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ARYA Atherosclerosis

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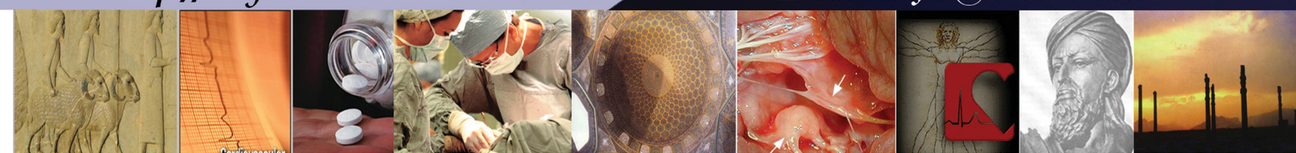

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2. Bonow RO, Mann DL, Zipes DP, Libby P. Braunwald's Heart Disease E-Book: A Textbook of Cardiovascular Medicine. 7th ed. Philadelphia, PA: Elsevier Health Sciences; 2007. p. 1976, 1981, 1982.

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ORIGINAL RESEARCH

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- **Special Articles** include data and generally focus on areas such as economic policy, ethics, law, or health care delivery. The text is limited to 3000 words, with an abstract, a maximum of 5 tables and figures (total), and up to 30 references.
- **Qualitative Researches** focus to clear underlying reasons, opinions, and motivations. It helps to develop ideas or hypotheses for potential quantitative research. The text is limited to 3500 words, with an abstract, a maximum of 5 tables and figures (total), and up to 30 references.
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* All the words of the article containing the references; each table is considered as 300 words.

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The effect of salusin-β on expression of pro- and anti-inflammatory cytokines in human umbilical vein endothelial cells (HUVECs)

Maryam Esfahani[(1)], Masoud Saidijam[(2)], Rezvan Najafi[(3)], Mohammad Taghi Goodarzi[(4)], Ahmad Movahedian[(5)]

Original Article

Abstract

BACKGROUND: Atherosclerosis is one of the predominant causes of cardiovascular disease (CVD). Several studies indicated the significant pathophysiological role of salusin-β in atherosclerosis. Cytokines are involved in all stages of atherosclerosis. Therefore, we aimed to assess the effect of salusin-β on interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 18 (IL-18) (as inflammatory cytokines) and interleukin 1Ra (IL-1Ra) (as anti-inflammatory cytokines) levels in human umbilical vein endothelial cells (HUVECs).

METHODS: The HUVECs were cultured in HUVEC completed medium and treated with different doses of salusin-β for 6 and 12 hours. For the investigation of nuclear factor ƙβ (NF-ƙβ) signaling pathway involvement, cells were treated in the presence or absence of Bay 11-7082 (as NF-ƙβ inhibitor). The mRNA expression and protein level of cytokines were measured by a real-time polymerase chain reaction (PCR) system and enzyme-linked immunosorbent assay (ELISA) method, respectively.

RESULTS: Salusin-β increased mRNA expression and protein level of IL-6, IL-8 and IL-18. This protein decreased mRNA and protein level of IL-1Ra in HUVECs. NF-ƙβ signaling pathway was involved in the up-regulatory effect of salusin-β on mRNA expression of pro-inflammatory cytokines. The down-regulatory effect of salusin-β on IL-1Ra expression could not be influenced by Bay 11-7082 pre-treatment.

CONCLUSION: It seems that salusin-β may participate in a cascade pathway in vascular inflammation. Our findings suggested that salusin-β has potential use as a therapeutic target for atherosclerosis.

Keywords: Atherosclerosis, Cardiovascular Diseases, Cytokines, Endothelial Cells, Inflammation, Salusin-Beta

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Introduction

Atherosclerosis is one of the predominant causes of cardiovascular disease (CVD). More than 17.5 million people die each year because of CVDs.[1] Due to the global growing rate of diabetes and obesity, it is expected that morbidity and mortality of CVD will increase.[2] Therefore, it is of great significance to understand the precise mechanisms which are involved in atherosclerosis. Atherosclerosis is now regarded as a chronic inflammatory disorder of large and medium arteries.[3] Cytokines are particularly significant in inflammatory processes,[4] and many of them are believed to be complicated in atherogenesis.[5] Several cytokines are detected in atherosclerotic plaque, on the other hand, all the cells involved in the disease can produce cytokines.[6] These proteins are involved in initial stages of atherosclerosis, recruitment and activation of leukocytes, foam cell and fatty streak formation, development of complex lesions, plaque stability and rupture.[2,4] It is noted that some cytokines have an anti-atherosclerotic effect.[2] Several signaling pathways are involved in cytokines expression, among which nuclear factor ƙβ (NF-ƙβ)

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transcription factor is an important signaling pathway inducing the expression of some cytokines.[7]

Salusin-β (with 20 amino acids) is a novel peptide which is discovered via bioinformatics analysis of a human full-length cDNA library.[8] This protein is synthesized from pre-pro-salusin which is expressed at a great level in human vascular smooth muscle cells (VSMCs) and endothelial cells.[8] It is indicated that salusin-β is released greatly from cell lines such as THP-1 (a human monocytic cell line) and U937 (a model cell line for the study of behavior and differentiation of monocytes), at the time of stimulation to be differentiated into macrophages.[9] Several data indicated that this protein has a pathophysiological role in atherosclerosis via overexpression of acyl-CoA, cholesterol acyltransferase-1 (ACAT-1) and increase in macrophage foam cell formation (an important step in atherosclerosis),[10] stimulation of the proliferation of VSMCs and fibroblasts and induction of the expression of c-myc, c-fos.[8] Also in vivo and in vitro studies have shown that Salusin-β accelerates inflammatory responses in vascular endothelial cells.[11]

As noted, salusin-β contributes to the pathogenesis of atherosclerosis, but little is known about the effect of this protein on pro- and anti-inflammatory cytokines expression. We aimed to assess the effect of salusin-β on interleukin 6 (IL-6), interleukin 8 (IL-8), interleukin 18 (IL-18) (as inflammatory cytokines) and interleukin 1Ra (IL-1Ra) (as anti-inflammatory cytokines) levels in human umbilical vein endothelial cells (HUVECs). Further, we assessed the probable involvement of NF-ƙβ signaling pathway in salusin-β effect on cytokines.

