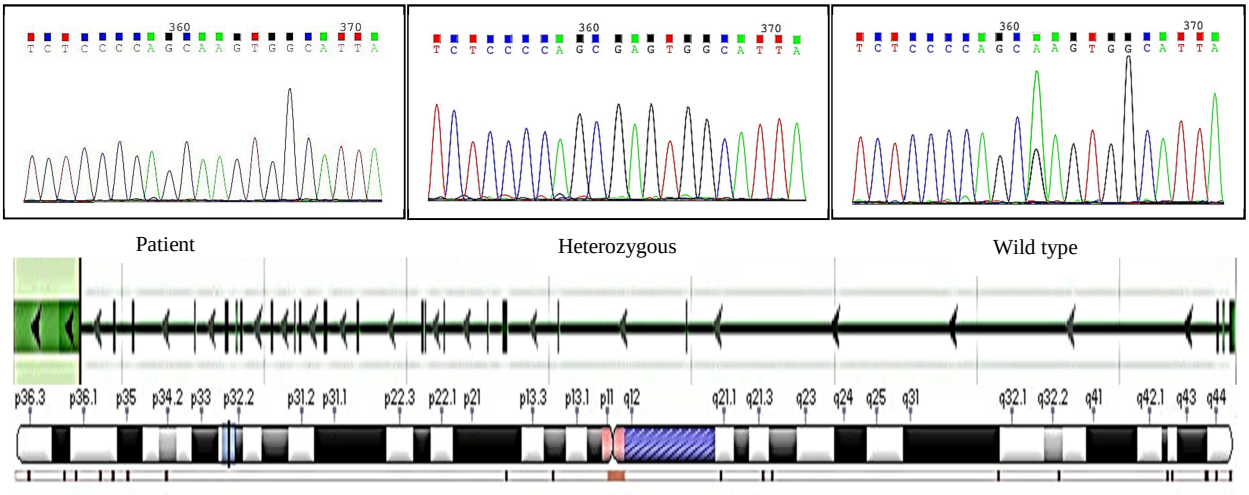

Figure 2. Truncated sequencing chromatogram of low-density lipoprotein receptor-related protein 8 (LRP8*)* gene of patients; the mutation point is indicated by an arrow.

In this study, WES helped us identify the cause of early-onset MI. The utility of exome sequencing as a fast and cost-effective technique in diagnosis of hereditary CAD, where the clinical diagnosis is uncertain, has been discussed.[9] The proband and other patients in our studied pedigree showed the missense mutation p.R952Q in LRP8 gene. LRP8 gene encodes LDLR which plays a critical role in lipoprotein metabolism and facilitates the clearance of LDL and very-low-density lipoprotein (VLDL) from plasma,[3] and is reported to be associated with early-onset and familial MI.[16,17] The LRP8 gene is highly expressed in the testes and brain; however, it is also expressed in the vascular smooth muscle cells, platelets, endothelial cells, and heart.[18] LRP8 gene encodes a member of the LDLRs family which play role as cell surface proteins. Signal transduction and receptor-mediated endocytosis of specific ligands for lysosomal degradation are the main role of LDLRs. Also, LDLRs play a critical role in the migration of neurons during development by mediating Reelin signaling,[19] and may be a marker for complex psychiatric disorders.[20] In work-up of patients in the present study, we found that patients had stressful lifestyle. However, we could not find history of psychiatric disorders in patients. Findings of this report show that the c.2855G > A (R952Q) mutation in LRP8 gene in homozygous state could be considered as a possible cause of early-onset familial MI. In this study, we found nine patients with early-onset CAD and MI that five patients died before the age of 50 years old. Parents in this family had consanguineous marriage. Shen et al. genotyped and analyzed a single-nucleotide polymorphism (SNP) (rs5174) of LRP8 in 381 patients with familial early-onset CAD, 183 patients with MI, and 560 controls. Results of their study showed that the c.2855G > A (R952Q) mutation in LRP8 gene conferred a significant risk of familial early-onset CAD/MI.[21] Also, Shen et al. studied multiple independent populations in 2007, which showed that genetic variants in LRP8 might contribute to the development of premature CAD and MI in familial form of the disease.[18] A case-control study by Martinelli et al. in the Italian cohort suggested that the c.2855G > A (R952Q) variant might have an additive effect to apolipoprotein E (APOE) genotype in determining APOE concentrations and risk of premature CAD and MI.[17] However, Asif et al. sequenced regions of a SNP (rs5174) of LRP8 in 100 patients with MI and 100 age-matched controls. Results of their study showed that the c.2855G > A (R952Q) mutation in LRP8 gene was not significantly associated with MI.[3] To better understand the association between c.2855G > A mutation in LRP8 gene and familial MI, we need large population studies on familial MI.

Conclusion

There was a significant association between c.2855G > A (p.R952Q) mutation and premature CAD and familial MI. However, further research is required to identify other unknown genes that cause premature CAD and familial MI in patients without common premature CAD and MI risk factor.

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Conflict of Interests

Authors have no conflict of interests.

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Relationship between periodontitis and cardiovascular disease

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Letter to Editor

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Dear Editor

In Iran, as in most countries of the world, cardiovascular diseases (CVDs) have been the leading cause of death in recent years and have replaced infectious diseases.[1,2]

According to the latest Global Burden of Disease (GBD) statistics, Iran has one of the highest rates of CVDs in the world and there is not much difference between genders and between rural or urban individuals, and even recently, the prevalence of the disease in young people has increased drastically.[2]

Due to the increasing age of the Iranian population, it is expected that the prevalence of these diseases will increase over the next decade, and hence, they should be paid more attention.[1]

On the other hand, several studies have shown that periodontal diseases are very common in Iran, however they are preventable and treatable.[3] These diseases are caused by inflammation of the gums due to chronic microbial infection in the tissues around the teeth, which leads to inflammation and destruction of the gums and protective tissues of the teeth, finally damaging the teeth.[4]

Considering that atherosclerosis, the most important cause of CVDs, is the result of a disorder in metabolism and accumulation of the cholesterol due to inflammation, it has been hypothesized for years that there may be a link between gingivitis and its circulatory inflammatory mediators entering the bloodstream, to the development of atherosclerosis, and several studies have been conducted and are ongoing.[4,5]

Currently, there is a consensus that both diseases are multifactorial and many risk factors are common between them, such as smoking, diabetes, addiction, old age, etc.[5]

Although some studies have not found a direct link between periodontal disease and CVDs, several meta-analyses have confirmed that there may be a link between the prevention and treatment of oral diseases and reducing the risk of CVDs.[3,4,6]

Therefore, in addition to conducting clinical trials in our country on this issue, it is recommended that appropriate planning be conducted by health officials and health care providers to avoid the pressure caused by the burden and treatment costs of CVDs in coming years. This is possible by simply addressing preventable and treatable underlying causes such as periodontitis.

Conflict of Interests

Authors have no conflict of interests.

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