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Iranian Heart Journal

OFFICIAL PUBLICATION OF THE IRANIAN HEART ASSOCIATION

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Iranian Heart Journal

THE OFFICIAL PUBLICATION OF THE IRANIAN HEART ASSOCIATION

EDITORIAL

In the Name of God, the Most Beneficent, the Most Merciful

Dear colleagues and friends:

We are delighted to present to you Volume14, Number4 (Winter, 2014) issue of the Iranian Heart Journal (IHJ), which contains some interesting and new studies and case reports in the cardiovascular medicine and surgery domains from our colleagues across Iran.

IHJ is indexed in the Scientific Information Database (WWW.SID.IR) and IMEMR and Index COPERNICUS, SCOPUS, CINAHL, facilitating access to published literature. There is no doubt, however, that IHJ requires your opinions, ideas, and constructive criticism in order to accomplish its main objective of disseminating cutting-edge medical knowledge.

As ever before, we continue to look forward to receiving your latest research and cases.

Yours Truly,

A. Hussein Tabatabaie, M.D.

Editor-in-Chief,
Iranian Heart Journal

F. Noohi, M.D.

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ORIGINAL ARTICLES: BASIC AND CLINICAL SCIENCES

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Tissue Sphingosine Kinase 1 Gene Expression in Female Rats: Effects of Endurance Running and Pistachio-Atlantica (Bene) Supplementation

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Abstract

Sphingosine kinase is a key enzyme in modulating the levels of lipids and is emerging as an important and regulated enzyme. The aim of this study was to determine sphingosine kinase 1 (SK1) relative gene expression in the liver, small intestine, and kidney. Twenty Wistar rats (6-8 weeks old, 125-135 g) were used. Animals were randomly assigned into saline-control (SC), saline-training (ST), Bene-control (BC), and Bene-training (BT). Training groups was given exercise on a motor-driven treadmill at 25 m/min (0% grade) for 60 min/day and 5 days/week for eight weeks. Subjects were fed orally with Bene extraction and saline for four weeks. SK1 relative gene expression was detected via the Real-time PCR method. Results demonstrated that exercise and Bene extraction significantly increased SK1 relative gene expression in the liver, small intestine, and kidney (p value < 0.006, p value < 0.007, and p value < 0.023, respectively); the increase, however, was not significant in visceral fat tissue.

Keywords: Sphingosine kinase ■ Female rats; Treadmill exercise ■ **Pistachio-Atlantica**

Introduction

Coronary artery disease (CAD) is one of the major causes of death in most societies and is associated with the concentration of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C lower).[1] The role of high-density lipoproteins (HDL) as the best endogenous predictor of the development of CAD and cardiovascular mortality has been clearly established.[2-4]

During recent years, growing insight into the properties of HDL has changed our perception of HDL: from mere cholesterol carriers, they have become global molecular players that impact on many different facets of cellular behavior.

The most common molecular explanation for the cardiovascular protection conferred by HDL has been their fundamental role in the reverse cholesterol transport process, by which excess cholesterol is shuttled from peripheral cells to the liver either for elimination via biliary excretion or reutilization in the enterohepatic cycle.[5]

However, there is clear evidence that HDL possesses other biological functions than reverse cholesterol transport, which may independently contribute to the prevention of cardiovascular risk. Advanced protein analysis has shown that apart from the apolipoproteins apoAI, AII, AIV, E, CI to CIV, LI, M, F,D, and H that are mainly involved in lipid metabolism,

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the HDL particle contains a multitude of other proteins and enzymes[6, 7] that have diverse functions associated with immunity, acute phase response, and complement regulation.[6]
Lipid profiling has revealed that in addition to free and esterified cholesterol, a variety of different lipids are found in HDL, including phospholipids (phosphatidylcholine, phosphatidylethanolamine [PE], PE-based plasmalogen, lysophosphatidylcholine, and glycerophospholipid), free and esterified fatty acids (mono- and triacylglycerols), and different sphingolipids such as ceramide, sphingolipids/sphingomyelin species, sphingosine-1-phosphate (S1P), lysosulfatide, and sphingosylphosphorylcholine.[8-10] The major carrier of S1P in plasma is HDL, and plasma S1P levels positively correlate with HDL-C, apoAI, and apoAII levels.[11] All eukaryotic cells are surrounded by a lipid layer that includes glycerolipid, sphingolipids and sterols.12 Sphingolipids (SLs) are known bioactive lipids that are involved in regulating proliferation, differentiation, cell hypertrophy, and apoptosis.[13] Sphingolipids include sphinganine, sphingosine, sphingosine-1-phosphate (S1P), ceramide, and ceramides-1-phosphate. Sphingolipids have emerged as molecules whose metabolism is regulated, leading to the generation of bioactive products, including ceramide, sphingosine, and sphingosine-1-phosphate. The balance between cellular levels of these bioactive products is increasingly recognized to be critical to cell regulation whereby ceramide and sphingosine cause apoptosis and growth arrest phenotypes, and sphingosine 1-phosphate mediates proliferative and angiogenic responses. Sphingosine kinase is a key enzyme in modulating the levels of lipids and is emerging as an important and regulated enzyme.[14]
There are two known isoforms of SK designated as SK1 and SK2, each of which contains several splice variants.15 Mouse and human SK1 exhibit substantial homology.15 SK1 mRNA is most highly expressed in the brain, heart, thymus, spleen, kidney, and lung, [16] whereas SK2 is highest in the kidney and the liver.[17] SK1 and SK2 activities reside mostly in the soluble extracts of cells, although a small portion of the activities has been associated with the membrane component as well. A key step in the sphingolipid pathway is the formation of S1P. Two known isoforms of SK, namely sphingosine kinase 1 and 2 (SK1 and SK2), are responsible for the production of S1P from sphingosine.[18] SK1 is known to be regulated by a multitude of growth factors and cytokines, including platelet derived growth factor (PDGF),[19,20] vascular endothelial growth factor (VEGF),[21] nerve growth factor (NGF),22 insulin-like growth factor (IGF),[23] IGF binding protein 3 (IGFBP3),[24] lysophosphatidic acid (LPA),[25] lipopolysaccharide (LPS),[26] compliment 5a (C5a),[27] TNFα (28,29), and IL-1β.[29] However, no research has thus far examined the effect of exercise on SK1gene expression. Most research has examined the effect of exercise on S1P. Błachnio and colleagues[30] showed that long-term acute exercise increased S1P content in whole soleus muscle and red muscle of gastrocnemius. In another study, Baranowski et al. showed that 6 weeks of endurance training increased significantly the plasma levels of S1P.[31] The study noted that increased amounts of S1P could be one of the possible mechanisms of the beneficial effects of physical activity.[31]
Herbal medicine has fewer side effects and is now also recommended by the World Health Organization. The effects of dietary supplement on healthy men have already been demonstrated. For instance, walnuts oil has properties that decrease blood total cholesterol.[32] Silymarin was assessed in animals fed with high-fat diets and results showed that this plant exerted a positive effect on the plasma lipoprotein profile.[33]
Knowledge about the effect of Pistachio-Atlantica (Bene) on tissue ABCG5 expression is lacking. Pistachio-Atlantica (Bene) is a plant of Anacardiaceae family and is rich in antioxidants and unsaturated fatty acid. The

leaves of this plant are thought to contain anti-oxidative compounds that reduce the amount of free radicals.[34]

Material and Methods

Plant Material

The ripped fruit samples of Pistachio-Atlantica (Bene) were collected from the fields of Maibod in the Yazd Province of Iran, and were stored at −18 ° C until use. Plant material was identified by herbarium collection in the Department of Physical Education and Sports Science, University of Mazandaran, Baboulsar, Iran.

Preparation of the Extracts

The extract was prepared according to Hamdan et al. (2004).[35] Briefly, the ripped and dried fruit of Pistachio-Atlantica (Bene) (10g) was coarsely powdered and mixed with 150 ml of tap water and boiled for 45 minutes before it was cooled at room temperature. After cooling, the mixture was filtered twice by using a Watman filter (No. 4 filter). The volume of the filtered solution was increased to 100 ml with tap water so that 1 ml was equivalent to 100 mg of starting material. It should to be noted that we did not use distilled water on the basis of the herbalist's recommendation. A fresh extraction was orally given at a dose of 100 mg/kg (7.5ul/g of body weight) immediately at the end of the training session for six weeks. The control group was treated in same manner and volume.

Animals

All the experiments involving the animals were conducted according to the policy of the Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. The study protocol was approved by the Ethics Committee of the University of Mazandaran (UMZ) and Babol University of Medical Sciences (BUMS, Mazandaran, Iran). Twenty Wistar female rats (6-8 weeks old, 125-135 g) were acquired from Pasteur's Institute (Amol, Mazandaran) and maintained in the Central Animal House of the Faculty of Physical Education and Sports Science of UMZ. Five rats were housed per cage (46-L volume) with a 12-hour: 12-hour light-dark cycle. Temperature was maintained at 22°C ± 1.4°C. Diets (a pellet form) and water were provided ad libitum. The animals were randomly assigned into control (n = 10) and training (n = 10) groups. The rats were divided further into saline-control (SC), saline-training (ST), Bene-control (BC), and Bene-training (BT). The control group remained sedentary, whereas the training group underwent a moderate running exercise program.

Exercise Training Protocol

First, the animals were familiarized with the rat treadmill apparatus, every day and for 4 days. (The 14-lane motorized-driven treadmill was designed by the primary author [UMZ, Baboulsar, Mazandaran, Iran].) The exercise group was trained for 8 weeks using the same training methods previously described.[36, 37] The rats ran at 25 m/min for 60 minutes, 5 d/wk. The animals were killed 72 hours after the last exercise session. Food but not water was removed from the rat cages 4 hours before the sacrifices. The estrous cycle was determined in intact female rats by taking vaginal smears each morning by vaginal lavage. The smears were analyzed under a microscope to determine the type of cells present and the stage of the estrous cycle.[38] Only the female rats showing at least two consecutive 4- or 5-day estrous cycles were used. The established estrous cycle in each female was used to select the day of the experiment, at which time the estrous cycle stage was confirmed by vaginal smear.[39]

Tissue Biopsies

Seventy-two hours after the last training session, the rats were anesthetized with intra-peritoneal administration of a mixture of Ketamine (30– 50 mg / kg body weight) and Xylazine (3– 5 mg / kg body weight). The liver, small intestine, kidney, and visceral fat were excised, cleaned, divided into two pieces, washed in ice-cold saline, and immediately frozen in liquid nitrogen and stored at − 80 ° C until RNA extraction.

RNA Isolation, cDNA Synthesis and Real-Time PCR

Total RNA was extracted from 80 to 100 mg of tissue using RNA purification kits (AccuZol, Bioneer, Cat. No: k3090). Complementary DNA (cDNA) was extended from 1µl oligo-(dt)18 primers (0.25 µg per reaction) using the cDNA synthesis kit (Accu Power RT PreMix, Bioneer, Cat. No: k2041-B) according to the manufacturer's instructions. Complementary DNA concentration was 1 to 2 ng/25µl reaction. Real-time quantitative PCR was performed using Quanti Fast SYBR Green PCR Kit (Cat. No. 204052; Qiagen, GmbH, Germany) using 15 µl reaction containing 0.5µl single-strand cDNA,7.5µl Master Mix, 1µl of the each forward and reverse primers (5 pmol/µl) and 5µl dH2O.

SK1 sense primer was 5′-GGTTCTTCGTCCCAGAGT -3′ and antisense primer was 5′-AGAGCGCCCTCGTCATTC-3′. β-actin sense and antisense primers were 5′-TATCGGCAATGAGCGGTTCC-3′ and 5′-AGCACTGTGTTGGCATAGAGG-**3′** (NM_031144, 145 bp) used as normalizer genes.[40]

Real-time PCR reactions were performed using the Rotor Gene 3000 real time PCR system from Corbett using the following program: step1:95 °C for 5 min and step 2:40 cycle of 95 °C for 10 sec and 60°Cfor 30 sec. The last heating step in phase 2 was carried out for the generation of a melting curve of the product. The amplicons were melted at the rate of 0.1°C/s to generate the high-resolution melting profile.

Statistical Analysis

The relative levels of mRNA were analyzed by the 2–ΔΔC T method. CT for each sample was determined using Rotor-Gene 3000 Software. Briefly, Δ-CT value was calculated by taking the CT of the SK1 gene and subtracting it from the CT of β-actin. The ΔΔ-CT was calculated by subtracting the Δ-CT (sample) values from the Δ-CT (control). The relative quantification was then calculated by the expression 2-ΔΔCT.[41]

The Kolmogorov-Smirnov test was used to determine the normality of distribution, and the variables were found to be normally distributed. All the results are expressed as means ± SEM. The statistical analyses were performed using a One-Way Analysis of Variance. Least significant difference (LSD) post hoc test was employed in the event of a significant (p value < .05) F ratio. All the statistical analyses were performed with SPSS (Version 13; SPSS, Chicago, IL).

Results

SK1 relative gene expression in the liver, small intestine, kidney, and visceral fat was determined in the female rats. Data analysis revealed a significant difference in liver SK1 mRNA relative abundance between the groups (F=6.045; p value < 0.006) (Figure 1). Using a suitable following post hoc test, the data revealed that the liver relative expression of SK1 was higher in the BT group when compared with the other groups at the end of the program (Figure 1). A significant difference was also found in the small intestine relative mRNA expression of SK1 at the end of the treadmill running program (F=5.715; p value < 0.007). In this regard, the SK1 mRNA relative abundance was lower in the BC animals when compared with the

Bene-treated groups (Figure 2). Apropos the kidneys, the same results obtained regarding the liver and intestine were observed (F=4.182; p value < 0.023) (Figure 3). Visceral fat SK1 relative gene expression did change not significantly in all the groups (Figure 4).

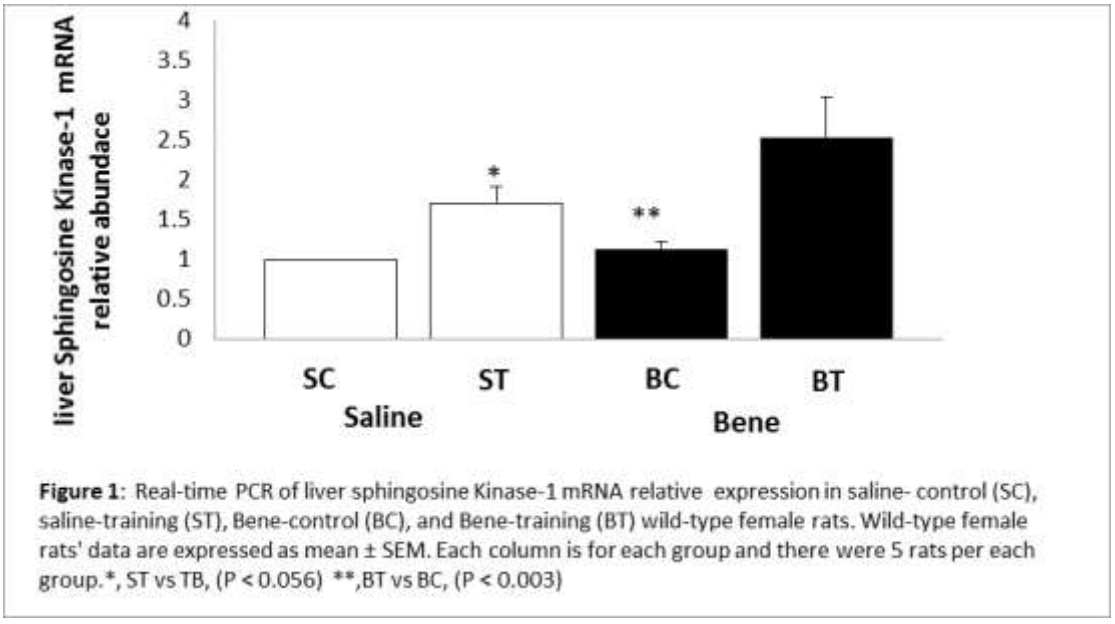

Figure 1: Real-time PCR of liver sphingosine Kinase-1 mRNA relative expression in saline- control (SC), saline-training (ST), Bene-control (BC), and Bene-training (BT) wild-type female rats. Wild-type female rats' data are expressed as mean ± SEM. Each column is for each group and there were 5 rats per each group.*, ST vs TB, (P < 0.056) **,BT vs BC, (P < 0.003)