Materials and Methods

This study was performed at Isfahan University of Medical Sciences, Iran, (grant number 394287) and Hamadan University of Medical Sciences, Iran, (2015-2016). Salusin-β was provided by PeptaNova (Cat No: 4417-s, Japan), and E)-3-(4-methylphenyl sulfonyl)-2-propenenitrile (Bay 11-7082) was supplied from Cayman chemical (CAY10010266, USA). All other substances were acquired with best attainable purity grade.

HUVEC (ATCC® CRL-1730) was purchased from Pasteur Institute, Tehran, Iran. The cells were cultured in cell culture treated flasks and grown in HUVEC completed medium, containing Dulbecco's modified Eagle medium: nutrient mixture F-12 (DMEM/F12), endothelial cell growth factor (ECGF), non-essential amino acid (NEAA), Heparin, Insulin, Nap and 10% fetal bovine serum, in a humidified 5% CO_2 incubator at 37 °C. The cells were pretreated with or without various concentrations of salusin-β (3, 10, 30, 90 nM) for 6 and 12 hours. Also, HUVECs were treated in the presence or absence of Bay 11-7082 (3 and 10 μM) as NF-ƙβ signaling pathway inhibitor.

The cell viability was assessed by the reduction of MTT to its insoluble formazan.[12] HUVECs (5000 cells/well) were seeded in 96-well microplates. After overnight incubation, the cells were treated with different concentrations of salusin-β (1, 3, 10, 30, 90 and 180 nM) for 24 hours. Afterward, the cells were incubated at 37 °C with 15 μl of MTT (5 mg/ml) in phosphate- buffered saline for 4 hours. Then, the medium was removed and 150 μl of dimethyl sulfoxide (DMSO) was added and the absorbance at 570 nm and 630 nm was measured by an enzyme-linked immunosorbent assay (ELISA) reader.

After RNA extraction by TRIzol® Reagent (Thermo Fisher Scientific, Cat No: 15596-026, USA) according to manufacturer's protocol, RNA concentration and purity were determined by microspectrophotometer (A & E Lab, UK). RNA was converted to first- strand cDNA by Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Cat No: K-1622, USA). The mRNA expression of IL-6, IL-8, IL-18 and IL-1Ra were measured by real-time polymerase chain reaction (PCR) system (BioRAD CFX 96) and Syber® Premix Ex TaqTMII (Takara, Cat.RR820L, Japan). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control gene. Real-time PCR data were quantified by $2^{-\Delta\Delta CT}$ method,[13] it should note that the PCR efficiency of the target gene was similar to the internal control gene. All samples and no template controls were examined in duplicates. All amplicons (Table 1) were confirmed by sequencing (sequencing service-Bioneer, South Korea).

The IL-6 level was determined by a human IL-6 ELISA kit, Affymetrix eBioscience (catalog No: 88-7066, USA) sensitivity < 2 pg/ml. The protein level of IL-8 was measured by Human IL-8 Elisa kit Affymetrix eBioscience (catalog No: 88-8086, USA), sensitivity 2 pg/ml. These ELISA kits were specifically engineered for accurate and precise measurement protein levels of IL-6 and IL-8, respectively. The protein level of IL-18 was quantified with Human IL-18 Elisa kit, Boster Bio (catalog No: EK0864, USA), with a sensitivity < 1 pg/ml. The protein level of IL-1Ra was determined by human IL-1Ra Elisa kit, Boster Bio (catalog No: EK0782, USA) with a sensitivity less than 2 pg/ml.

Table 1. Polymerase chain reaction (PCR) primers and the PCR product size

Gene	Accession number	Sequence	PCR product (bp)
GAPDH	NM_002046.5 (Variant 1) NM_001256799.2 (Variant 2) NM_001289745.1 (Variant 3) NM_001289746.1 (Variant 4)	F: AAGGCTGTGGGCAAGGTCATC R: GCGTCAAAGGTGGAGGAGTGG	248
IL-1Ra	NM_173842.2 NM_173843.2 NM_173841.2 NM_000577.4	F: GCAAGCCTTCAGAATCTGGGA T R: ACTTGACACAGGACAGGC ACA T	185
IL-6	NM_000600.3	F: CTGGATTCAATGAGGAGAC R: ATTTGTGGTTGGGTCAGG	206
IL-8	NM_000584	F: AACACAGAAATTATTGTAAAG R: CACTGATTCTTGGATACC	149
IL-18	NM_001562.3 NM_001243211.1	F: AACCTCAGACCTTCCAG R GCATTATCTCTACAGTCAG	292

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; IL-1Ra: Interleukin 1Ra; IL-6: Interleukin 6; IL-8: Interleukin 8; IL-18: Interleukin 18; F: Forward; R: Reverse; bp: Base pair; NM: mRNA accession number; PCR: Polymerase chain reaction

These ELISA kits were specific for natural and recombinant IL-18 and IL-1Ra, respectively. The procedures were done according to manufacturer's instructions. All cytokine level was measured in cell culture supernatant treated with salusin-β after 6 and 12-hour treatments. All samples were analyzed in duplicates.

Data were presented as the mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) test. Bonferroni post-hoc test was done to access statistical differences between groups. The normality test was controlled by Kolmogorov-Smirnov test. The results supported normality of our variables. $P < 0.050$ was considered as statistically significant. The statistical analyses were accomplished by SPSS software (version 24, IBM Corporation, Armonk, NY, USA).

Results

The effect of salusin-β on HUVECs: HUVECs viability was assessed by the MTT assay. The results indicated that salusin-β had no cytotoxic effect in HUVECs in the concentration range of 1-180 nM.

The effect of salusin-β on mRNA expression of IL-6, IL-8, IL-18 and IL-1Ra: To assess whether salusin-β could exert an inflammatory effect on endothelial cells via modulating cytokines expression, mRNA expression of pro- and anti-inflammatory cytokines were determined. The results were reported base on Bonferroni post-hoc test. The mRNA expression of IL-6 was increased by salusin-β at 30 nM (2.86 ± 0.20, $P < 0.001$) and 90 nM (1.82 ± 0.02, $P = 0.007$) for 6-hour treatment, and 90 nM (2.17 ± 0.20, $P = 0.013$) for 12-hour treatment. Also, we observed that mRNA expression of IL-8 was increased at 10 nM (1.92 ± 0.08, $P = 0.021$), 30 nM (2.68 ± 0.19, $P = 0.001$) and 90 nM (5.44 ± 0.24, $P < 0.001$) for 6-hour treatment, and at 90 nM (2.00 ± 0.09, $P = 0.002$) for 12-hour treatment. The mRNA expression of IL-18 was increased at 30 nM (2.55 ± 0.01, $P = 0.014$) and 90 nM (3.17 ± 0.40, $P = 0.003$) for 6-hour treatment, and at 90 nM (1.70 ± 0.11, $P = 0.012$) for 12-hour treatment.

Salusin-β reduced mRNA expression of IL-1Ra at 10 nM (0.41 ± 0.08, $P = 0.005$), 30 nM (0.57 ± 0.11, $P = 0.021$) and 90 nM (0.60 ± 0.03, $P = 0.026$) for 6-hour treatment. The mRNA expression of IL-1Ra was decreased at 10 nM (0.22 ± 0.03, $P = 0.006$), 30 nM (0.26 ± 0.01, $P = 0.008$) and 90 nM (0.37 ± 0.04, $P = 0.017$) for 12-hour treatment (Table2).

Table 2. The expression ratio of each target gene normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in treated cells with different doses of salusin-β compared to untreated cells

Target Gene	Salusin-β							
	(3 nM)		(10 nM)		(30 nM)		(90 nM)	
	6 hour	12 hour	6 hour	12 hour	6 hour	12 hour	6 hour	12 hour
IL-6	0.89 ± 0.04	1.20 ± 0.20	1.31 ± 0.02	0.91 ± 0.18	2.86 ± 0.20***	1.26 ± 0.04	1.82 ± 0.02**	2.17 ± 0.20*
IL-8	1.60 ± 0.09	1.26 ± 0.13	1.92 ± 0.08*	0.71 ± 0.11	2.68 ± 0.19**	1.03 ± 0.02	5.44 ± 0.24***	2.00 ± 0.09**
IL-18	1.26 ± 0.20	1.50 ± 0.12	1.97 ± 0.11	0.96 ± 0.13	2.55 ± 0.01*	0.94 ± 0.06	3.17 ± 0.40**	1.70 ± 0.11*
IL-1Ra	0.58 ± 0.05	0.61 ± 0.15	0.41 ± 0.08**	0.22 ± 0.03**	0.57 ± 0.11*	0.26 ± 0.01**	0.60 ± 0.03*	0.37 ± 0.04**

Data are shown as mean ± standard deviation (SD); One-way analysis of variance and Bonferroni post-hoc tests are used; * $P < 0.050$, ** $P < 0.010$, *** $P < 0.001$; IL-6: Interleukin 6; IL-8: Interleukin 8; IL-18: Interleukin 18; IL-1Ra: Interleukin 1Ra

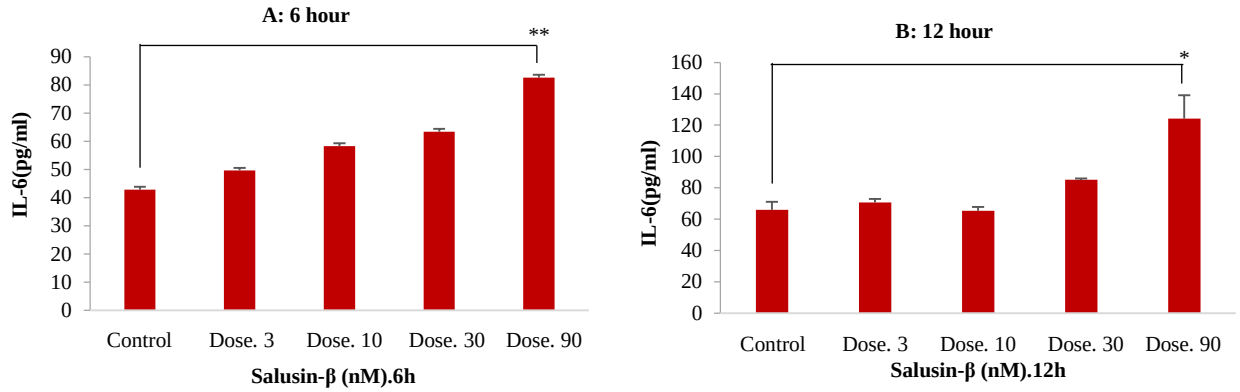

Figure 1. The effect of salusin-β on protein level of interleukin 6 (IL-6) at different time points and concentrations. Salusin-β (90nM) increased IL-6 protein level at 6-hour (A) and 12-hour (B) treatment; * P < 0.050, ** P < 0.010
IL-6: Interleukin 6