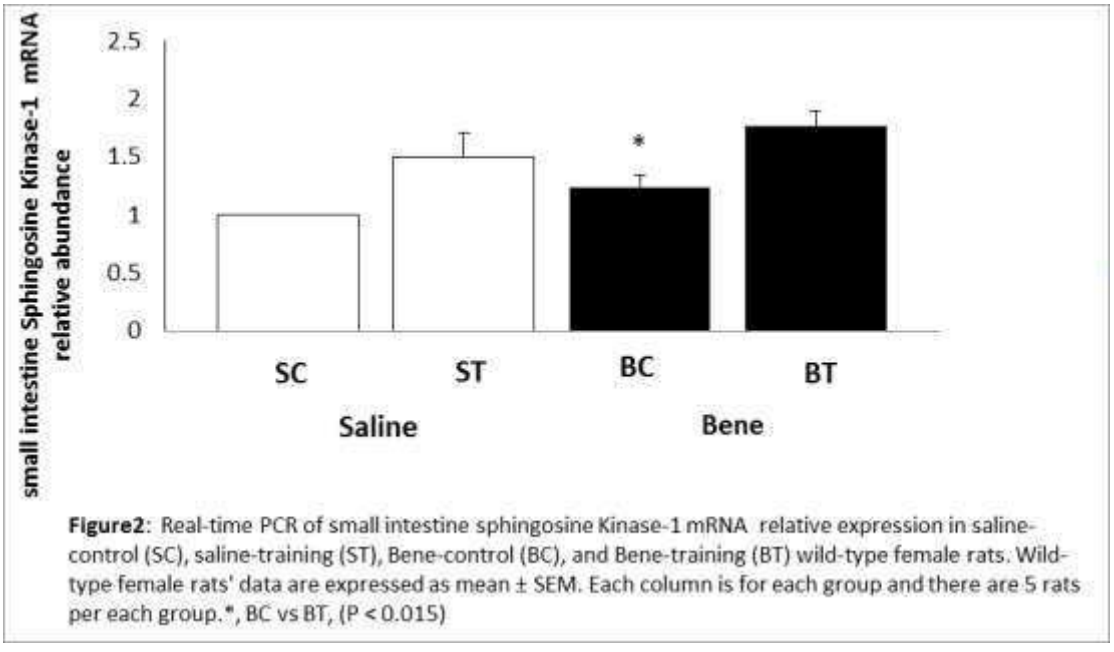

Figure2: Real-time PCR of small intestine sphingosine Kinase-1 mRNA relative expression in saline-control (SC), saline-training (ST), Bene-control (BC), and Bene-training (BT) wild-type female rats. Wild-type female rats' data are expressed as mean ± SEM. Each column is for each group and there are 5 rats per each group.*, BC vs BT, (P < 0.015)

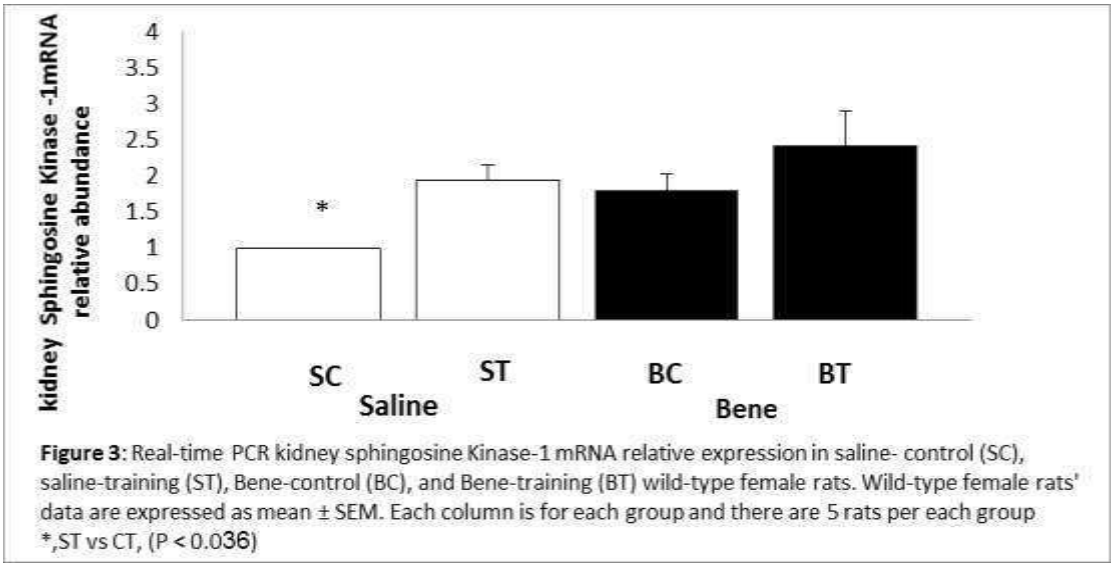

Figure 3: Real-time PCR kidney sphingosine Kinase-1 mRNA relative expression in saline- control (SC), saline-training (ST), Bene-control (BC), and Bene-training (BT) wild-type female rats. Wild-type female rats' data are expressed as mean ± SEM. Each column is for each group and there are 5 rats per each group
*,ST vs CT, ($P < 0.036$)

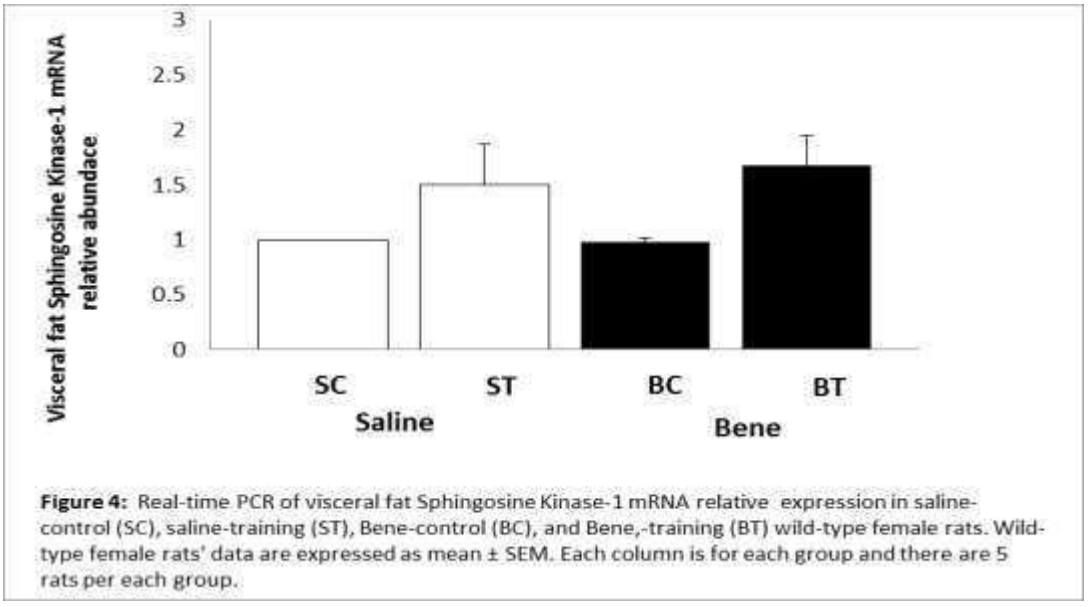

Figure 4: Real-time PCR of visceral fat Sphingosine Kinase-1 mRNA relative expression in saline-control (SC), saline-training (ST), Bene-control (BC), and Bene,-training (BT) wild-type female rats. Wild-type female rats' data are expressed as mean ± SEM. Each column is for each group and there are 5 rats per each group.

Discussion

This study was conducted to investigate the effect of the exercise on treadmill running program with or without a liquid Pistachio-Atlantica (Bene) extraction on SK1 gene expression in female rat tissues. To our knowledge, this is the first report to demonstrate the alterations of SK1gene expression in female rat tissues in response to a treadmill running and Bene crud extraction regime. The main findings of the present study were (1) SK1 was expressed in liver, small intestine, kidney, and visceral fat tissues, (2) exercise training increased SK1 mRNA expression in the liver, small intestine, kidney, and visceral fat tissue, and (3) the administration of Pistachio-Atlantica extraction (Bene) at the given dose was able to increase SK1 mRNA expression in the liver, small intestine, kidney, and visceral fat tissue.

Błachnio and colleagues[30] showed that long-term acute exercise increased S1P content in whole soleus muscle and red muscle of gastrocnemius. In another study, Baranowski et al. reported that 6 weeks of endurance training increased significantly the plasma levels of S1P.[31]The study noted that increased amounts of S1P could be one of the possible mechanisms of the beneficial effects of physical activity.[31] Formigli in another study showed that the enzyme activity of the endogenous levels of SK1 and S1P were significantly increased in damaged tissue.[42] Platelets can store a great deal of S1P, which is released during stimulation and increases activity.43 Exercise will result in changes in platelet number and function.[44] Various mechanisms such as increase in plasma epinephrine and norepinephrine and changes in adrenergic receptors in platelets can increase platelet activity after vigorous exercise acutely.[45]

Bene is oil seed and has abundant fatty acids. Analysis of the Pistachio-Atlantica var mutica essential oil by GC-MS method has shown that it is composed of α-pinene (70%), β-pinene (1.94%), 3-carene (0.2%), carveol (2.18%), epoxypinene (2.15%), limonene oxide (9%), myrtenol (5.31%), limonene (0.62%), citral (5.72%), α-phellandrene (0.2%), and β-myrcene (0.3%).46 GC-MS analysis of Pistachio-Atlantica in our study showed that it is composed of α-pinene (0.71%), limonene (0.54%), hexadecenoic acid (7.52%), palmitic acid (28.86%), trans-oleic acid (49.28%), n-octadecanoic acid (3.87%), oleic acid (0.2%), 9-octadecenoic acid (Z) (0.18%), 3-pentadecyl-phenol (2.69%), phenol, 3-pentadecyl (0.84%), 3-pentadecyl- phenol (1.58%), 4, 5: 9, 10-dibenzo-1, 3, 6, 8-tetraazat ricyclo [4. 4. 1. 1 (3, 8)] dodecane 3-butyl-thiophene-1, 1-dioxide 3-(2, 2-dideuterobutyl)-thiophene-1, 1-dioxide (0.86%), 4, 5: 9, 10-dibenzo-1, 3, 6, 8-tetraazat ricyclo [4. 4. 1. 1 (3, 8)] dodecane Cinnamyl cinnamate (0.28%), (acetoxymethyl) methyl [(trimethylsilyl) methyl] silane Cinnamyl cinnamate (0.34%), 2, 4-diphenyl glutaronitrile 2-methyl-4, 5-diphenyl-4, 5-dihydroo xazole Cinnamyl cinnamate (0.36%), phenol, 3-pentadecyl-acetic acid, 4-methylphenyl ester acetic acid, 4-methylphenyl ester (0.62%), phenol, 3-pentyl (0.36%), ribitol, pentaacetate N-propenyl-2-methoxy-6-methylbenza mide 6, and 7-dimethoxyisatin (0.15%). The total amount of essential oil obtained was 22% v/w, which is higher than any other species of the genus pestacia.[47]

According to Hu et al.,[48] quantitative real-time PCR demonstrated that palmitate (PAL) increased sphingosine kinase 1 (SK1) mRNA by approximately four-fold. This was accompanied by a two to threefold increase in sphingosine kinase enzyme activity. This up-regulation did not occur upon treatment with oleate, suggesting some levels of specify for PAL. These findings were recapitulated in the diet-induced obesity mouse model, in which high-fat feeding increased SK1 message in skeletal muscle over two to threefold. Moreover, these findings identify PAL as a novel regulatory stimulus for SK1. Palmitate increases sphingosine-1-phosphate in C2C12 myotubes via the up-regulation of sphingosine kinase message and activity.48 Zabielski et al.[49] reported that the content of ceramide and sphingosine decreased in the high-fat diet (HFD) group at each time point. The rats were fed HFD or standard diet for 7 days prior to the partial hepatectomy (PH). A decrease in the content of ceramide and sphingosine was accompanied by elevated contents of sphingosine-1-phosphate and sphinganine-1-phosphate. A drop in SPHK activity in cytosol after partial hepatectomy was inversely correlated (r=-0.7538) with a rise in S1P, which suggested translocation of SPHK to plasma membrane. Shingosine-1-phosphate to ceramide ratio was higher in the rats fed HFD.[49]

Bruce and et al.[50] showed that high-fat diet (HFD) for 6 weeks increased SphK activity in skeletal muscle. In this study, transgenic (Tg) mice that overexpressed SphK1 were fed a standard chow or high-fat diet (HFD) for 6

weeks before undergoing several metabolic analyses. SphK1 Tg mice that were fed HFD displayed increased SphK activity in skeletal muscle, which was associated with an attenuated intramuscular ceramide accumulation compared with wild-type (WT) littermates. Accordingly, skeletal muscle and whole-body insulin sensitivity were improved in SphK1 Tg, compared with WT mice, when fed HFD.[50]

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Descriptive Analysis of Endoscopic versus Traditional Open Vein Harvest Technique for Coronary Artery Bypass Graft Surgery: Report of 1974 Cases

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Abstract

Background: Coronary artery disease (CAD) is one of the most common types of heart disease and the leading cause of death in the United States in both men and women. Coronary artery bypass grafting (CABG) has been used for patients with significant CAD. Successful CABG depends on many factors and one of them is the choice of graft conduit. The aim of this study is to report a descriptive analysis of endoscopic (EVH) versus traditional open vein harvest (OVH) technique for CABG from 2010 to 2011 in Mashhad, northeast of Iran.

Methods and Materials: This is a cross-sectional study conducted on 1974 CABG operations in Javad-al-Aeme Hospital in Mashhad, from January 2010 to July 2011. Totally, 989 patients underwent the traditional OVH and the other 985 patients had the EVH. Data analysis (including demographical and operational) in addition to parametric and nonparametric tests were undertaken using the SPSS 16 software. A P value < 0.05 was regarded as statistically significant.

Results: Mean age of the EVH group was 62.1±3.5 years in comparison with 64.2±3.9 years in the OVH group, which means no significant difference was revealed between the two groups. A significant difference between the groups in hospital stay days was detected, with the EVH and OVH groups being 5.6±1.2 and 6.5±1.8 days, respectively (P=0.041). Patient's Pain was measured by the use of a visual analog scale (VAS); we found significant differences between the groups at 2nd day, 5th day, and 40th day after CABG (P<0.05). We also found a noticeable difference in cosmetic satisfaction and less wound complication in the EVH group in comparison with the OVH group (P<0.05).

Conclusions: In this study, we found that EVH brings fewer postoperative wound complications and less postoperative pain and could reduce analgesic usage after the operation. It also results in shorter hospital stay and of course better cosmetic outcome in the patient's view.

Keywords: Coronary artery disease; Endoscopic vein harvest; Open vein harvest

Introduction

Many people around the world suffer from coronary artery disease (CAD), which causes enormous morbidity and mortality. Coronary artery bypass grafting (CABG) is the optimal cardiac surgical modality in these patients (1).

This surgery is performed on individuals with each of the following diseases: 1) left main coronary artery disease; 2) three-vessel disease; 3) three-vessel disease in diabetics; 4) severely depressed heart function; and 5) heart conditions in addition to CAD, e.g., replacement of valves or reconstruction of the heart muscle (1).

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More than 300000 CABG operations are performed in the North America annually (2). Furthermore, it has been reported that over 10000 patients require CABG every year in Iran (3).

Endoscopic greater saphenous vein harvesting (EVH) decreases the wound complications related with open techniques (4). In order to decrease the considerable morbidity and wound complications associated with the extensive incisions made in the traditional approach to vein harvest, minimally invasive techniques such as endoscopic vein harvest (EVH) are recommended (5). The use of such minimally invasive methods can reduce postoperative complications.

In the traditional methods of greater saphenous vein harvesting, large incisions must be made; however, local pain in the leg, dysmobility, wound infection, wound bleeding, prolonged hospital stay, and insufficient cosmetic results could happen (6, 7). A meta analysis showed that EVH is safe and reduces the rates of wound complications, leg wound infection, wound hematoma, and postoperative pain, compared to traditional open techniques (5).

One of the main debates regarding the EHV versus OVH is the graft patency. Allen KB et al. reported that a five-year follow-up of a prospective RCT displayed that the use of the EVH did not influence event-free survival (4). Some other scientists believe that the EVH is independently associated with vein-graft failure (8).

In Iran, the minimally invasive approach of the endoscopic greater saphenous vein harvesting is currently not widely used and only some open-heart surgery centers use this technique. The aim of this study is to review 1974 patients who underwent CABG via the EVH or the OVH for vein harvesting in Mashhad, Northeast of Iran.