The effect of salusin-β on protein level of IL-6, IL-8, IL-18 and IL-1Ra: In line with the changes in cytokines mRNA, we observed that protein level of pre- and anti-inflammatory cytokines were changed. The production of IL-6 was increased at 90 nM for 6-hour and 12-hour treatments (82.62 ± 4.81 pg/ml vs. 42.89 ± 4.11 pg/ml in untreated cells, P = 0.004 and 118.06 ± 15.02 pg/ml vs. 65.92 ± 5.21 pg/ml in untreated cells, P = 0.040, respectively) (Figures 1A and 1B). The results indicated that salusin-β at 90 nM increased protein level of IL-8 (238.82 ± 3.98 pg/ml vs. 178.10 ± 1.80 in untreated cells) for 6-hour treatment (P = 0.036); however, 12-hour treatment had no effect on protein level of IL-8 (dose 3 nM: P = 0.074, dose 10 nM: P = 0.841, dose 30 nM: P = 0.188, and dose 90 nM: P = 0.072) (Figure 2A and 2B). Treatment of HUVECs with different doses of salusin-β increased protein level of IL-18 at 90 nM (84.82 ± 0.76 pg/ml vs. 35.49 ± 1.41 pg/ml untreated cells) (P < 0.001) for 12-hour treatment. It was noted that 6-hour treatment had no effect on protein level of IL-18 (dose 3 nM: P > 0.999, dose 10 nM: P > 0.999, dose 30 nM: P = 0.330, dose 90 nM: P = 0.055) (Figures 3A and 3B). The protein level of IL-1Ra was decreased at 30 and 90 nM (7.33 ± 1.20 pg/ml and 5.82 ± 0.76 pg/ml, respectively vs. 16.52 ± 0.70 pg/ml in untreated cells) for 12-hour treatment (P = 0.020 and P = 0.010, respectively). Salusin- β at 6-hour treatment had no effect on IL-1Ra protein level, (dose 3 nM: P > 0.999, dose 10 nM: P > 0.999, dose 30 nM: P > 0.999, dose 90 nM: P = 0.594) (Figure 4A and 4B).

The role of NF-kβ signaling pathway in Salusin-β treatment: To better understand the mechanisms of salusin-β-triggered cytokine expression, we measured mRNA expression of cytokines in the pretreatment of Bay 11-7082 (3, 10 μM) and salusin-β treatment. The results indicated that Bay 11-7082 (10 μM) can suppress the up-regulatory effect of salusin-β on mRNA expression of IL-6, 0.67 ± 0.11 (P = 0.002), Figure 5A. Also, Bay 11-7082 (10 μM) inhibited the up-regulatory effect of salusin-β on mRNA expression of IL-18 (0.95 ± 0.09) (P = 0.023) (Figure 5B).

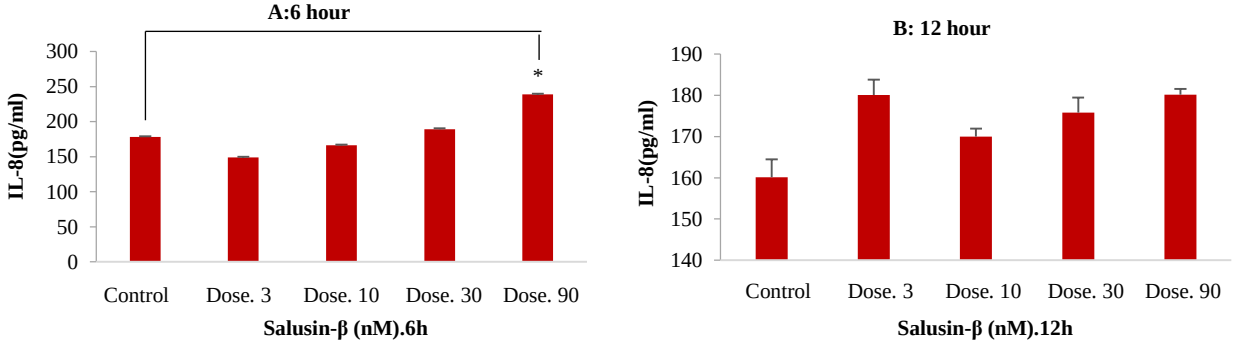

Figure 2. The effect of salusin-β on protein level of interleukin 8 (IL-8). Salusin-β (90 nM) increased protein level of IL-8 at 6-hour treatment (A), and had no effect at 12-hour (B) treatment; * P < 0.050
IL-8: Interleukin 8

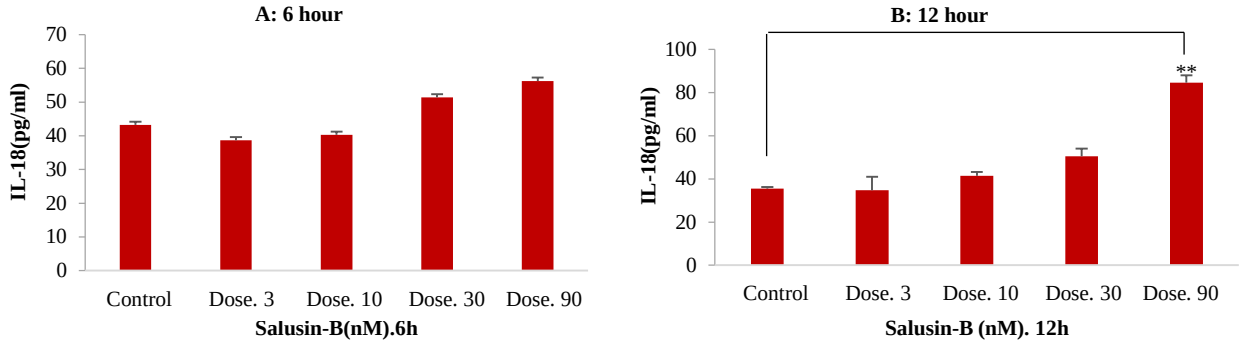

Figure 3. The effect of salusin-β on protein level of interleukin 18 (IL-18) at different time points and concentrations. Salusin-β (90nM) increased IL-18 protein level at 12-hour treatment (B), with no effect at 6-hour treatment (A); ** P < 0.001
IL-18: Interleukin 18

Also, we observed that Bay 11-7082 (3 and 10 μM) inhibits IL-8 mRNA expression induced by salusin-β (0.37 ± 0.09 and 0.45 ± 0.06, respectively) (P < 0.001) (Figure 5C). This inhibitor had no effect on the down-regulatory effect of salusin-β on IL-1Ra mRNA expression, data not indicated.

Discussion

Endothelial cells have a fundamental function in the inflammatory response. These cells can synthesize various pro-inflammatory cytokines in response to different stimuli.[14] The present study, for the first time, demonstrated that salusin-β can increase mRNA and protein level of pro-inflammatory cytokines including IL-6, IL-8 and IL-18 and decrease mRNA and protein level of IL-1Ra in HUVECs. Several studies confirmed that salusin-β has a pro-atherogenic effect,[15,16] considering the prominent role of cytokines in key pathogenic events in atherosclerosis, it is worth to recognize the relationship between salusin-β and cytokines.

IL-8 is an atherogenic chemokine. The high level of this protein has been reported in the arterial atherosclerotic wall, atherosclerotic plaques and macrophages.[17] IL-8 is involved in firm adhesion of monocytes to vascular endothelium under flow condition, a crucial step in atherosclerosis initiation.[18] Also, IL-8 can promote monocytes and neutrophils activation.[19] Because of specific biochemical properties of IL-8, this cytokine is a perfect choice for sites of inflammation.[20] IL-8 is a mitogenic and chemotactic factor for VSMCs, which up-regulates mRNA expression and production of matrix metallopeptidase 2 (MMP2) and matrix metallopeptidase 9 (MMP9) in endothelial cells.[21] It also inhibited tissue inhibitor of metalloproteinase 1 (TIMP-1) expression which leads to an imbalance between matrix metalloproteinase and TIMP-1; consequently, resulting in atherosclerotic plaque rupture and thrombosis.[22] Therefore, IL-8 can accelerate the initiation, progression and plaque destabilization.

Very recent studies revealed that salusin-β can elevate migration and intimal hyperplasia of VSMCs via reactive oxygen species (ROS)/NF-ƙβ/MMP-9 pathway.[23]

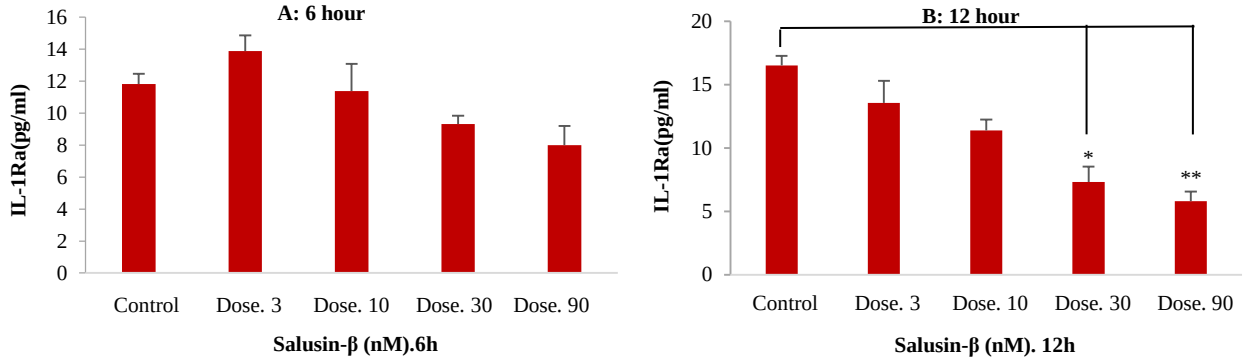

Figure 4. The effect of salusin-β on protein level of interleukin 1Ra (IL-1Ra) at different time points and concentrations. Salusin-β (90 and 180 nM) reduced protein level of IL-1Ra at 12-hour treatment (B), without any effect at 6-hour treatment (A); * P < 0.050, **P < 0.010
IL-1Ra: Interleukin 1 Ra

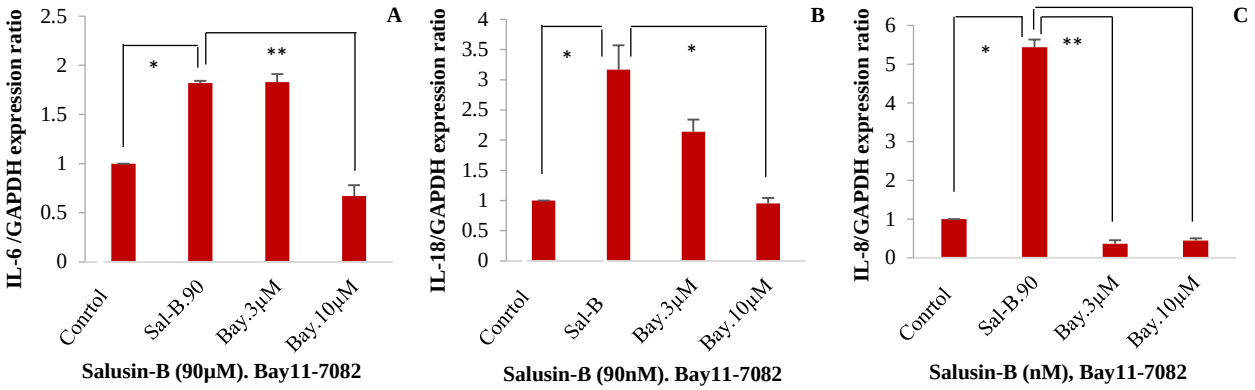

Figure 5. The involvement of nuclear factor ƙβ (NF-ƙβ) signaling pathway in up-regulating effect of salusin-β on interleukin 6 (IL-6) (A), interleukin 18 (IL-18) (B), and interleukin 8 (IL-8) (C) mRNA expression; * $P < 0.050$, ** $P < 0.010$
IL-6: Interleukin 6; IL-18: Interleukin 18; IL-8: Interleukin 8; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Our results indicated that salusin-β increases mRNA and protein level of IL-8 in HUVECs. As noted above, salusin-β accelerates oxidative stress leading to activation of NF-ƙβ signaling pathway in endothelial cells.[11] Furthermore, salusin-β induces mRNA expression of IL-1β.[11] It is well known that these factors are involved in IL-8 expression.[24,25]