Methods and Materials

Between January 2010 and July 2011, 1974 patients underwent CABG in Mashhad, Northeast of Iran. This is a retrospective cross-sectional study analyzing the above-mentioned patients' demographic information (age, gender, educational level, marital status, income, and occupation), family history of CAD, smoking habits, FBS level, serum lipid profile, and wound complications such as inflammation, cellulitis, lymphangitis, drainage, and necrosis.

Pain Evaluation

Leg pain severity was measured using the visual analogue scale (Figure1) on the 2nd, 5th, and 40th days after CABG.

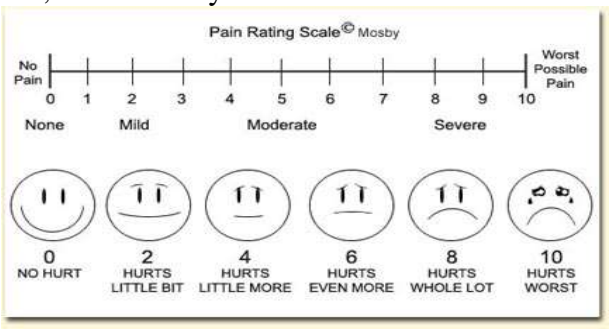

Figure 1: Visual linear analog scale (VAS) (0-10 NUMERIC PAIN DISTRESS SCALE)

Cosmetic Satisfaction

Cosmetic satisfaction was judged by the patient on a scale graduated as follows: unacceptable = 1; not satisfied = 2; satisfied = 3; very satisfied = 4; and extremely satisfied = 5.

Endoscopic Operative Technique

Endoscopic dissection and excision of the saphenous vein has the advantages of requiring smaller skin incision, which heals better. Our EVH method is based on the CO2 technique. A small incision is made 1.5-2.5 cm below/above the knee to build the entrance of the probe, which then continues its path toward the groin region. For dividing the branches, we used a bipolar cauterizer and by the use of scissors, we made a punctured incision to clamp, ligate, and divide the vein, followed by

ligating the side branches with 7.0 monofilament Prolene suture (Figure 2).

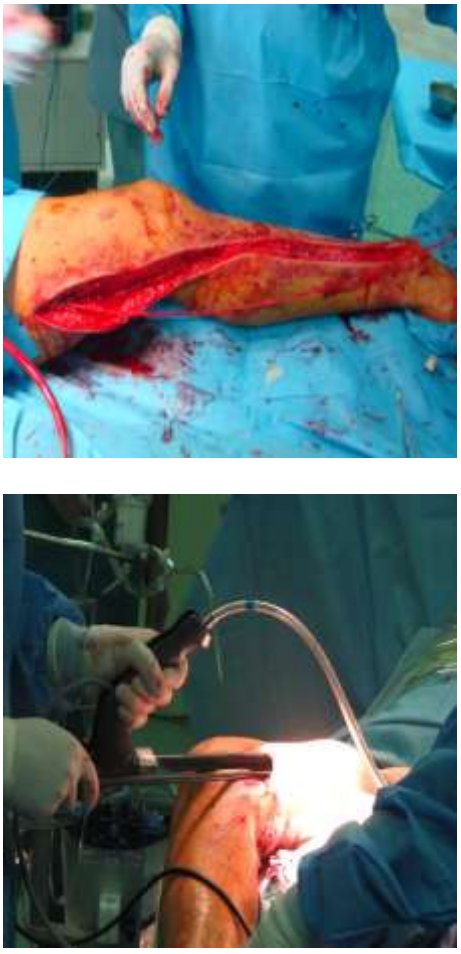

Figure 2: EVH and OVH procedure

Open Operative Technique

First, the leg is abducted and rotated laterally by placing a roll under the knee. After a long incision is made over the saphenous vein, the side branches are ligated /clipped. The vein is then removed and prepared after the closure of the incision site in layers with absorbable suture and the leg wound is covered with cotton gauze dressing, in addition to applying an elastic ace to the entire leg (Figure 2).

Statistical Analysis

The statistical analyses were conducted using the Statistical Package for Social Sciences (version 16). Descriptive statistics (frequency, mean, and standard deviation) were determined for all the variables. Differences between the two techniques' categorical variables (EVH vs. OVH) were analyzed by the chi-squared test. A p value < 0.05 was considered significant.

Results

In this study, we reviewed 1974 cases of CABG, which were divided into two groups of EVH (n=985) and OVH (n=989). Table 1 depicts the demographic and characteristics of both groups.

The mean age was 62.1±3.5 years in the EVH group and 64.2±3.9 years in the OVH group; there was no significant difference between the two groups (P=0.874).

The preoperative tests such as FBS, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride of the two groups were almost similar (P>0.05) (Table 1). In addition, no significant difference was found between the two groups in HTN, DM, or smoking habits (P>0.05) (Table 1).

We found a noticeable difference between the groups in the days of hospital stay, with the EVH and OVH groups being 5.6±1.2 days and 6.5±1.8 days, respectively (P=0.041) (Table 1).

We also asked patients to assess their pain by the use of the VAS; we found a significant difference between the groups at 2nd, 5th, and 40th days after CABG (P<0.05) (Table 1). In addition we asked the patients to rate their cosmetic satisfaction with what they imagined and as a result we found another great difference between the groups (P=0.037). Wound complications occurred in both groups: while the rate of prevalence was 4% in the EVH group, it was 14.1% in the OVH group. As a result, a marked difference was found between the groups in wound complications (P=0.006) (Table 1).

Table 1: Characteristics data from all subjects in each group

Variable		EVH (n=985)	OVH (n=989)	P Value
Age(year) Mean ± SD		62.1 ± 3.5	64.2 ± 3.9	0.874
Sex (No. %)	Male	640 (65)	632 (64)	0.941
	Female	345 (35)	357 (36)	
Smoking (No, %)	Current	177 (18)	158 (16)	0.835
	Former	197 (20)	148 (15)	
	Never	611 (62)	683 (69)	
Diabetes Mellitus (No. %)	Yes	78 (8)	128(13.5)	0.541
	No	907(92)	861(86.5)	
Education Level	Primary school (No.(%))	118(12)	178(18)	0.374
	High school (No.(%))	778(79)	702(71)	
	Higher education level (No.(%))	89(9)	109(11)	
Family income	<150$/month	206(21)	257(26)	0.360
	150-350$/month	659(67)	573(58)	
	>350$/month	120(12)	159(16)	
Marital status	Single	168(17)	158(16)	0.835
	Married	611 (62)	683(69)	
	Divorced/widow/widows	206(21)	148(15)	
Family history of CAD	Positive (No.(%))	246(25)	207(21)	0.98
	Negative (No.(%))	739(75)	782(79)	
FBS(mg/dl) Mean ± SD		118.27±51.30	121.05±66.60	0.741
TC(mg/dl) Mean ± SD		192.60±38.68	160.59±46.32	0.653
LDL-C(mg/dl) Mean ± SD		120.83 ± 32.07	117.75 ± 40.07	0.425
HDL-C(mg/dl) Mean ± SD		43.95 ± 8.61	46.39 ± 31.62	0.864
TG (mg/dl) Mean ± SD		138.73±62.98	135.01±60.15	0.799
Hypertension (No. %)	Positive	98(10)	158(16)	0.554
	Negative	887(90)	831(84)	
Vein Harvesting time Mean ± SD		35±3.65	45±5.1	0.042*
Wound complication	Inflammation (No. %)	5(0.5)	33(3.3)	0.006*
	Cellulitis (No. %)	30(3.0)	33(3.3)	
	Lymphangitis(No. %)	0(0.0)	42(4.2)	
	Drainage (No. %)	0(0.0)	10(1)	
	Necrosis (No. %)	5(0.5)	33(3.3)	
Hospital Stay(Day) Mean ± SD		5.6±1.2	6.5±1.8	0.041*
Pain Score	2^{nd} day(median(IQR))	1(3)	2(4)	0.042*
	5^{th} day(median(IQR))	1(2)	2(3)	0.038*
	40^{th} day(median(IQR))	1(1)	2(2)	0.032*
Cosmetic satisfaction (median(IQR))		4(2)	2(1)	0.037*

FBS: Fasting blood Sugar, TC: total cholesterol, HDL-C: High density lipoprotein- cholesterol, LDL-C: Low density lipoprotein- cholesterol, TG: triglyceride, IQR: interquartile range

*: P<0.05 and it mean significant different between groups.

Discussion

Our data demonstrate that the EVH technique resulted in a significantly reduced postoperative pain score and hospital stay.

Chronic wounds or post operation complicated wounds are associated with increased morbidity and mortality and pose a serious economic burden on the health care system. It has been estimated that nearly $25 billion is spent annually in the United States to treat ulcers (9). Underlying confounding factors such as old age, DM, microcirculation impairment, systemic hypoxia, atherosclerosis, and malnutrition cause chronic wound (10, 11). In the EVH group, the complicated wounds are easier to handle and it reduces late interventions (12). This finding was similar in our long-term complications of wound.

Most CABG candidates' causes of chronic wounds are related to old age, DM, microcirculation impairment, and systemic hypoxia; therefore, it is logical to find the best method and the minimally invasive technique for vein harvesting in these patients. Kiaii B, et al. in 2002 showed that the EVH technique reduced postoperative leg wound complications, including infection, and improved patient satisfaction as compared with the conventional harvesting technique (13). Similar results were shown in this study.

Surgeons' fatigue before they reach the main part of the operation procedure is crucial. Most of the time in OVH is gained in the closure of the wound (12), whereas EVH is performed at a satisfactory speed (14).

In 2008, Andreasen JJ and his colleagues found better cosmetic results with a substantial reduction in the EVH method compared with OVH (15). In addition, Schurr UP et al. (2002) reported better cosmetic results in EVH versus OVH (16). Cosmetic satisfaction is acceptable in the EVH group and had significant difference with the OVH group. Santo VJ and his colleagues found that hospital stay was 5 days for EVH and 7 days for OVH, and the difference was statistically significant ($P<0.001$) (17). We found the same results inasmuch hospital stay was 5.6 days for the EVH group and 6.5 days for the OVH group.

Conclusion

In this study, we found that the EVH technique makes fewer postoperative wound complications and less postoperative pain and can reduce analgesic usage after the operation. It also results in shorter hospital stay and of course better cosmetic outcome in the patient's view.

Conflict of Interest Statement

The authors declare no potential conflict of interests.

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A Comparative Study of Two Methods of Thrombus Aspiration and Adenosine Injection on No-Reflow Phenomenon

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Abstract

Introduction- Lack of myocardial reperfusion after an ischemic period despite reperfusion therapy is called the no-reflow phenomenon.This study aimed to compare no-reflow phenomenon outcomes (ECG and enzyme changes) in Adenosine injection and thrombosis aspiration methods.

Methods- A quasi-experimental study was performed on patients undergoing percutaneous coronary intervention in three selected Tehran hospitals (April 2011 to June 2012). Forty patients (24 men and 16 women) were included in the study. ST-segment elevation and cardiac enzymes changes were compared in Adenosine injection and thrombus aspiration in patients with the no-reflow phenomenon 24 hours after percutaneous coronary intervention.

Findings- The Adenosine and thrombus aspiration groups did not show significant differences in cardiac enzyme changes (positive troponin, or CKMB elevation). Also, the Adenosine and thrombus aspiration groups did not show significant difference in ST-segment elevation (P>0.05).

Conclusions- This study confirms the results of positive restore coronary perfusion in Adenosine infusion and thrombosis aspiration. Now the question is why enzyme and ECG changes have been reported more in the thrombus aspiration method despite the improvement in cardiac perfusion to Adenosine injection? The response seems to be related to more aggressiveness of the thrombus aspiration method, but this explanation should be confirmed by more investigations.

Keywords: Cardiac enzyme, Adenosine injection, Thrombus aspiration, ECG, PTCA, No-reflow

Introduction

Cardiovascular disorders are the main cause of death in developed countries and occur more due to coronary artery diseases.[1] Coronary artery disease is the most common cause of the acute coronary syndrome.

The acute coronary syndrome consists of myocardial infarction with or without S-segment elevation and unstable angina.[2]

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Reperfusion interventions are the basic techniques for treatment and limit myocardial infarction area.[3] Lack of myocardial reperfusion after an ischemic period despite reperfusion therapy in the ischemic area is called no-reflow. However, the pathophysiological mechanisms leading to this phenomenon have not been well identified.[4] This phenomenon was first described by Krug (1966) in experimental models5 and was then defined by Kloner in 1974.6 Despite the lack of epicedium vascular occlusion, this phenomenon reduces the blood flow of the coronary artery, causes coronary spasm, dissection, or local thrombosis.[7] This phenomenon was completely described in 1985 by the TIMI Study Group.[8] The angiographic flow grade (0 or 1) is called no-reflow and higher grade is considered as a slow-flow phenomenon. In proportion to the occlusion in the coronary artery, no-reflow has a greater risk for the patient.3, 7 Butler (2008) studied 5286 PCI cases and estimated this phenomenon to be about 4.8%.8 A less common form of this phenomenon was reported after PCI in stable angina and acute coronary syndrome without ST-segment changes in the Abbo (1995) study.3 Reperfusion is generally conducted by thrombolytic and antiplatelet agents or in combination with PCI. The success of reperfusion in emergency PCI and in thrombolytic therapy was reported to be about 95% and 81%, respectively. Also, PCI had the better outcome for patients and is considered as the first method of choice.[9]

Adenosine is commonly used in the no-flow condition.[8] Distal coronary infusion of Adenosine improves coronary perfusion in many patients.[10] Adenosine is a vasodilator used routinely to detect significant occlusion in the coronary blood flow. In this regard, there is a risk of potential side effects such as hypotension and bradycardia. In animal models, Adenosine has improved microvascular condition in atherosclerotic plaque rupture.[11] The studies have been confirmed by the usefulness of Adenosine in reducing no-reflow and also ventricular function improvement in patients with acute myocardial infarction undergoing emergency balloon angioplasty.12 Kloner (2006) with a study on 2118 PTCA procedures showed that the use of intra-coronary Adenosine reduced one and 6 months' mortality, heart failure, and relapse rates of myocardial infarction.[13] Butler (2011) determined that the use of intravenous Adenosine at a dose of 50-70 mg/kg/min in primary coronary stenting in patients with acute myocardial infarction could reduce the size of the infarct and improve left ventricular ejection fraction within 4 weeks.[8] Micari (2005) compared the outcomes and no-reflow incidence after bypass graft in low and high doses of Adenosine grafts. Although Adenosine had no effect on this phenomenon, there was a significant improvement in TIMI criteria in the administration of Adenosine into grafts.[14]

Adenosine is the best vasodilator for the treatment of the no-reflow phenomenon; however, further clinical studies are necessary to evaluate its clinical outcomes in intact arteries and saphenous vein graft interventions.[15] There has yet to be an investigation to compare simultaneously thrombus aspiration and intra-coronary Adenosine administration. Most of the studies conducted hitherto have focused on the patient outcome.

The aim of this study was to determine the essential points of the no-reflow phenomena in electrocardiographic and enzyme changes in two commonly used percutaneous interventions (i.e., Adenosine injection and thrombosis aspiration).

Methods

This quasi-experimental study was performed on patients undergoing percutaneous coronary intervention. Samples were selected from Vali-Asr, Tehran and Khatam Hospitals (April 2011 to June 2012). Inclusion criteria were patients with the no-reflow phenomenon after percutaneous coronary interventions.

Exclusion criteria were the contraindications of PCI. Data collection instruments were demographic sheet, recording the degree of myocardial reflow based on the standard TIMI score 8 (Box1); recorded ECG changes (ST-segment elevation of at least ≥1mm), and cardiac enzymes changes (troponin, CK-MB) 24 hours after percutaneous coronary intervention.

Box 1. TIMI score

Grade	Definition
0	There is no dye penetration to the distal point of the obstruction.
1	Contrast material passes from the point of the obstruction but does not completely distribute into the vascular bed.
2	There is filling and clearance of the contrast, but it is slower than normal.
3	There is no normal flow.

Patients were randomized to a thrombus aspiration group and a group injected with Adenosine. In the thrombus aspiration group, aspiration of clots and debris discharge resulted from atherosclerotic plaque. The Export Aspiration Catheter (Medtronic, Fr 6, diameter 145 cm) was used for this procedure. This catheter was connected to a 20-cc syringe which aspirated clots at a speed of 1 cc /sec. In the second group, 75 mg of Adenosine was injected into the coronary artery. Both groups received the same bolus dose of Nitroglycerin (100 mcg) and Integrin (10 mg). These two procedures are routinely performed in the catheterization lab and do not impose additional charges on the patients. Patient evaluation was based on TIMI (degree of myocardial perfusion), ECG changes, and cardiac enzyme changes (positive troponin or two-fold increase in CK-MB) 24 hours after intervention.

This study was approved by the Medical Ethics Committee (Code: 175189/41) of Shahed University. Data were analyzed by SPSS software (ver. 16) using descriptive statistics and the chi-squared test.

Results

Forty patients (24 men and 16 women) were included in the thrombus aspiration and Adenosine infusion groups. The chi-squared test did not show significant differences between the two groups based on age, sex, and underlying diseases. Twenty-eight (70%) patients with STEMI, 8 (20%) patients with unstable angina NSTEMI, and 4 (10%) patients experienced stable angina (Table1). In the Adenosine infusion group, the most involvement was reported in the LAD (N=11, 55%), and the RCA (N=10, 50%) was the most involved artery in thrombus aspiration (Table 2). In the thrombus aspiration group, reflow was reported in all the cases. In contrast, in the Adenosine group, reflow was unacceptable in 2 (10%) patients: in these two patients the grade of myocardial perfusion increased from zero to one and one of them died. In the thrombus aspiration group, the degree of perfusion in 5 (25%) patients was 2 and 15 (75%) patients had perfusion in grade 3 and no patients showed grade 1. During Adenosine infusion, 2 (10%) patients showed grade 1, 8 (40%) patients were in grade 2, and 10 (50%) patients were in grade 3. The chi-squared test did not show a significant difference in the myocardial perfusion grade between the two methods (P=0.343) (Table 3). Similarly, the Adenosine and thrombus aspiration groups did not show a significant difference in cardiac enzyme changes (positive troponin or CKMB elevation) (P=0.482). Also, the Adenosine and thrombus aspiration groups did not demonstrate significant differences in ST-segment changes (P=0.342) (Table4).

Table 1. Frequency in both groups of patients according to the type of coronary disease

Adenosine infusion group	N	%
STEMI	13	65
NSTMI/UA	4	20
Stable Angina	3	15
Thrombus aspiration group	N	%
STEMI	15	75
NSTMI/UA	4	20
Stable Angina	1	5

Table 2. Coronary artery involvement in the Adenosine and thrombus aspiration groups

Groups	LAD	LCX	RCA
Adenosine infusion	11(55%)	4(20%)	5(25%)
Thrombus aspiration	6(30%)	4(20%)	10(50%)

Table 3. Degrees of myocardial perfusion in the Adenosine and thrombus aspiration groups

Myocardial perfusion grade	1	2	3	P value
Groups				
	N (%)	N (%)	N (%)	
Adenosine infusion	2 (10)	8 (40)	10 (50)	P=0.343
Thrombus aspiration	-	5 (25)	15 (75)	

Table 4. Cardiac enzyme changes in the Adenosine infusion and thrombus aspiration groups

Enzyme change	Positive	Negative	P value
	N (%)	N (%)	
Adenosine infusion	8 (40)	12 (60)	0.482
Thrombus aspiration	11 (55)	9 (45)	
ECG change	↑ ST	↓ ST	
	N (%)	N (%)	
Adenosine infusion	3 (40)	5 (60)	0.342
Thrombus aspiration	4 (37)	7 (63)	

Discussion

Compared to the studies conducted so far, the present research seems new insofar as it compares the effect of two therapeutic methods on the no-reflow phenomenon. This study confirms the previous studies [5, 6, 14- 20] about the positive effects of Adenosine infusion and thrombosis aspiration to restore high-grade coronary perfusion. In this study, the thrombus aspiration group experienced 100% improvement in coronary perfusion, and in the Adenosine group, 90% recovery of the coronary blood flow was recorded. Although it represents a more effectiveness of thrombus aspiration, there was no significant difference with the Adenosine group. This finding may be related to an improvement in the ruptured plaque and materials released from thrombosis. In the Adenosine method, the material was not removed completely and it moved at a small vascular bed. Finally, by removing debris and plaque thrombosis, a reduction in distal embolization can be expected. Locally produced vasomotor factors are more effectively removed by aspiration thrombosis, leading to reduced small vascular bed contraction and better coronary circulation.[10] In addition, other probable causes are related to an unspecified dose of Adenosine because a higher dose of Adenosine may yield a better outcome. On the other hand, the correct route of drug injection is unclear. The no-reflow phenomenon is the main cause of elevated cardiac enzymes after PTCA.16 The average rate of cardiac enzyme elevation in patients undergoing angioplasty is 3% to 5%. This amount differs with the no-reflow phenomenon.[10] In the present study, 35% of the patients undergoing thrombus aspiration experienced enzyme changes. Perhaps we can link it to the invasive method and the transient disturbances of the coronary flow during catheter insertion in the small vessels. The lack of support and protection in the vascular bed of the small vessels and collateral branches, the probability of thrombus embolization during aspiration, and a longer time to reperfusion can contribute to the unsuccessful results. This means that it might maintain perfusion despite transient myocardial ischemia. In comparison to the standard method, thrombus aspiration has shown less creatine kinase release and lower risk of distal embolization and no-reflow phenomenon.[18] The slight improvement of the enzyme in the Adenosine group confirms the previous studies.[19] Also, ECG changes were somewhat consistent with the enzyme changes having occurred in 40% of the patients in the Adenosine injection and 55% in the thrombus aspiration group.

Previous studies have shown ST-segment elevation improvement in thrombus aspiration.[20] In this study, only 4 patients in the thrombus aspiration and 3 patients in the Adenosine infusion group showed ST-segment elevation. However, because the patients were often in the context of myocardial infarction, it is not easy to judge about the ECG changes.[21]

Meanwhile, we must consider differences in coronary artery involvement in the thrombus aspiration and Adenosine injection methods. The question is why enzyme and ECG changes have been reported more in thrombus aspiration despite the improvement in cardiac perfusion in Adenosine injection? The response seems to be related to the more aggressiveness of the thrombus aspiration. Be that as it may, this explanation requires further confirmation through future studies.

Conclusion

According to the results of this study, there was no significant difference between the two methods of thrombus aspiration and injection of Adenosine in the no-reflow phenomenon as well as enzyme and ECG changes after

percutaneous coronary intervention. However, these results might have been affected by confounding factors such as comorbidities (e.g., DM, hypertension, and renal failure). According to the recent descriptions of no-reflow, its rare incidence, and limited previous studies in this area, the mechanism and causes of this phenomenon are not well understood. This has given rise to many ambiguities when trying to determine therapeutic approaches; challenges such as the best time for treatment or the best method to treat no-reflow. Due to the limited experience in this field, the present study may be the key to opening horizons to ideal treatment among Iranian investigators.

Recommendations

We suggest that factors such as underlying disease, psychological conditions, and medications be included in data analysis. Also, we also recommend that future research be undertaken to change the dose of Adenosine and compare its outcome with thrombus aspiration. It would be desirable to evaluate PTCA procedures separately in various contexts of heart diseases such as myocardial infarction and unstable angina.

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Evaluation of Plasma Myeloperoxidase Levels in Patients with Premature Myocardial Infarction

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Abstract

Introduction- The most significant consequence of coronary artery disease is myocardial infarction (MI), which accounts for most cardiac deaths. Differences between premature and non-premature MI based on the inflammatory enzyme, myeloperoxidase (MPO), have not been fully elucidated. Therefore, we sought to investigate whether plasma MPO could differentiate patients with premature or non-premature MI as compared to control subjects.

Methods- The participants included 42 patients with premature MI, 42 patients with non-premature MI, and 84 control subjects. The plasma concentration of MPO was determined with an enzyme immunoassay. Data were entered into SPSS-16 and analyzed by ANOVA, chi-squared, and Pearson correlation tests.

Results- Plasma MPO levels were significantly elevated in both premature and non-premature MI groups as compared to the control subjects ($P < 0.01$), but there were no differences between the patients and the controls by themselves ($P > 0.05$).

Conclusions- Our findings demonstrated that plasma MPO levels could not differentiate between premature and non-premature acute MI, although it could differentiate patients from control subjects.

Keywords: Myocardial infarction; Premature; Myeloperoxidase

Coronary artery thrombosis is the main cause for coronary obstruction, leading to acute myocardial infarction (MI) and many sudden cardiac ischemic deaths (1).

The most significant clinical cardiac ischemic consequences result from the instability of the fibrous cap overlying the atherosclerotic plaque (2).

Based on pathological studies, it has been shown that coronary artery culprit lesions have high macrophage content and prothrombotic materials (3). These plaques are predisposed to thinning and then the breakdown of the overlying fibrous cap.This causes the exposure of bared plaque to blood stream, which in turn leads to thrombus formation, luminal obstruction, and ischemia (4).

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Myeloperoxidase (MPO) is a white blood cell enzyme that has been demonstrated to have complex mechanistic relations with vulnerable plaque development (5). Many lines of evidence demonstrate that MPO may enhance plaque instability and thrombus formation. MPO activates matrix metalloproteinases, thereby causing plaque fissuring and thrombus formation (6). MPO promotes endothelial cell apoptosis (7) and causes superficial coronary artery erosions and as such plays an important role in MI (8). The results of some studies demonstrate that systemic MPO concentration is a predictor of plaque vulnerability in individuals at risk for major adverse cardiac events (9, 10). MPO appears as a potential prognostic index of near-term coronary artery disease risk (11). Plasma MPO has been reported to independently predict the early risk of MI in patients with chest pain and the risk of major adverse cardiac events in patients with acute coronary syndrome (12, 10).

According to the above literature, it appears that plasma MPO levels may have implications in many cardiovascular diseases such as MI. The implication of MPO in premature MI has yet to be comprehensively evaluated. It is uncertain whether MPO is different among patients with premature or non-premature MI in comparison with control subjects. Therefore, in the present study, we sought to find out whether plasma MPO could differentiate patients with premature or non-premature MI as compared to control subjects.

Methods

Patients: This observational case-control study, conducted at Noor and Aliasghar Hospitals in Isfahan, Iran, recruited 42 patients with premature MI, 42 patients with non-premature MI, and 84 control subjects to investigate the diagnostic value of MPO. All the subjects were categorized based on age and disease: 1) premature MI patients, who were < 50 years old; 2) non-premature MI patients, who were ≥ 50 years old; and 3) control subjects for each group of the patients that were age and sex-matched. The control subjects had no coronary artery disease. All the patients had enzymatically and electrocardiographically approved MI. In addition, patients with hepatic and renal disease, chronic or acute inflammatory diseases, or malignancies were not included in the study. Written informed consent was obtained from all the subjects. This research was approved by the hospital's Ethics Committee.

Biochemical Analysis

EDTA plasma samples were prepared from each patient during a 6-hour period after MI. Lipemic or hemolyzed plasma was excluded from the study. Overnight fasting EDTA plasma samples were also collected from all the control subjects. After plasma preparation, sample aliquots were stored frozen at −70°C until analysis was performed. The plasma concentration of MPO was determined with an enzyme immunoassay (Immunology Consultants Laboratory, Inc., Newberg, OR 97132, USA). Intra-assay and inter-assay coefficients of the variation of the assay were 2.8 and 6.8%, respectively. Each plasma sample was measured in duplicate.

Other clinical risk factors of the subjects such as hypertension, diabetes mellitus, smoking, and medication were also determined. Hypertension was assigned based on systolic blood pressure ≥ 140 mm Hg and diastolic blood pressure ≥90 mm Hg on two tandem occasions, or consumption of antihypertensive drugs. Diabetes mellitus was determined as a fasting blood sugar ≥ 126 mg/dl and hemoglobin A1c ≥ 6.5%, or administration of hypoglycemic medications. Family history of CAD was recognized as cardiovascular diseases in first-degree relatives at < 55 years of age in men or < 65 years in women. Smoking was elucidated as the use of tobacco and cigarettes in any quantity.

Statistical Analysis

Variables were assigned as mean ± SD or counts and percentages. The normal distribution of the data was evaluated using the Kolmogorov-Smirnov test. Differences in

MPO levels were assessed with the one-way ANOVA and post-hoc multiple comparisons by the Bonferroni test. The categorical data were compared using the chi-squared test. The correlation between the continuous variables was evaluated using the Pearson correlation coefficient. All the analyses were accomplished using SPSS version 16.0 (SPSS Inc., Chicago, Illinois, USA). A values of $p<0.05$ was assigned significant.

Results

The clinical characteristics of the study participants are depicted in Table 1. As is shown in the table, the patients and control subjects were age and sex-matched and the clinical risk factors had the same prevalence in the patient groups.

Table 1. Clinical characteristics of the study participants

Variable	AMI patients ≥ 50 years	AMI patients < 50 years	Control subjects ≥ 50 years	Control subjects < 50 years	P value
Age (year)	61.55 ± 7.39	43.57 ± 3.84	61.05 ± 7.57	43.74 ± 4.78	< 0.01†
Male	24(57.1)	25(59.5)	26(61.9)	24(57.1)	0.96
MPO (ng/mL)	74.82 ± 19.42	71.11 ± 19.39	24.92 ± 5.59	23.03 ± 4.34	< 0.01‡
Hypertension	20(47.6)	19(45.2)	4(9.5)	0(0)	< 0.01‡
Diabetes mellitus	20(47.6)	11(26.2)	2(4.8)	0(0)	< 0.01
Smoking	15(35.7)	8(19.0)	2(4.8)	5(11.9)	< 0.05‡
Family history of CAD	20(47.6)	18(42.9)	5(11.9)	4(9.5)	< 0.01‡
Aspirin	23(54.8)	11(26.2)	1(2.4)	0(0)	< 0.01

Data are expressed as mean ± SD or number (percentage).
AMI, acute myocardial infarction; MPO, myeloperoxidase; CAD, coronary artery disease
† Between two groups of patients
‡ Between patients and control subjects

Plasma MPO levels were significantly elevated in both premature and non-premature MI groups as compared to the control subjects ($P < 0.01$, Table 1), but there were no differences between the patients or controls by themselves (Figure 1).

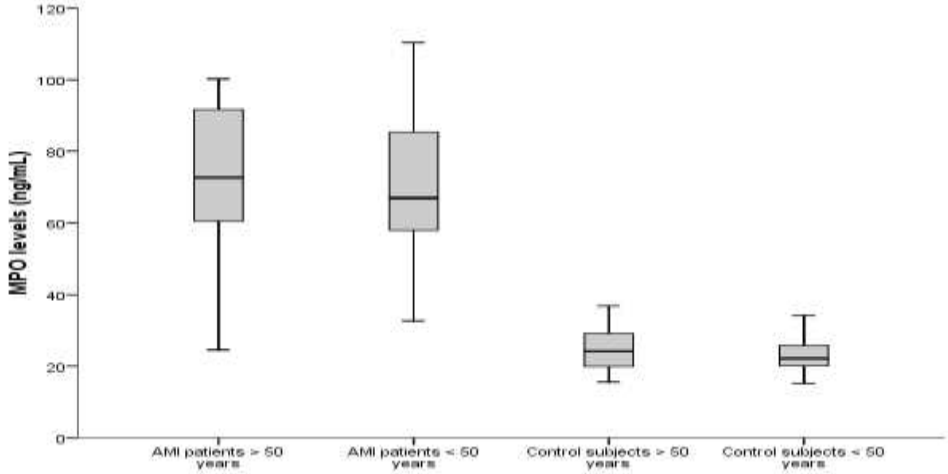

Figure 1. Plasma myeloperoxidase (MPO) levels in acute myocardial

infarction (AMI) patients and control subject

There were no differences of plasma MPO levels between men or women in all groups as whole (Figure 2).