IL-18 is an important player in atherosclerotic processes. This cytokine is highly expressed in macrophages, endothelial cells and smooth muscle cells (SMCs) of atherosclerotic lesions. Recombinant IL-18 accelerates atherogenesis and increases cytokines level such as IL-1β, IL-8, IL-6,[26-28] also intensify adhesion molecules expression such as vascular cell adhesion protein 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) in endothelial cells and fibroblasts.[29] Thus, IL-18 results in endothelial dysfunction.[28] IL-18 has a close association with interferon gamma (IFN-γ) induction and it is proposed that pro-atherogenic effect of IL-18 is mediated by this cytokine. IFN-γ has a key role in atherosclerosis especially in foam cell formation via up-regulation of scavenger receptor for phosphatidylserine and oxidized lipoprotein (SR-PSOX).[30] IFN-γ inhibits collagen synthesis by SMCs, thereby can modulate collagen content of atherosclerotic plaques.[31] IL-18 deficiency is associated with decreased recruitment of macrophages.[2] Apoptosis of endothelial cells and acceleration of migration and proliferation of SMCs is mediated by IL-18.[32,33] In vivo studies indicated that endogenous inhibitor of IL-18 impeded fatty streak development in the thoracic aorta and has slowing effect on progression of advanced atherosclerotic plaque.[34]

IL-18 is a part of IL-1 superfamily; therefore, this cytokine has structural and functional similarity with IL-1β. It also has the identical signaling cascade in common with IL-1β.[35] Salusin-β increased mRNA expression of IL-1β and this study showed that salusin-β increased mRNA and protein level of IL-18 in HUVECs, suggesting a crosstalk between salusin-β, IL-1β and IL-18.

IL-6 is expressed in human atherosclerotic plaque and increases inflammatory cascade.[36,37] IL-6 is involved in the expression of acute phase proteins in SMCs, also in migration and differentiation of activated macrophages.[38] IL-6 accelerates the proliferation of VSMCs and enhances the permeability of endothelial cells.[14] These events are included in onset and development of atherosclerosis. Animal studies have shown that recombinant IL-6 caused atherosclerotic lesion development.[39] IL-6 is a pro-coagulant cytokine,[36] which can activate the tissue factor production.[40] Therefore, IL-6 has a role in plaque stability and rupture. It is demonstrated that endothelial cells can express ICAM-1 when are exposed to several inflammatory cytokines such as IL-6.[39] The studies indicated that salusin-β can increase mRNA level of ICAM-1. This study revealed that salusin-β increased mRNA and protein level of IL-6 in HUVECs. In line with our results, in vivo and in vitro studies indicated that salusin-β increased protein level of IL-6; however, these studies did not measure mRNA expression of IL-6.[41]

IL-1 is an influential pro-inflammatory cytokine in vascular hemostasis. This cytokine induces the production of some cytokines and chemokines,[42] and stimulates adhesion molecule expression which accelerates monocyte recruitment and permeation into the arterial wall.[43] Also, IL-1 participates in the development of tissue damage via inducing cell proliferation and matrix metalloproteinases release.[43,44]

IL-1Ra is a negative regulator of IL-1 signaling and has a role in maintaining vascular hemostasis.[44,45] It is proved that treatment of apolipoprotein E (ApoE) / mice with recombinant IL-1Ra is an impressive therapy for atherosclerosis.[45] Several lines of evidence indicated that IL-1Ra has an anti-atherosclerotic effect.[5,46] Animal studies indicated that lack of IL-1β causes less atherosclerotic lesions development;[42] On the other hand, partial deficiency of IL-1Ra changes the composition of atherosclerotic plaques with a higher level of membrane cofactor protein 1 (MCP-1), ICAM-1 and VCAM-1 mRNA and accelerates vascular inflammation.[42] Salusin-β increased mRNA level of MCP-1, ICAM-1, VCAM-1 and IL-1β in HUVECs.[11] Our results indicated that salusin-β decreased mRNA/ protein level of IL-1Ra in HUVECs. Because the balance between IL-1 and IL-1Ra may have a role in atherogenesis development,[42] we suggested the other role for salusin-β via disturbance in IL-1 and IL-1Ra equilibrium.

NF-ƙβ is an important transcription factor in inflammatory processes.[47] This transcriptional factor regulates transcriptions of several genes with a well-known function in atherosclerosis including cytokines, chemokines and adhesion molecules.[48] The promoters of IL-6 and IL-8 genes have functional NF-ƙβ binding sites which have been proven to be essential for the transcriptional activation of these genes.[14] Also, several studies confirmed the involvement of NF-ƙβ signaling pathway in IL-18 expression.[48-50] The studies demonstrated that salusin-β accelerated vascular inflammatory responses via NF-ƙβ signaling pathway.[11] We found that this complex protein involved in the up-regulatory effect of salusin-β on pro-inflammatory cytokines.

This study was performed with some limitations. We only studied mRNA expression of cytokines in the involvement of NF-ƙβ signaling pathway and further studies are necessary to confirm these results. Also, we could not study another cytokine-associated signaling pathway. Regarding no involvement of NF-ƙβ signaling pathway on the down-regulatory effect of salusin-β on IL-1Ra, it seems that the other cytokine-associated signaling pathways must be investigated.

Conclusion

Primary prevention of atherosclerosis, which is important for the management of atherosclerotic CVD, requires comprehensive assessment and modification of molecular cardiovascular risk factors. Cytokines are a central player in atherosclerotic processes. To the best of our knowledge, this is the first study to indicate the association of salusin-β with pro- and anti-inflammatory cytokines. This work indicated that salusin-β increased mRNA/protein level of IL-6, IL-8 and IL-18 as pro-inflammatory cytokines and decreased mRNA/protein level of IL-1Ra as an anti-inflammatory cytokine in HUVECs. The previous studies demonstrated the accelerator effect of salusin-β on vascular inflammation. It seems that salusin-β may participate in a cascade pathway in vascular inflammation (Figure 6). Our novel results can help to open up a new vista into the potential use of salusin-β as a therapeutic target for the prevention of atherosclerosis. Prospective studies to determine the mechanisms latent down-regulatory effect of salusin-β on IL-1Ra and in vivo researches are recommended.