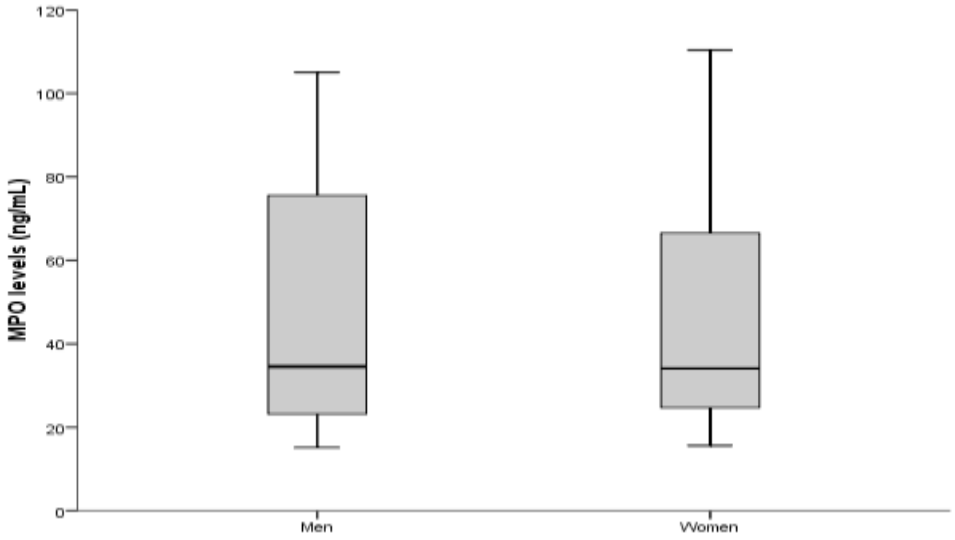

Figure 2. Plasma myeloperoxidase (MPO) levels between men or women in all the participants

Also, there was no significant correlation between plasma MPO levels and age in all the subjects (r = 0.014, P = 0.86).

Discussion

To the best of our knowledge, the present study is the first of its kind to exquisitely compare the plasma MPO levels in premature AMI and non-premature AMI patients in comparison with related control subjects. The results of the present study elucidated that plasma MPO levels were elevated in both AMI patient groups as compared with the control groups, but there was no significant differences between the patients with premature or non-premature MI.

So far, baseline plasma MPO levels have been introduced as an independent predictor of MI in patients with acute coronary syndrome (17). Also, the elevation of plasma MPO levels in coronary artery disease patients has been associated with the total mortality in the patients (12). The elevation of plasma MPO levels in our AMI patients in comparison with their related control subjects is in good agreement with the findings of the aforementioned studies.

Given the importance of MPO in plaque rupture and instability (18), it is conceivable that MPO would be elevated in any acute setting such as MI regardless of other contributors. Therefore, our findings that MPO could not differentiate between patients with premature and non-premature AMI may be reasonable. However, further studies are needed to corroborate this finding.

It is probable that many other risk factors may be responsible for the differences between premature and non-premature AMI. For example, genetic factors may be involved in premature MI (19), and MPO may not be a specific index for differentiating between the two groups of patients.

In summary, our results demonstrated that plasma MPO levels could not differentiate between premature and non-premature AMI, although it could diagnose patients from

control subjects, because the differences between patients and control subjects was about 4-fold.

One limitation of the present study was its sample size. Therefore, the results need to be corroborated in a larger population. However, our data followed a normal distribution and the subjects were selected in a randomized double-blind fashion.

Acknowledgements

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Myocardial Dysfunction in Thalassemia Major: Comparison between Echocardiography and MRI

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Abstract

Background- Iron overload contributes to cardiac dysfunction in patients with beta thalassemia major. T2* magnetic resonance is the gold- standard test for detecting iron overload in myocardial cells. Tissue Doppler imaging (TDI) has been developed to evaluate ventricular function in beta thalassemic patients.

Aim- We aimed to assess the efficacy of TDI in predicting myocardial iron load in beta thalassemic patients using T2* magnetic resonance imaging (MRI) as the gold standard test.

Methods- During a period of one year (2011- 2012), 27 known beta thalassemic patients were included in the study to be evaluated by TDI and T2* MRI.

Results- There were 15 females and 12 males at an age range of 18 to 39 years (23.89±5.19). Patients in the age group of 18-25 years old comprised 63% of the subjects. Serum ferritin level ≤1500 ng/ml was observed in 20 (74%) cases. T2* MRI > 20 ms was detected in 22 (81.5%) patients. Abnormal T2*MRI (T2*≤ 20 ms) had a significant correlation with increased serum ferritin levels (P = 0.007), increased left ventricular end diastolic dimension (P = 0.039), decreased E/A filling ratio (P = 0.022), and decreased early and late diastolic myocardial velocities at the lateral left ventricular wall (P = 0.028, and P = 0.023, respectively).

Conclusions- The present study findings suggested that even in a population of young, asymptomatic, and well-chelated thalassemic patients; impairment of myocardial function can be observed and this damage can be detected by echocardiographic techniques.

Keywords: Echocardiography; Cardiac dysfunction; Thalassemia major; T2* MRI

Introduction

Despite iron chelation therapy, congestive heart failure due to iron accumulation is still the leading cause of death in thalassemia major (TM) (1). Congestive heart failure occurs during the second decade of life. Early recognition of patients at risk of heart failure has been difficult because global ventricular function and exercise capacity in chronically transfused patients with iron overload may remain normal until late in the disease process (2). Patients may silently accumulate cardiac iron despite apparently adequate chelation therapy as judged by serum ferritin level. Monitoring of chelation therapy is a very important part of the management of transfusion-dependent patients. The ultimate aim of monitoring chelation therapy is to ensure that iron loading resulting from transfusion is kept to a minimum in order to prevent tissue damage,

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organ dysfunction, and adverse outcomes (3). Quantifying myocardial iron content has become possible using magnetic resonance imaging (MRI). The gold standard noninvasive diagnostic technique best able to detect iron accumulation is T2* cardiovascular MRI. MRI operates like many imaging modalities in that it transmits a signal into the body and creates an image from the signal returning from the body after it has interacted with the organ. The MRI scanner can refocus the returning signal either using a special radiofrequency pulse (spin echo) or using special small magnets known as gradients (gradient echo). The time constant for a spin echo is known as T2 and for a gradient is known as T2*. The greater the tissue iron, the shorter the signal half lives, and the smaller the T2 and T2* time (4).The development of the T2* technique using cardiovascular magnetic resonance has allowed physicians to have a reliable method for measuring cardiac iron to guide chelation therapy. However, T2* MRI is expensive, not widely available in many centers, and interpretation needs an expert. This will limit the application of this technique, especially in the developing countries where thalassemia is more common (5). Echocardiography is more widely available. Tissue Doppler imaging (TDI) is an echocardiographic technique that enables quantitative assessment of myocardial tissue velocities. TDI uses the amplitude of the echocardiographic signal returning from the examined myocardial tissue, which is much stronger than the echoes backscattered by blood. TDI M-mode enables a quantitative analysis of local myocardial velocities as a function of time. Analysis of regional ventricular function is of particular importance in the early detection of local abnormalities, .i.e. before any change is apparent in global function measurements (6). Previous studies have reported that TDI can detect early myocardial dysfunction related to iron overload (7, 8, 9).

In this study, we aimed to assess the efficacy of TDI in predicting myocardial iron load in thalassemic patients using T2* MRI as the gold standard diagnostic test.

Patients and Methods

During a one-year period between January 2011 and December 2012, we examined 27 patients attending a hematologic clinic for thalassemia in Shohada Hospital. These patients were referred to the Pediatric Cardiology Clinic of Shahid Modarres Hospital for echocardiography. Patients were ≥ 18 years old, without clinical signs and symptoms of congestive heart failure at the time of examination, and were all included in the study. All the subjects underwent a complete history taking, physical examination, and electrocardiography. All the participants gave their informed consent to the procedure and the Ethics Committee of the hospital approved the study. None of the subjects enrolled in the study had systemic hypertension (defined as diastolic blood pressure ≥ 90 mm Hg or systolic blood pressure ≥ 140 mm Hg or both). Two patients suffered from diabetes and were under treatment. Seven patients reported palpitation but had no documented arrhythmias and were not on specific therapy. Splenectomy had been performed in 17 (63%) cases.

Transthoracic echocardiography was performed with the subjects in partial left decubitus position by an expert pediatric cardiologist using a 3.5-MHz transducer with a system 5 sector scanner (Vingmed). Conventional echocardiography evaluation from the parasternal long-axis view included left ventricular (LV) end-diastolic and end-systolic diameter, interventricular septum thickness, and LV posterior wall thickness at the end of diastole and systole. Also, LV ejection fraction and shortening fraction were measured. All Doppler echocardiography recordings were obtained in the apical four-chamber view. The pulsed Doppler sample volume was placed at the mitral valve tips. The following parameters of LV diastolic function were measured: early (E) and late (A) peak

velocities (m/s) and their ratio; E wave deceleration time (DT) (ms); and isovolumic relaxation time (IVRT) (ms). The TDI program was set to the pulsed wave Doppler mode. Filters were set to exclude high-frequency signals, and the Nyquist limit was adjusted to a velocity range of 15-20 cm/s. The sample volume was placed at the apical four-chamber view on the lateral corner of the mitral annulus, the medial (septal) corner of the mitral annulus, and the lateral corner of the tricuspid annulus. Tissue velocity imaging measures included systolic myocardial velocities at the lateral LV wall, septum, and right ventricular (RV) free wall (LV-Sm, Septum-Sm, and RV-Sm, respectively); early and late diastolic myocardial velocities (Em, and Am, respectively) at the same segments (i.e., LV-Em, and LV-Am for the lateral LV wall, Septum Em, and Am, RV-Em, and RV Am for the RV free wall); and LV E/ Em ratio. All the measurements were achieved on three consecutive heart beats, and the average of the three measurements was calculated.

All the subjects enrolled in the study underwent MRI within 30 days of TDI using a 1.5 T-edge scanner. ECG-gated cardiac MR was obtained for T2* calculation, and short-axis images were prepared in different sequences. T2* and iron load values were calculated by "CMR Tools" software. Patients with myocardial T2* > 20 ms were categorized as having normal myocardial iron load or group A, and patients with myocardial T2* ≤ 20 ms were categorized as having abnormal myocardial iron load or group B. Analysis of the MRI data by the radiologist was performed blind to the echocardiography measurements, and analysis of the echocardiographic data by the cardiologist was performed blind to the results of the MRI study.

All the statistical analyses were accomplished using SPSS software (version 16). All the continuous data were expressed as mean ± SD, and the categorical variables as percentage. The Student t-test was used to compare the mean values of all the variables. Logistic regression analysis was used to disclose possible correlations between cardiac T2* and echocardiographic data. A P-value of < 0.05 was considered to indicate statistical significance.

Results

During the study period, 27 thalassemic patients (15 females and 12 males) were examined. Of the 27 patients enrolled in the study, 21 suffered from thalassemia major and 4 from thalassemia intermedia. The patients' age ranged from 18 to 39 years (23.89 ± 5 .19 years). The age group of 18-25 years old was the most common 17 (63%), followed by 25-32 years old 9 (33.3%) and 32-39 years old 1 (3.7%). The average serum ferritin levels for the same year were available for all the patients. The serum ferritin levels were from 80.30 to 4889 ng/ml (1221.39 ±1113.30 ng/ml). The serum ferritin level of < 1500 ng/ml was the most common 20 (74%), followed by 1500-2500 ng/ml in 3(11.2%) and > 2500 in 4 (14.8%) cases. Of the 27 patients in the study population, 10 were receiving iron chelation therapy with Deferoxamine, 4 were on the oral chelator, and 13 were switched from Deferoxamine to oral chelator. The range of T2* MRI in the patients was from 5.77 to 49.40 ms (29.6 ± 11.55 ms). In 22 cases, T2* MRI was > 20 ms, and in 5 cases, T2* MRI was ≤ 20 ms. Eighteen cases of the 20 patients with serum ferritin level < 1500 ng/ml had T2* MRI > 20 ms.

Table 1 summarizes the results of age, serum ferritin level, and echocardiographic data in the two groups of patients and compares them with each other. As is shown in this table, the serum ferritin level in group B was significantly higher than that in group A, and LV end diastolic dimension in group B was also significantly higher than that in group A. Table 2 shows the results of linear regression analysis. As is evident in this table, the T2* MRI value decreased by increasing the serum ferritin level (P = 0.007), increasing LV

dimension in diastole (P = 0.039), decreasing lateral LV wall early and late velocities (P = 0.028 and P = 0.023, respectively), and decreasing E/A ratio (P = 0.022). We did not find a significant correlation between the T2* MRI values and the other echocardiographic parameters.

Table 1. Demographic and echocardiographic characteristics of the study population

Variables	All patients	Group A T2*(>20 ms)	Group B T2*(<20 ms)	*P*-value(A vs. B)
Patients	27	22 (81.5%)	5 (18.5%)	NA
Female (%)	15 (55.6%)	12 (80%)	3 (20%)	NA
Age (year)	23.89±5.58	24.41±5.49	21.6±2.97	NS
Serum ferritin (ng/ml)	12214±1113.3	960.39±835.33	2369.8±1539.42	0.008
LVD diastole (cm)	4.73±0.46	4.24±0.26	4.84±0.43	0.006
IVS diastole (cm)	0.84±0.16	0.84±0.15	0.88±0.18	NS
LVPW diastole (cm)	0.80±0.22	0.79±0.22	0.86±0.24	NS
LVD systole (cm)	3±0.43	3.05±0.43	2.78±0.38	NS
IVS systole (cm)	0.98±0.19	0.97±0.19	0.99±0.23	NS
LVPW systole (cm)	0.97±0.3	0.94±0.26	1.12±0.43	NS
EF (%)	72±0.1	73±0.096	67±0.12	NS
SF (%)	35±0.07	36±0.07	32±0.09	NS
E velocity (m/s)	1.14±0.18	1.13±0.15	1.21±0.27	NS
A velocity (m/s)	0.72±0.14	0.73±0.14	0.69±0.14	NS
E/A ratio	1.62±0.49	1.55±0.37	1.91±0.86	NS
IVRT (ms)	71.6±24.49	68.5±24.28	85.23±22.73	NS
DT (ms)	189.03±68.19	191.89±66.34	176.46±82.92	NS
Lateral LV Sm (m/s)	0.11±0.04	0.12±0.04	0.10±0.03	NS
Lateral LV Em (m/s)	0.15±0.04	0.16±0.04	0.11±0.02	NS
Lateral LV Am (m/s)	0.09±0.03	0.10±0.29	0.06±0.01	NS
Septum Sm (m/s)	0.11±0.03	0.11±0.03	0.11±0.02	NS
Septum Em (m/s)	0.13±0.04	0.13±0.03	0.14±0.03	NS
Septum Am (m/s)	0.09±0.02	0.09±0.02	0.08±0.02	NS
Lateral RV Sm (m/s)	0.16±0.03	0.16±0.03	0.15±0.01	NS
Lateral RV Em (m/s)	0.17±0.04	0.17±0.04	0.16±0.02	NS
Lateral RV Am (m/s)	0.13±0.04	0.13±0.04	0.13±0.04	NS
LV E/ Em ratio	9.37±2.50	9.43±2.51	9.06±2.75	NS

LVD = left ventricular diameter, IVS = Inter ventricular septum, LVPW = left ventricular posterior wall, EF = ejection fraction, SF = shortening fraction, IVRT = isovolumic relaxation time, DT = deceleration time, NA = not available, NS= not significant.

Table 2. Logistic regression analysis of variables

Variables	r	P-value
Serum ferritin	-0.507	0.007
LVD diastole	- 0.400	0.039
E/A ratio	0.439	0.022
Lateral LV Em	0.423	0.028
Lateral LV Am	0.437	0.023

r= correlation, LVD= Left ventricle dimension in diastole

Discussion

Our data indicated that 22 (81.5%) patients had myocardial T2* > 20 ms (group A), which was considered the lower limit of the normal range. Serum ferritin level was ≤ 1500 ng/ml in 20 (74.1%) subjects, and 18 of the 20 had been placed in group A. There was a significant correlation (P = 0.007) between serum ferritin level and myocardial T2*.