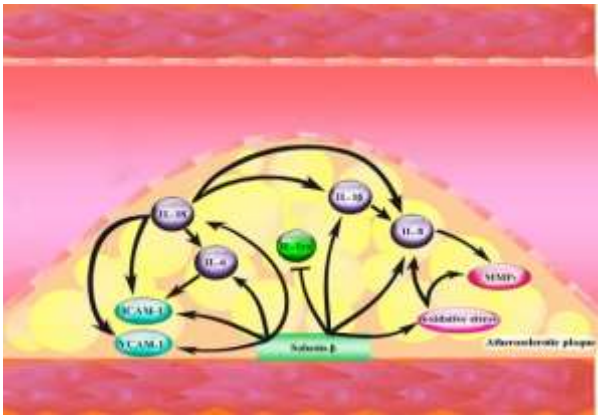

Figure 6. Salusin-β intensifies vascular inflammation via several mechanisms. It increases oxidative stress, adhesion molecules and inflammatory cytokine expression. On the other hand, salusin-β decreases anti-inflammatory cytokine level

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

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The relationship between serum vitamin D levels and ankle-brachial index in patients with metabolic syndrome

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Original Article

Abstract

BACKGROUND: Vitamin D deficiency is a prevalent condition in Iran and previous studies have shown that a low level of serum vitamin D is related to low ankle-brachial index (ABI). In the present study, the relationship of the serum level of vitamin D with ABI, as an index for atherosclerosis of peripheral arteries, was evaluated.

METHODS: In this cross-sectional study, data on 91 patients with metabolic syndrome (Mets) from the Isfahan Cohort Study (ICS) were analyzed in order to evaluate the association between serum 25(OH) vitamin D level and ABI. The participants were divided into two groups; group A with desirable serum vitamin D level and group B with abnormal serum vitamin D level. ABI was measured and compared between these groups.

RESULTS: A crude and adjusted model showed no association between vitamin D level and ABI in patients with MetS.

CONCLUSION: It can be concluded that serum vitamin D level could not affect ABI in patients with MetS.

Keywords: Vitamin D, Ankle Brachial Index, Metabolic Syndrome

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Introduction

Metabolic syndrome (MetS) is characterized by the clustering of cardiovascular risk factors including adiposity, hyperglycemia, hypertension, and dyslipidemia. It has become one of the major public health challenges in developed and developing countries and the management of these risk factors can change with community trial.[1] MetS has been linked to increased arterial stiffness and thickness.[2] Different studies show that the stage of arterial stiffness was significantly more pronounced in patients with MetS.[3] The majority of literature have demonstrated that diverse inflammatory and oxidative stress markers correlate with arterial damage leading to arterial stiffness and thickness.[4-5]

Vitamin D is a secosteroid which is attained by the body through exposure to sunlight and dietary sources. Although 1,25 (OH)2D has been recognized as the active form of vitamin D, the 25(OH)D level is a marker of more clinical importance.[6] Studies have provided evidence of the involvement of vitamin D in bone metabolism. There is also evidence of the role of vitamin D in glucose levels, insulin resistance (IR), and prevalence of type 2 diabetes mellitus (DM).[7] Other studies have found that vitamin D plays a role in systemic inflammation, the immune system, and lipid metabolism to reduce the risk of cardiovascular diseases (CVD).[8] However, few investigations have reported an association between vitamin D and ankle-brachial index (ABI) in MetS. Hence, it is necessary to investigate the relationship between vitamin D and ABI.

Both MetS and abnormal vitamin D serum level are prevalent in our community.[9-13] Therefore, this study was designed to investigate the possible association between vitamin D and ABI as an indicator of peripheral artery atherosclerosis in patients with MetS.

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Materials and Methods

This cross-sectional study was conducted in Isfahan Cardiovascular Research Center, Iran, in 2014. Patients with MetS were enrolled to participate in the study. MetS was diagnosed based on the National Cholesterol Education Program/Adult Treatment Panel, based on the presence of at least three of the factors of central obesity (i.e., waist circumference [WC] > 102 cm for men and > 88 cm for women), high blood pressure (BP) (i.e., systolic BP [SBP] ≥ 130 mmHg or diastolic BP [DBP] ≥ 85 mmHg), hyperglycemia (i.e., fasting glucose ≥ 110 mg/dl), hypertriglyceridemia (i.e., fasting triglycerides [TGs] ≥ 150 mg/dl), and low high-density lipoprotein (HDL)-cholesterol (i.e., HDL-cholesterol < 40 mg/dl for men and < 50 for women).[14-15] The exclusion criteria were chronic renal failure, prior grafting or stenting of lower limb arteries, and abnormal coronary or peripheral angiography.

The study participants consisted of 91 patients from Isfahan Cohort Study (ICS).[16] Patients with MetS diagnosed by an endocrinologist were included in the study based on the inclusion and exclusion criteria. The data on all 91 patients with MetS was complete. The study was approved by the Ethics Committee of Isfahan Cardiovascular Research Center, and written informed consent forms were obtained from all participants.

The diet of the participants consisted of a balanced diet for 3 days and fasting overnight for 12 hours. Body mass index (BMI) was calculated through the division of weight by height squared (kg/m^2). The participants' systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. The collected blood samples were frozen and reserved at -80 °C until analysis. Using an automatic biochemical analyzer, fasting blood sugar (FBS), total cholesterol (TC), TG, low-density lipoprotein (LDL), and HDL were measured. Moreover, some variables such as sex, age, physical activity, smoking, and education were evaluated as confounding variables.

25(OH)D was measured using an Elisa Kit (Calbiotech Inc, USA) and automated analyzer. The various states of serum vitamin D levels were defined as abnormal (< 75 nmol/l) and desirable (≥ 75 nmol/l).

To perform ABI measurements, the subjects were asked to lie in the supine position. The Doppler instrument was used for this purpose. The blood pressure cuff of the Doppler device was wrapped around the patient's upper arm and was inflated until no brachial pulse was detected. Then, the cuff was slowly deflated until the pulse returned to measure brachial systolic blood pressure (BSBP). The cuff was then placed on the distal calf and the Doppler device was placed over the dorsalis pedis or the posterior tibial artery to measure ankle systolic blood pressure (ASBP) and the same measures were repeated on the leg.[17]

Descriptive statistics such as mean ± standard deviation (SD) and absolute number (percentage) for categorical variables are reported in the present text for continuous and categorical variables. Chi-square and independent t tests were used to evaluate differences between the two groups for categorical and continues variables, respectively. Moreover, logistic regression analysis was used to evaluate the relationship between ABI and vitamin D. All statistical analyses were performed in SPSS software (version 22, IBM Corporation, Armonk, NY, USA). All P-values of less than 0.05 were considered significant.