This finding suggested that an effective chelation therapy had been administered for the patients. A previous study (8) showed that LV global function was preserved in patients with serum ferritin level < 2500 ng/ml, and the majority of our patients were also in this category. LV diastolic dysfunction is still considered an early marker of myocardial damage, but there is no agreement on which echocardiographic indices best predict tissue iron overload (10, 11). Leonardi et al. (2008) reported a poor correlation between diastolic function parameter and myocardial T2* values. And they suggested that these parameters were not suitable for risk stratification (12). The results of a preliminary study by Gregorio et al. (2010, Italy) on 14 asymptomatic TM patients revealed that 12 of the 14 had normal (> 20 ms) T2* values. They found that TDI was not able to discriminate patients with low T2* (< 20 ms) from those with normal values. In contrast, conventional echocardiography parameters like short isovolumic relaxation time, E/A > 2, and atrial enlargement were correlated with myocardial iron overload (13). The results of a research by Silvidairat et al. (2008, Thailand) on 31 asymptomatic TM patients indicated that myocardial diastolic dysfunction was absent in all patients with serum ferritin levels < 2500 ng/ml and was present in all patients with serum ferritin > 5000 ng/ml. TDI parameter E/Em had a significant correlation with the serum ferritin level. They concluded that LV systolic and diastolic functions were preserved in patients with serum ferritin < 2500/ng/ml (8). In the present study, there was a significant correlation (P = 0.039) between increased LV end diastolic diameter and abnormal T2*MRI. Decrease in the ratio of early to late diastolic filling (E/A) and decrease in the velocities of early and late diastolic myocardial function of the lateral LV wall indicated impaired relaxation. Magri et al. (2008, Italy) studied 30 asymptomatic TM patients. They found impairment in myocardial function in even well-chelated patients. This mild impairment was detected by advanced TDI and strain echocardiography (7).Vogel et al. (London, 2002) reported 52 asymptomatic TM patients aged 29.2 (14.2 - 43.1 years). Ninety-six percent of the patients had normal ejection fraction. Thirty-eight cases had T2* < 20 ms and in 14 patients T2* was > 20ms. Septal motion abnormality was observed in 28 patients. They concluded that wall motion abnormality might be the early sign of myocardial iron load despite preserved global function (5). According to our findings, cardiac global function was preserved in all the patients but there was a significant correlation between lower T2* values and decreased lateral LV wall early and late diastolic velocities.

Larussi et al. (2003, Italy) used TDI for the evaluation of 30 asymptomatic TM patients. They found that the TM patients with normal systolic function had diastolic dysfunction such as increased E velocity and increased size of the left atrium. The above findings were suggestive of increased preload (14). In our patients, the pattern of diastolic dysfunction was decreased E/A ratio and increased LV end diastolic dimension. In a previous research by Aypar et al. (2010, Turkey), 33 asymptomatic TM patients aged 18 ± 6 (6 – 31 years) with normal LV global systolic function were evaluated. Twenty-five cases (86%) had T2* < 20 ms. They found that TDI echocardiography was a sensitive and specific technique for the prediction of myocardial iron load and could be employed for the screening

of TM patients (1). In a study by Amoozgar et al. (2011, Iran), 51 thalassemia intermedia with normal M-mode and two-dimensional echocardiography were examined by TDI technique. The authors found significant changes in the systolic velocity of the septum and E velocity of the tricuspid valve (15). In a previous report by Hamdy et al. (2007, Cairo), 27 asymptomatic thalassemic patients were evaluated. The researchers found that TM patients had regional systolic dysfunction in the lateral LV wall, and diastolic dysfunction in the septum and RV free wall (2). Another study by Hamdy et al. (2007) on 30 TM patients showed that patients with serum ferritin levels > 2000 ng/ml were at risk for RV systolic dysfunction and pulmonary hypertension (16). We found neither abnormal tissue velocities in the RV free wall nor pulmonary hypertension in our cases.

The limitation of our study was its small sample size. Multicentre studies with larger populations can scrutinize our findings.

In conclusion, in the present study, mild diastolic dysfunction as impaired relaxation was detected by Doppler and TDI imaging, and this finding had a correlation with iron overload observed by T2* MRI.

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Renal function following On-pump versus Off-pump CABG

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Abstract

Introduction- Despite the success of on-pump coronary artery bypass grafting (CABG) in the treatment of coronary artery disease, morbidity and mortality due to renal failure is a well-known complication of this method. Currently, it is believed that off-pump CABG can be done with the same result and fewer adverse renal effects due to a lack of the use of the cardiopulmonary bypass machine. The aim of this study was to compare postoperative renal function between off-pump CABG and on-pump CABG.

Method- Sixty-seven consecutive candidates for elective isolated CABG were enrolled in this study and randomized into two groups. The on-pump group consisted of 34 patients and the off-pump group included 33 patients. All the patients were operated on via the same technique and surgeon. Blood samples were obtained before surgery and 6, 24, and 48 hours after surgery to measure the serum creatinine and assess creatinine clearance. Either 20% increase in the serum creatinine level or 20% decrease in creatinine clearance was considered as renal dysfunction.

Results- In the off-pump group, 5, 9, and 4 patients developed renal dysfunction 6, 24, and 48 hours postoperatively, respectively in comparison with 13, 22 ,and 11 patients of the on-pump group at the same time. There was a significant difference between the two groups regarding renal dysfunction 6 and 24 hours postoperatively (p value=0.038 and 0.003, respectively), but no significant difference was observed at 48 hours after surgery. We found no relationship between age and weight and postoperative renal dysfunction in either of the groups. Preoperative hypertension and diabetes had no effect on postoperative renal dysfunction.

Conclusions- Renal function was better preserved in the patients undergoing off-pump CABG than on-pump CABG. Old age was not a risk factor for renal function but it could be a matter of debate and must be studied in future experiments.

Keywords: Coronary artery bypasses graft surgery; Renal dysfunction; Cardiopulmonary bypass

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Introduction

Coronary artery bypass grafting (CABG) is a frequent performed surgery and is a major option for the treatment of coronary artery disease (CAD). Despite the improvement and advances in anesthesia and surgery techniques and protocols, mortality and morbidity of this operation is still a major concern. One of the causes of mortality and morbidity after cardiac surgeries is postoperative acute renal failure. Today many cardiac surgeons have become interested in doing CABG in the beating heart because they think that renal failure may occur due to systemic inflammatory response after cardiopulmonary bypass (CPB).

Renal dysfunction has been reported to occur at a rate of 8%–30% after procedures performed under CPB and is associated with mortality rates of 7% to 38%. When postoperative CPB leads to an acute renal failure which requires dialysis, the mortality rate rises to 60%.[1–3]

There is a dearth of data on the function of the renal system after on and off-pump CABG (OPCAB) in the Iranian population. The present prospective study sought to compare the renal function between on-pump CABG and off-pump CABG.

Methods

This prospective study was performed on 67 patients candidated for elective primary isolated CABG and met our inclusion criteria. The study protocol was approved by the local Ethics Committee of Mashhad University of Medical Sciences. Patients with any concomitant cardiac repair, any history of renal failure, serum creatinine level > 1.3, and a history of cardiac catheterization within the last 3 days were excluded from the study.

The patients were randomized into two groups: patients undergoing OPCAB (Group 0) and those undergoing on-pump CABG (Group 1). All the patients were operated on by a one surgeon, and the surgical and anesthetic protocols were identical between the two groups. Blood samples were obtained before surgery as well as 6, 24, and 48 hours after CABG to measure the level of serum creatinine and determine creatinine clearance. Either 20% increase in the serum creatinine level or 20% decrease in creatinine clearance was considered renal injury.

Operative Techniques

OPCAB: All the operations were performed through median sternotomy. The left internal mammary artery (LIMA) and saphenous vein were harvested. Heparin was given at a dose of 150 units/kg, and the activated clotting time was maintained above 250 seconds. Pericardial traction sutures elevated the left posterior pericardium. The site of the distal anastomosis of the coronary artery was stabilized with an Octopus stabilizer (Medtronic, Inc., Minneapolis, MN). The LIMA to left anterior descending coronary anastomosis was usually the first anastomosis to be done. The patient's position was changed to the Trendelenburg position. The control plaque was placed proximal to the anastomosis location, the coronary artery incision was made longitudinally by the 11th blade, and the anastomosis was performed by standard technique. All the distal anastomoses were constructed prior to proximal anastomoses. The proximal anastomosis was performed by means of a side-biting clamp. ACT was reversed by protamine at the end of the procedure.

On-pump CABG: Median sternotomy was performed as was described in the OPCAB technique. CPB was established after aorto-bicaval cannulation. Cardiologic solution was administered following mild hypothermia and cross-clamping of the aorta. The distal and proximal anastomosis was constructed as was previously described. Weaning from CPB was done following homeostasis. The chest wall was closed in layers by standard technique.

Statistical Analysis

The data are expressed as mean±SD. Statistical analysis was performed using SPSS 15.0 software. The chi-squared text or the Fisher exact test was used for the categorical data, and unpaired Student t-tests were employed to compare the continuous variables. A p value<0.05 was considered statistically significant.

Results

A total of 67 patients were enrolled in this trial and randomized into two groups (on-pump=34 and off-pump=33). There was no difference between the two groups in terms of age, weight, gender, and prevalence of diabetes and hypertension. The mean age was 58.5 ± 10.71 years and 58.91 ± 9.39 in the on-pump group and off-pump group, respectively. The mean weight was 65.00 ± 11.90 kg in Group 0 versus 69.41 ± 12.36 kg in Group 1. A statistical difference was observed in the ejection fraction (EF) of the patients between the two groups, so logistic regression model was used to eliminate the confounding effect of the EF on our results. The mean CPB and cross-clamp time was 85.62 ± 22.04 and 53.09 ± 23.53, respectively. Post-surgical renal dysfunction was observed in 5, 9, and 4 patients of the off-pump group at the 6th, 24th, and 48th hour, respectively, compared to in 13, 22, and 11 patients of the on-pump group at the same time points.

The logistic regression model revealed a statistically significant difference between the two groups in terms of postoperative renal impairment at the 6th and 24th postoperative hours (p value=0.038 and 0.003 respectively). There was no difference in the development of postoperative renal impairment between the two groups 48 hours after surgery, and nor was there a significant relationship between postoperative renal impairment and age, diabetes, and hypertension.

Discussion

In this trial, we sought to compare postoperative renal function between two groups of on-pump CABG and off-pump CABG.

Systemic ischemia and oxidative stress might be the results of CABG with CPB.[9–11] CPB is well known to induce a generalized inflammatory response associated with complement activation and release of cytokines and free radicals, potentially damaging the renal brush-border membrane.[12] It is assumed that in 30% of patients undergoing cardiac surgeries with CPB, moderate transient impairment of renal function (serum creatinine level >1.5 mg/dL, but <5.0 mg/dL) will occur.[4,8]

The results of our study showed that renal function was more impaired in the on-pump CABG group than in the off-pump CABG group insofar as at the 24th post-CABG hour. However the renal function was the same between these two groups on the second postoperative day. In the Mitsugu et al. study[12], renal impairment was detected in 12.7% of the off-pump group, whereas it was 18.5% in the on-pump group. Although these differences were not statistically significant between these two groups, postoperative hemodialysis requirement was significantly higher in the on-pump group. Thus, CPB might be one of the risk factors of renal impairment following on-pump CABG. Nevertheless, these changes occur only in the acute phase of the postoperative period.

Even though the difference was not statistically significant at the 48th postoperative hour, it was clinically important and should be the subject of further research. Prolonged CPB time is thought to be a risk factor for post-CPB renal dysfunction. We, however, observed no significant relationship between CPB time and postoperative renal dysfunction. This may be related to the low CPB time in our series, which was less than 90 minutes. Old age and diabetes are reported to be risk factors for the

development of post-CABG renal dysfunction, but in the present study we found no significant difference. This might be due to the small sample size, which was estimated for postoperative renal dysfunction and could be a subject for further studies on larger populations.

Table 1- Prevalence of renal injury in the on-pump versus off-pump CABG groups

		Off-pump group (n=34)	On-pump group (n=33)	P-value
Renal injury*	6th hour	5 (15.2%)	13 (38.2 %)	**0.032**
	24th hour	9 (27.3%)	22 (64.7 %)	**0.002**
	48th hour	4 (12.1%)	11(32.4 %)	0.061

CABG, Coronary artery bypass grafting surgery

*** Either 20% increase in the serum creatinine level or 20% decrease in creatinine clearance was considered as renal injury**

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Difficult Tracheal Intubation in a Child with Cornelia De Lange Syndrome: A Case Report

Mohsen Ziyaeifard[1], MD; Zia Totonchi[1], MD; Seyed Mohamad Mahdavi[2], MD; Rasoul Azarfarin[3], MD;

Abstract

Cornelia de Lange syndrome (CdLS) is a rare multiple congenital abnormalities with dominantly inherited and developmental delay. Regarding craniofacial deformities in these patients, airway management may be challenging for anesthesiologists. In these patients, pre-anesthetic evaluation must be done with exact assessment of the airway, including physical examination and radiography, CT, or MRI of the head and neck. An anesthetist should prepare the "difficult intubation set", including a flexible fiberscope, and may need to consult with an ear-nose and throat (ENT) specialist if necessary for unexpected emergency situations. In this case-report, we describe a 7-year-old boy with CdLS with prominent maxilla, mouth opening restriction, and micrognathia who underwent complete repair of ventricular septal defect and pulmonary valve stenosis surgery. Anesthetic and airway management and postoperative period were uneventful.

Keywords: Tetralogy of Fallot repairing■ Cold blood cardioplegia■ Crystalloid cardioplegia

Introduction

Airway management is one of the most important tasks of an anesthesiologist for careful airway assessment before the induction of anesthesia.[1] Difficult airway management is constantly a significant issue.

Hypoxia because of ventilation failure is a major cause of fatality and leads to neurological deficit in patients with a difficult airway.2 Cornelia de Lange syndrome (CdLS) is a rare multiple congenital disease with dominantly inherited and developmental delay.

It is essentially characterized by craniofacial, limb and developmental anomalies, hirsutism, and motor and intellectual disability. [3] A range of other anomalies (cardiac, gastrointestinal, genital, renal, and ocular) is frequently present. Regarding maxillofacial deformities in these patients, airway management may be challenging for anesthesiologists. There are limited reports that discuss this issue and explain clinical scenarios of difficult tracheal intubation and also gastro-esophageal reflux during the induction of general anesthesia in patients with CdLS.[4, 5]

In this case-report, we describe a 7-year-old boy with CdLS and double-outlet right ventricle (DORV),

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ventricular septal defect (VSD), and pulmonary valve stenosis (PS) who underwent complete corrective surgery.

Case Report

A 7-year-old boy with a known history of CdLS was scheduled for complete repair of DORV, VSD, and PS under general anesthesia. The patient was 97 cm tall and 13 kg in weight. On physical examination, the patient had psychomotor retardation, hirsutism (Figure 1), congenital anomalies of hands and feet (Figure 2), craniofacial skeletal malformation, micrognathia (Figure 3), short and stiff neck, and restricted opening of the mouth (Figure 4), which orientated us to the probability of difficult airway (Mallampati score=3). We, therefore, prepared a "difficult intubation set", including a flexible fiberscope.

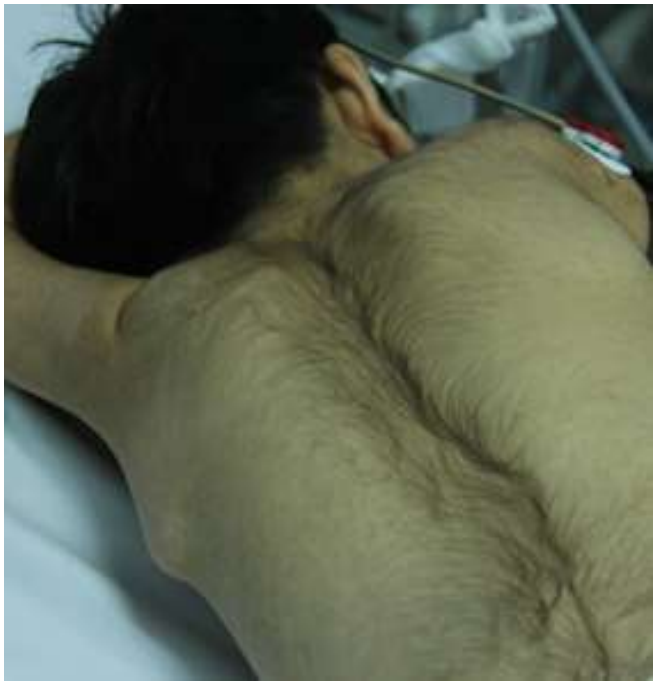

Figure 1. hirsutism in the patient

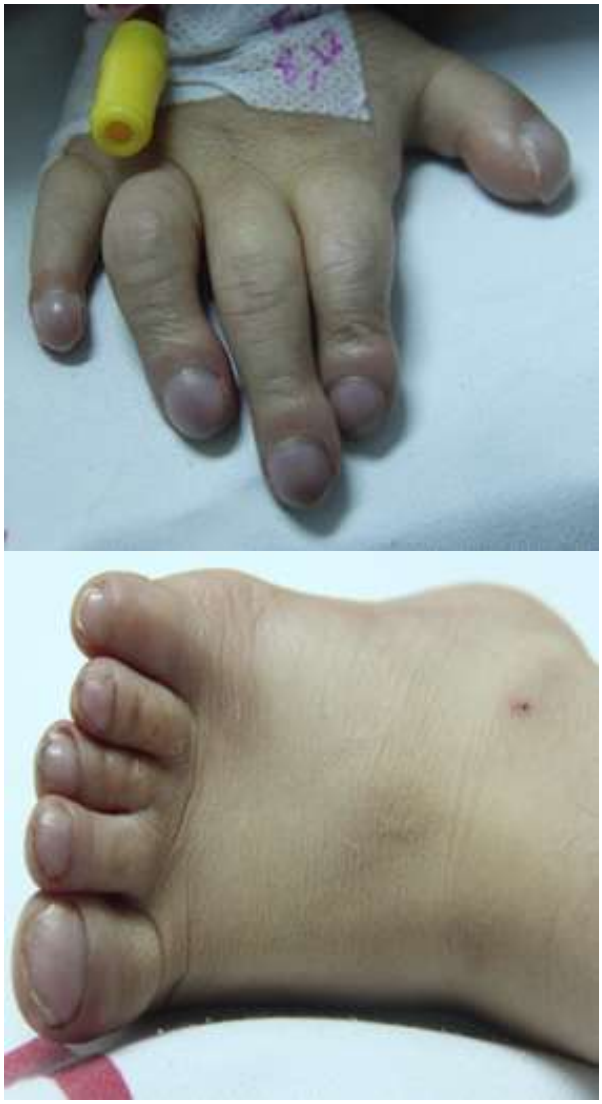

Figure 2.Hands and feet deformity in the patient

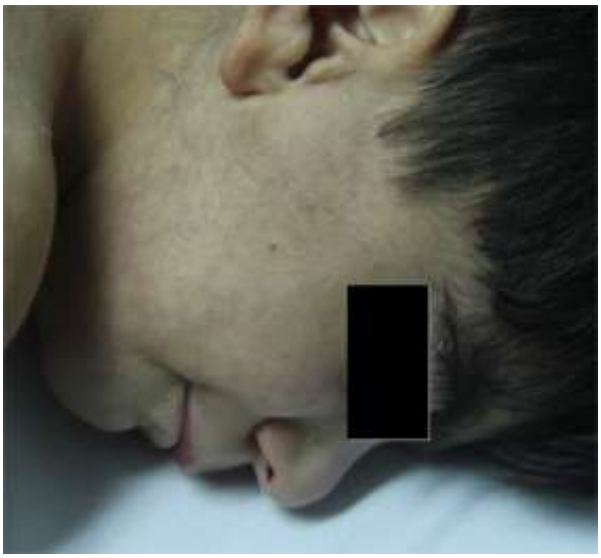

Figure3.Micrognathia in the patient

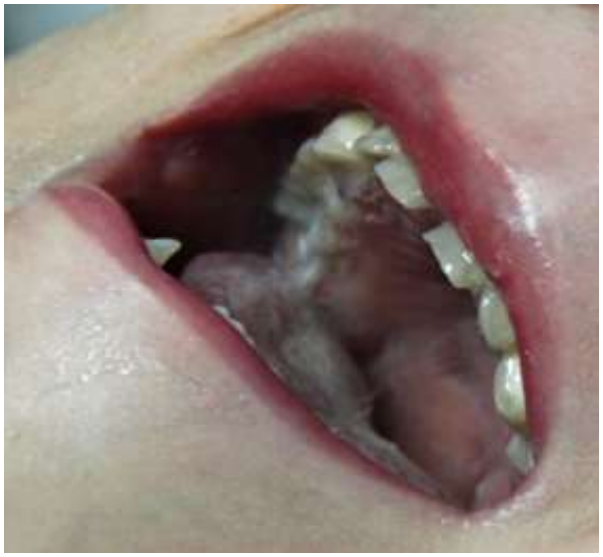

Figure 4. Maxillofacial deformity and restricted mouth opening in the patient.

The anesthesiologist in preoperative visit ordered oral Diphenhydramine (1 mg/kg) as premedication 2 hours before the operation. In the operating room, the patient was monitored with electrocardiography, peripheral oxygen saturation (SpO2), and noninvasive blood pressure monitoring. The patient was initially sedated by Sevoflurane and 100% oxygen inhalation with a face mask. Then, a peripheral cannula (20 G) was inserted and an IV infusion of Ringer's solution was initiated at 10 ml/kg. The induction of anesthesia was completed by Midazolam (0.05 mg/kg), Fentanyl (4 µ/kg), and Pancuronium bromide (0.15 mg/kg). The lungs were ventilated with a face mask without difficulty. At the first attempt by an anesthesia resident, tracheal intubation was difficult and unsuccessful. At the second attempt by an anesthesia attending, tracheal intubation was successful but was not easy (laryngoscopy grade=3) and was done by using an introducer (buggy). The patient's trachea was intubated with an ID=5.5 mm cuffed endotracheal tube (ETT). The cuff inflation pressure was adjusted to 25 cmH2O by using a "cuff pressure gauge". A central venous line catheter was inserted in the right internal jugular vein. Anesthesia was maintained by continuous infusion of Midazolam (1 µ/kg/min), Fentanyl (5 µ/kg/hr), and bolus doses of 0.05 mg/kg of Pancuronium bromide. VSD closure and correction of PS were done by using cardiopulmonary bypass. Aortic crass-clamp time was 70 minutes, and operation time was approximately 4 hours.

After the completion of surgery, the patient was transferred to the ICU. In the ICU, the weaning process from mechanical ventilation was uneventful, and the patient's trachea was extubated after 8 hours. The patient was discharged after 3 days' stay from the ICU and was transferred to the ward. Finally, the patient was discharged from the hospital after 10 uneventful days.

Discussion

CdLS was first described in 1933.5 CdLS is also identified as Brachmann- de Lange syndrome and is a rare congenital genetic dominantly inherited disorder. This syndrome presents mainly with mental and skeletal developmental delay.[6] It is essentially characterized by craniofacial, limb anomalies, hirsutism, and motor and intellectual disability.[7] A range of other anomalies, including cardiac, gastrointestinal, genital, renal, and ocular, is frequently present. Congenital cardiac defects that are frequently reported with this syndrome are atrial septal defect (ASD), VSD, patent ductus arteriosus (PDA), left ventricular (LV) hypoplasia, and abnormal ECG such as LV and RV hypertrophy. CdLS patients experience some pulmonary abnormalities (i.e., pulmonary or

lobular hypoplasia).[3] Acute bronchitis or pneumonia has been reported as the main cause of mortality in this syndrome. These patients are very sensitive to respiratory infections and have irritable airways, so endotracheal intubation should be done carefully so as to keep adequate depth of anesthesia.[1, 5, and 7]

Airway management in special circumstances has always been of great significance to the physicians of all eras.[8] Nowadays, emphasis is placed on the education and proper success in airway management [9] by using proper instrument, [10] especially in pediatric patients undergoing cardiac procedures.[11] There are various types of craniofacial anomalies in pediatric patients that make airway management very challenging for anesthesiologists. Some etiologies of this deformity are congenital laryngeal stenosis, Goldenhar's syndrome, Pier Robin sequence, Treacher-Collin's, hemifacial microsomia, Cleft palate, Down's, Klippel-Feil, epiglottitis,[12] lingual tonsillitis, abscess, laryngeal trauma, achondroplasia, angioedema, Turner, Noonan, Thalassemia major, Stevens-Johnson, Marfan, Crouzon, Apert, and finally CdLS.[13,14]

There are limited reports discussing this issue and explain the clinical scenarios of difficult orotracheal intubation during anesthesia induction in patients with CdLS.[7] Our patient was scheduled for complete repair of DORV, VSD, and PS under general anesthesia. The difficulty of airway management of this patient mainly was due to craniofacial skeletal malformation, short and stiff neck, restricted opening of the mouth (Mallampati score=3); accordingly, we prepared a "difficult intubation set", which included a flexible fiberscope. After the induction of anesthesia, the lungs were ventilated with a face mask without difficulty. Tracheal intubation was unsuccessful in the first attempt. At the second attempt, tracheal intubation was not easy (laryngoscopy grade=3) and was done by using an introducer (buggy).

In such patients (prediction of difficult airway), pre-anesthetic evaluation must be done by complete assessment of the airway anatomy with physical examination and radiography or other imaging modalities. Consultation with an ENT specialist may be necessary for further evaluation of the throat and larynx anatomy.

As anesthesia induction in these patients can simply occlude the airway, administration of hypnotics and opioides must be titrated to maintain the spontaneous breathing of the patient. Also, slow induction with inhalation anesthetics (i.e., Sevoflurane and Isoflurane) is suitable because of the better control of airway management. However, Madan et al. found that intravenous induction was preferable to the inhalational one. Muscle relaxant should be administered cautiously and be considered for similar reasons.[4,5] Tsukazaki reported that orotracheal intubation with direct laryngoscopy may complicate CdLS.[15]

In pediatric patients with difficult airway, intubation with the aid of a flexible fiberscope and local anesthetics and mild sedation is the choice method for tracheal intubation in limited mouth opening. The smallest size of a fiberoptic bronchoscope is 1.8 mm, which can pass through a 2.5-mm ID endotracheal tube. This technique permits simultaneous oxygen delivery directly into the trachea, as well as airway suctioning.[16] Another option for these patients is stylet-guided endo-intubation after anesthesia induction. Introducing a Fastrach laryngeal mask through a laryngeal mask airway (LMA) has been suggested a substitute

procedure for tracheal intubation in these patients.[7] Tracheostomy must be done only in emergency situation or when other alternative measures unsuccessful. [13]

In conclusion, in patients with craniofacial anomalies such as CdLS, anesthesia and airway management often is challenging and exact preoperative evaluation and preparation of a "difficult intubation set" can potentially reduce airway management complications.

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Rupture of Sinus of Valsalva Aneurysm Associated with Infective Endocarditis in a 39-Year-Old Patient with Congenital Ventricular Septal Defect and Subvalvular Pulmonary Stenosis: A Case Report

Hossein Azarnik [1], MD, FACC; Syedeh Leila Rezvani [2*], MD; Gholamreza Omrani [3], MD; Gita Rezvani [4], DMD;

Abstract

Aneurysm of the sinus of Valsalva is a rare congenital lesion that may be associated with other congenital lesions such as ventricular septal defect (VSD). Less common lesions can occur secondary to trauma, infective endocarditis, or syphilis. The majority of these aneurysms originate from the right coronary sinus. Patients with unruptured aneurysms usually remain asymptomatic. Rupture of the aneurysm usually causes the appearance of a continuous murmur in the left sternal border. Locations of the common ruptures include the right ventricle, the right atrium, and less commonly the left atrium. Surgical repair of the tear usually has optimal outcomes.

We describe a 39-year-old man with a history of congenital VSD, subvalvular pulmonary stenosis, and rupture of the right sinus of Valsalva aneurysm complicated by infective endocarditis.

Keywords: Myocardial infarction■ Collateral coronary vessels■ Electrocardiography

Case

A 39-year-old asymptomatic man was referred to Rajaie Cardiovascular, Medical and Research Center for surgery and repair of ventricular septal defect (VSD) and ruptured aneurysm of the right coronary sinus to the right ventricular outflow tract. The patient had a history of congenital VSD and had been under medical supervision until age 18 and was completely symptom free. One year previously, he had developed prolonged fever, weight loss, loss of appetite, and night sweat for 8 months with frequent medical visits and with no definitive diagnosis. He had empirically been treated with various antibiotics, but his symptoms did not improve. Then cardiology consultation was done and with a high suspicion of infective endocarditis, transesophageal echocardiography was performed and VSD, rupture of the right coronary sinus to the right ventricular outflow tract with left-to-right shunting and vegetations of endocarditis were reported.

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The patient was managed with appropriate antibiotics for 5 weeks with complete improvement. Then, he was referred to our center for surgical repair.

After admission, the patient was totally asymptomatic and in good condition. On examination, he was alert and oriented and had no respiratory distress. Blood pressure at both arms were 120/80 mm Hg and heart rate was 80 per/minute and regular. Oxygen saturation was 95% in room air. Jugular vein pulse was normal. On cardiac examination, a continuous murmur with 3/6 intensity was heard on the left sternal border, and there was a clear trill. Lungs were clear on auscultation, and there were no abnormal findings in the examination of the abdomen and the extremities. Distal pulses were symmetric. Routine lab data, including renal function tests, liver function tests, lipid profiles, and blood biochemistry, were normal. The ECG showed normal sinus rhythm, normal axis, and diastolic overload of the left ventricle.

Transthoracic and transesophageal echocardiographic examinations were performed and revealed left ventricular ejection fraction of 50%, healed vegetations (on the aortic and pulmonary valves, close to the VSD and on the location of the ruptured sinus of Valsalva), right coronary sinus of Valsalva rupture with a continuous flow to the right ventricular outflow tract, and sub-aortic VSD (Figure 1-3).

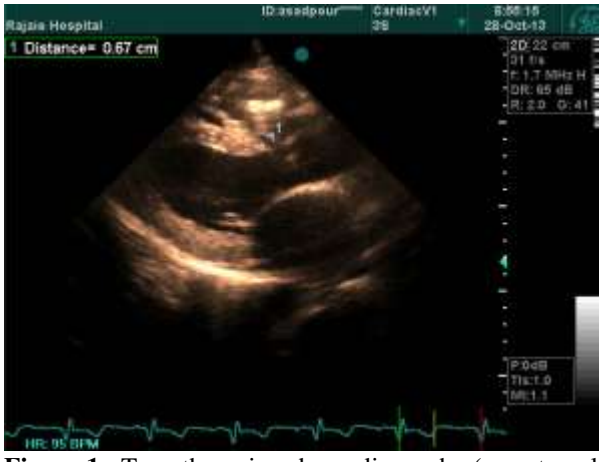

Figure 1. Transthoracic echocardiography (parasternal long-axis view), demonstrating ventricular septal defect

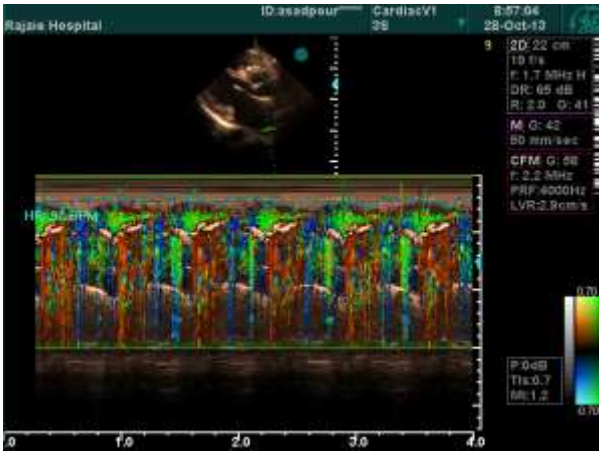

Figure 2. Color M-mode in transthoracic echocardiography, showing a continuous flow in systole and diastole, which is due to concomitant ventricular septal defect and ruptured sinus of Valsalva

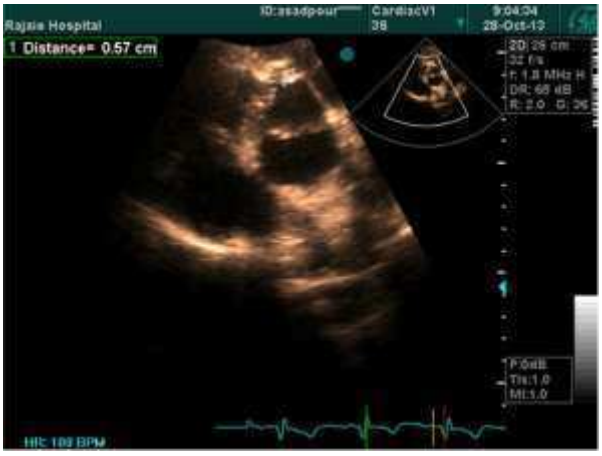

Figure 3. Transthoracic echocardiography (parasternal short-axis view), showing the ruptured sinus of Valsalva

The patient underwent cardiac catheterization, which demonstrated sub-valvular pulmonary stenosis, a high VSD and rupture of the right Valsalva sinus to the right ventricular outflow tract, and a left-to-right shunt with QP / QS of 2.8.

Finally, elective surgery was performed. The rupture of the sinus of Valsalva was repaired, the VSD was closed, pulmonary stenosis was relieved, and the sinus of Valsalva rupture was repaired. The patient was discharged home with good general condition.

Discussion

Aneurysm of the sinus of Valsalva is rarely associated with VSD and endocarditis.

However, our patient had muscular subvalvular pulmonary stenosis, a large VSD, and ruptured sinus of Valsalva aneurysm complicated by infective endocarditis.

Sinus of Valsalva rupture usually occurs in the aneurysmal dilatation. The Valsalva sinus aneurysm is caused by a local weakness in the wall of the sinus Valsalva.(1) It seems to be due to a lack of integration and incomplete connection between the media layer of the sinus of Valsalva and the media of the aortic annulus.(2)

Congenital aneurysm of the sinus of Valsalva can be associated with other congenital heart defects such as VSD, atrial septal defect, patent ductus arteriosus, Tetralogy of Fallot, and coarctation of the aorta.(3) This lesion is significantly more common in men (4:1), and the incidence is higher in the Asian population (2). Two thirds of the aneurysms originate from the right coronary sinus, one quarter from the non-coronary sinus, and the remaining originate from the left coronary sinus (1). If the aneurysm originates from the right coronary or non-coronary sinuses, it is more likely to be accompanied by VSD. It is probably because of the same congenital origins of these lesions.(2)

Aneurysm of the sinus of Valsalva can be acquired and is caused by factors such as infective endocarditis, syphilis, trauma, or iatrogenic reasons (caused by cardiac catheterization), which is a less common cause of acquired rather than congenital causes.(4)

Aneurysms often grow over time and remain symptom free until they do rupture. However, even in the absence of rupture, there can be complications such as obstruction of the right ventricular outflow tract, destruction of the aortic valve which results in aortic insufficiency, myocardial ischemia due to the compression of the left coronary artery, and compression of the cardiac conductive system which can result in conduction disturbances, or even complete heart block.(1) Thrombus may be formed within the aneurysm and may lead to thromboembolic events.(5) These aneurysms can be opened in any of the chambers of the heart and followed by any type of fistula between the sinus and the cavities of the heart. The most common type of tear is the rupture of the right coronary sinus to the right ventricle, especially if there is simultaneous VSD.(1)

Side effects of aneurysms are related to their size and function. These aneurysms grow slowly; therefore, symptoms are rare in infancy or early childhood. The average age of the onset of symptoms resulting from a ruptured aneurysm is 31 years old. If the aneurysm ruptures, the size of the fistula and consequently the value of the shunt, and the chamber that fistula opens to, will determine the clinical presentations. Based on this, the rupture of the aneurism to the left heart does not cause a left-to-right shunt, whereas rupture to the right heart may cause varying degrees of left-to-right shunting.(1) Since infective endocarditis is a complication of small fistula, it is difficult to determine whether aneurysm is congenital or is caused by endocarditis. (1)

Before rupture, aneurysm of the sinus of Valsalva is diagnosed accidentally while imaging for other lesions.(1) If the aneurysm ruptures and creates a large shunt, CHF symptoms appear quickly. But if there is a smaller fistula, it may take many months to develop heart failure. Approximately 20% of the patients are asymptomatic (similar to our case). A small fistula may create a continuous heart murmur with maximum intensity in the third and fourth intercostal space at the left sternal border. A large chronic fistula will cause hypertrophy of the receiver chamber. Sometimes, symptoms of myocardial ischemia or conduction disturbances due to the compression of the coronary arteries and conduction system are evident in the ECG.(1)

Echocardiography has an important role in the diagnosis of a ruptured aneurysm of the sinus of Valsalva. Transthoracic and transesophageal echocardiographic examinations show the site of rupture and also can show associated lesions whose existence will affect the surgical procedure. (5)

When these aneurysms rupture, the mean survival, if untreated, is reduced to one to two years and patients progressively develop heart failure.(4) Thus, early diagnosis and treatment of these aneurysms is very important and provides better long-term results. Prognosis is excellent after surgery and recurrence is rare. (6)

Conclusions

Based on this study, it seems that, even asymptomatic sinus of Valsalva aneurysm associated with VSD, ultimately will be complicated during life time. As a result, it is more advisable to revise the surgical indications of VSD and they should not be limited to symptomatic cases in large shunts (QP / QS> 1.5 / 1), pulmonary artery pressure > 50 mm Hg, increased size of the left ventricle or left atrium, reduced left ventricular ejection fraction, or recurrent endocarditis. Elective surgery should be performed in all cases of VSD in early childhood.

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- Arrange manuscripts as follows: 1) title page, 2) abstract, 3) text, including introduction, material or patients and methods, results, discussion, conclusion, 4) references, 5) tables, and 6) legends. Number pages consecutively, beginning with the title page as number 1 and ending with the legend page. Page numbers should be at the bottom center of each page.

- Average length for original articles is five printed pages, equivalent to 20 double-spaced manuscript pages: 1 title page, 1 abstract page, 10 pages of text, 4 tables or illustrations, 1 page of figure legends, and not more than 20 references. Text for case reports should be no more than 4 double-spaced typewritten pages, and correspondences no more than 2 double-spaced manuscript pages.

- The title page should include the title, authors and their academic degrees, name and location of the institutional affiliation or department (no more than two), and address, telephone number, fax number and email address for reprint requests at the bottom of the page. If more than one institution is named, indicate which authors are affiliated with each.

- **Titles** should be as short as possible (fewer than 95 letters and spaces). Also submit a short title of 40 characters to be used as a running title.

- **Abstracts** should be no longer than 250 words and should contain four sections in the following order: Background, Methods, Results, and Conclusions. Abstracts for case reports and correspondences should not be structured and must be shorter (50 to 75 words). Include key words at the end of the abstract.

- **Text** should be organized as follows: Introduction, Methods, Results Discussion and Conclusion. Methods should include the statistical analysis. Cite references, illustrations and tables in numeric order in the text. Give all measurements and weights in standard metric units. Credit suppliers of drugs, equipment, and other brand-name material mentioned in the article in parentheses, giving company name and location.

- **Acknowledgement** All contributors who do not meet the criteria for authorship may be mentioned in the acknowledgement section. It should include persons who provided technical help, writing assistance and departmental supervision who only provided general support. Financial and material support should also be acknowledged.

- **Conflicts of interest** Authors must acknowledge and declare any sources of funding and potential conflicting interest, such as receiving funds or fees by, or holding stocks and shares in, an organization that may profit or lose through publication of your paper. Declaring a competing interest will not lead to automatic rejection of the paper, but must be declared.

- **Ethical guidelines** Ehtical considerations must be addressed in the Materials and Methods section. 1) Please state that informed consent was obtained from all human adult participants and from the parents or legal guardians of minors. Include the name of the appropriate institutional review board that approved the project. 2) Indicate in the text that the maintenance and care of experimental animals complies with National Institutes of Health guidelines for the humane use of laboratory animals, or those of your institute or agency.

- **References** should be identified by using superscript numbers without changing font size (for example,[1,2,3]). Do not cite personal communi-cations, manuscripts in preparation or unpublished data. Type references double-spaced on a separate sheet and number consecutively in the order in which they are mentioned in the text. List all authors if 6 or fewer; otherwise list first six and add "et al." Style and punctuation of references should conform to Vancouver style or *Index Medicus* format as in the examples below:

Journal article:

1. Burt VL, Cutler JA, Higgins M, Horan MJ, Labarthe D, Whelton P, et al. Trends in the prevalence, awareness, treatment and control of hypertension in the adult US population. Hypertension 1995; 26: 60-69.

Chapter in Book:

2. Ross DN, Martelli V, Wain WH: Allograft and autograft valves used for aortic valve replacement. In: Ionescu MI, (ed.). Tissue Heart Valves. London: Butterworth, 1979: pp. 319-29.

- **Tables** should be typed double-spaced on separate sheets, each with a table number and title above the table and explanatory notes and legends below. Tables should be self-explanatory and the data should not be duplicated in the text or figures. If tables provide repetitive information, they will be deleted.

- **Legends** to illustrations should be typed double-spaced on a separate sheet. Numbers should correspond to the order in which they appear in the text. Give the type of stain and magnification power for photomicrographs. Patients should not be recognizable in illustrations unless written consent is supplied.

- **Illustrations** should be submitted digitally (preferable), or in three sets of glossy prints. High-quality laser artwork is also acceptable, but photocopies are not. Write the first author's last name, figure number

and an arrow indicating the top of the figure on the back of each illustration in pencil. All illustrations will be published in black and white unless color prints are specifically requested by the author(s). The author must be prepared to pay a fee for publication of color photographs.

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All authors are ***strongly encouraged*** to submit their manuscripts on floppy disc as a document file using Microsoft Office Word (97 or higher) in order to allow more rapid editing and preparation for publication. The author should retain copies of all files as backup. Diskettes should each be labeled with the author's name, short title of the article, and operating system used. Diskettes should be packed in cardboard. All material on diskettes must be in full conformity with the standard information for authors on the previous pages to be considered for publication.

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Editor in Chief
Iranian Heart Journal
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Tehran 19974 Iran
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Fax: (009821) 22048174
E-mail: iha@rhc.ac.ir

Forthcoming Meetings 2014

SATURDAY, FEBRUARY 1, 2014

New Cardiovascular Horizons-Mobile

Grand Hotel Marriott Resort
One Grande Boulevard
Point Clear, AL 36564
United States
For information, contact:
New Cardiovascular Horizons Foundation
registration@ncvh.org
Phone: 337-993-7920
www.ncvh.org/mobile

Monday, February 3, 2014 to Friday, February 7, 2014

Fundamentals in Cardiac Surgery Part I

EACTS House
Madeira Walk
Windsor
United Kingdom
For information, contact:
European Association for Cardio-Thoracic Surgery
Phone: +44 (0)1753 832 166
http://www.eacts.org/academy/2014-programme/

WEDNESDAY, FEBRUARY 5, 2014 TO SATURDAY, FEBRUARY 8, 2014

34th Annual Cardiothoracic Surgery Symposium (CREF 2014)

Hilton San Diego Bayfront Hotel
San Diego, CA
United States
For information, contact:
info@crefmeeting.com
Phone: 1 805 541-3118
http://www.crefmeeting.com

SUNDAY, FEBRUARY 9, 2014 TO THURSDAY, FEBRUARY 13, 2014

nternational Congress on Endovascular Interventions 2014

The Arizona Biltmore
2400 E. Missouri Ave.
Phoenix
United States
For information, contact:
International Society of Endovascular Specialists
Phone: (337) 993-7920
http://isesonline.org/icon-2014.html

SUNDAY, FEBRUARY 9, 2014

Vein Experts International Phebology Symposium

Arizona Biltmore
2400 E. Missouri Ave.
Phoenix, AZ 85016
United States
For information, contact:
American Society of Cardiovascular Phlebologists
info@ascvp.org
Phone: 337-993-7920

SUNDAY, FEBRUARY 9, 2014 TO WEDNESDAY, FEBRUARY 12, 2014

43rd Annual Meeting of the German Society for Thoracic and Cardiovascular Surgery

Messe Freiburg

Germany
For information, contact:
Prof. Dr. Dr. Friedhelm Beyersdorf
friedhelm.beyersdorf@universitaets-herzzentrum.de
http://www.dgthg-jahrestagung.de

THURSDAY, FEBRUARY 13, 2014 TO FRIDAY, FEBRUARY 14, 2014

1st European Transoesophageal Echocardiography Course on Congenital Heart DiseaseSheraton
UCL Institute of Child Health
London
United Kingdom
For information, contact:
info@ichevents.com
Phone: +44 (0)20 7905 2675

Friday, February 14, 2014 to Saturday, February 15, 2014

Functional Mitral and Tricuspid Regurgitation
EACTS House
Madeira Walk
Windsor
United Kingdom
For information, contact:
EACTS
Phone: +44 (0)1753 832 166

SATURDAY, FEBRUARY 15, 2014 TO TUESDAY, FEBRUARY 18, 2014

14th Annual International Symposium on Congenital Heart Disease
Renaissance Vinoy Resort
501 5th Avenue NE
St. Petersburg, FL
United States
For information, contact:
Suzanne Anderson
suzanne.anderson@allkids.org

3 - 5 OCTOBER 2013
RIDGEDALE, MO UNITED STATES
[ICALENDAR]

The Steven R. Hall, MD, Trauma Symposium, Trauma Continuum of Care: Challenges in Trauma
Big Cedar Lodge
CME available
For information, contact:
Mary Eberwein, UMKC
Continuing Medical Education Office
612 Devil?s Pool Road
Ridgedale, Missouri 65739
Phone: 1 816 235-6808
Fax: 1816 235-6812
Email: cmeoffice@umkc.edu
Additional information:
https://cmetracker.net/UMKC/Catalog

THURSDAY, FEBRUARY 20, 2014 TO SUNDAY, FEBRUARY 23, 2014

The 3rd International Congress on Cardiac Problems in Pregnancy (CPP 2014)
Hilton Molino Stucky
Venice
Italy
For information, contact:
secretariat@cppcongress.com
http://www.cppcongress.com

FRIDAY, FEBRUARY 21, 2014 TO SUNDAY, FEBRUARY 23, 2014

17th Conference of the Indian Association of Cardiovascular Thoracic Anaesthesiologists
Grand Hyatt

Mumbai
India
For information, contact:
Doctor Uday Gandhe
secretariat@iacta2014.com
http://www.iacta2014.com

WEDNESDAY, FEBRUARY 26, 2014 TO FRIDAY, FEBRUARY 28, 2014

INTERNATIONAL SYMPOSIUM ON UNIPORTAL VATS-WETLAB AND LIVE SURGERY

Coruña University Hospital
Coruña
Spain
Phone: +44 1753 832166
Email: louise.mcleod@eacts.co.uk or info@eacts.co.uk

WEDNESDAY, FEBRUARY 26, 2014 TO FRIDAY, FEBRUARY 28, 2014

2nd EACTS Certified Course in Cardio-Thoracic Robotic Surgery Part 2 of 3

EACTS House
Madeira Walk
Paris
France
For information, contact:
EACTS
Phone: +44 (0)1753 832 166

THURSDAY, MARCH 6, 2014 TO FRIDAY, MARCH 7, 2014

Aortic Valve Repair: A Step by Step Approach

L'Institut Mutualiste Montsouris
Paris
France
For information, contact:
Sabine Ruck
s.ruck@kelcon.de
Phone: 0049 6189 94666 27
Fax: 0049 6182 94666 44
www.caviaar.com

THURSDAY, MARCH 6, 2014 TO SATURDAY, MARCH 8, 2014

The Houston Aortic Symposium: Frontiers in Cardiovascular Diseases, the Seventh in the Series

The Westin Oaks Hotel
Houston, TX
United States
For information, contact:
Promedica International CME
rlaw@promedicacme.com
Phone: 1 760 720-2263
Fax: 1 760 720-6263
http://promedicacme.com/

FRIDAY, MARCH 7, 2014 TO SUNDAY, MARCH 9, 2014

Florida Valve International-Transforming Valvular Therapy

Four Seasons Hotel Miami
Miami, FL
United States
For information, contact:
Carole Erickson
floridavalve@upmc.edu
Phone: 305-358-3535
www.floridavalve.com

SATURDAY, MARCH 8, 2014

New Cardiovascular Horizons-Baton Rouge

Baton Rouge Marriott
5500 Hilton Avenue
Baton Rouge, LA
United States
For information, contact:
New Cardiovascular Horizons Foundation
registration@ncvh.org
Phone: 337-993-7920

SUNDAY, MARCH 9, 2014 TO FRIDAY, MARCH 14, 2014

Interventional Cardiology 2014: 29th Annual International Symposium

The Westin Snowmass
Snowmass, CO
United States
For information, contact:
Promedica International CME
rlaw@promedicacme.com
http://promedicacme.com

THURSDAY, MARCH 13, 2014 TO SUNDAY, MARCH 16, 2014

10th International Congress of Update in Cardiology and Cardiovascular Surgery

Kaya Palazzo Congress Center
Antalya
Turkey
For information, contact:

Bengu Tokatlioglu
info@uccvs2014.org
Phone: 0090 212 292 88 08
Fax: 0090 212 292 88 07
www.uccvs2014.org

FRIDAY, MARCH 14, 2014 TO SATURDAY, MARCH 15, 2014

Symposium Management of Laryngotracheal ProblemsCentara Medical University of Vienna Lecture Hall Center
AKH Wien Floor 7, Lecture Hall 3
Waehringer Guertel 18-20, 1090 Vienna
Austria
For information, contact:
Monika Puehringer
laryngotrachealvienna2014@meduniwien.ac.at
Phone: +43 (0)1 40400 - 5644
Fax: +43 (0)1 40400 - 5100
www.meduniwien.ac.at/laryngotrachealvienna2014

WEDNESDAY, MARCH 19, 2014 TO FRIDAY, MARCH 21, 2014

Advanced Module: Open and Endovascular Aortic Therapy

EACTS House
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