Results

91 patients completed the study. The patients were divided into two groups. Group A with desirable vitamin D level and group B with abnormal vitamin D level. These groups were compared in terms of demographic and clinical variables. The mean age in group A was greater than group B [59.8 ± 10.1 v 54.9 ± 7.4 years; P = 0.010]. Furthermore, the number of patients with high LDL level in group A were more than group B [7 (21.9%) vs. 3 (5.1%); P = 0.014]. However, there was no significant difference between the two groups in terms of the other variables such as ABI (low ABI was 23.7% in group B and 28.1% in group A) (P = 0.645) (Table 1).

Each group (A and B) was evaluated in terms of ABI and they were divided into normal ABI and low ABI. Other variables were evaluated in the two groups. It was found that in group A, mean age was greater in individuals with low ABI [66.5 ± 9.4 v 57.1 ± 9.3 (years); P = 0.016] and also BMI was greater in this group than individuals with normal ABI [35.9 ± 10.5 v 30.5 ± 3.5 kg/m^2; P = 0.036]. Nevertheless, high TG (≥ 150 mg/dl) was more prevalent in individuals with normal ABI than individuals with low ABI, but no significant difference was observed between normal ABI and low ABI in terms of the other variables. In group B, abnormal BP was more prevalent in individuals with normal ABI [31 (68.9%) v 14 (100%); P = 0.017], but there was no significant difference in terms of other variables between individuals with normal ABI and low ABI (Table 2).

Table 1. The frequency of studied variables based on vitamin D levels

Variable		Group A Vitamin D ≥ 75 (nmol/dl) (n = 32)	Group B Vitamin D < 75 (nmol/dl) (n = 59)	P
		Mean ± SD	Mean ± SD	
Age (year)		59.8 ± 10.1	54.9 ± 7.4	0.010*
BMI (kg/m^2)		32.0 ± 6.6	30.9 ± 4.3	0.332
Physical activity(MetS/week)		803.1 ± 669.5	758.8 ± 418.9	0.699
		n (%)	n (%)	
Sex (Man)		8 (25.0)	18 (30.5)	0.579
Education	Illiterate	4 (12.5)	9 (15.3)	0.088
	Primary school	20 (62.5)	23 (39.0)	
	Higher than primary school	8 (25.0)	27 (45.8)	
Low ABI (< 0.9)		9 (28.1)	14 (23.7)	0.645
Smoking		1 (3.1)	5 (8.5)	0.419
High BS [FBS ≥ 110 (mg/dl)]		7 (21.9)	19 (32.2)	0.298
Triglyceride ≥ 150 (mg/dl)		30 (93.8)	55 (93.2)	0.923
High density lipoprotein (≤ 40 mg/dl for men or ≤ 50 for women)		24 (75.0)	45 (76.3)	0.892
LDL ≥ 100 (mg/dl)		7 (21.9)	3 (5.1)	0.014*
Total cholesterol ≥ 200 (mg/dl)		12 (37.5)	22 (37.3)	0.984
Systolic blood pressure ≥ 130 (mmHg) or Diastolic blood pressure ≥ 85 (mmHg)		25 (78.1)	45 (76.3)	0.841
Waist circumference [men ≥ 102 (cm) or women ≥ 88 (cm)]		25 (78.1)	42 (71.2)	0.473

* P < 0.050

ABI: Ankle-brachial index; BMI: Body mass index; FBS: Fasting blood sugar; LDL: Low-density lipoprotein; MetS: Metabolic syndrome

The results of logistic regression showed no significant association between ABI and vitamin D levels even after adjustment for age, sex, physical activity, and smoking (P = 0.875) (Table 3).

Table 2. The frequency of studied variables based on low ankle-brachial index and normal ankle-brachial index and vitamin D levels

Variables		Group B Vitamin D < 75 (nmol/dl)			Group A Vitamin D ≥ 75 (nmol/dl)		
		Low ABI (n = 14)	Normal ABI (n = 45)	P	Low ABI (n = 9)	Normal ABI (n = 23)	P
		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Age (year)		57.7 ± 7.3	54.0 ± 7.3	0.101	66.5 ± 9.4	57.1 ± 9.3	0.016*
Physical activity (MetS/week)		811.3 ± 482.0	742.4 ± 401.9	0.595	667.3 ± 416.1	856.2 ± 747.1	0.482
BMI (kg/m^2)		29.5 ± 3.2	31.3 ± 4.5	0.171	35.9 ± 10.5	30.5 ± 3.5	0.036*
		n (%)	n (%)		n (%)	n (%)	
Sex (Man)		7 (50.0)	11 (24.4)	0.070	3 (33.3)	5 (21.7)	0.496
Education	Illiterate	3 (21.4)	6 (13.3)	0.762	2 (22.2)	2 (8.7)	0.378
	Primary school	5 (35.7)	18 (40.0)		6 (66.7)	14 (60.9)	
	Higher than primary school	6 (42.9)	21 (46.7)		1 (11.1)	7 (30.4)	
Smoking		2 (14.3)	3 (6.7)	0.583	0	1 (4.3)	> 0.999
High BS [FBS ≥ 110 (mg/dl)]		6 (42.9)	13 (28.9)	0.329	3 (33.3)	4 (17.4)	0.327
Triglyceride ≥ 150 (mg/dl)		12 (85.7)	43(95.6)	0.201	7(77.8)	23(100)	0.020*
HDL [≤ 40 (mg/dl) for men or ≤ 50 (mg/dl) for women]		9 (64.3)	36 (80.0)	0.227	7 (77.8)	17 (73.9)	0.820
LDL ≥ 100 (mg/dl)		1 (7.1)	2 (4.4)	0.564	2 (22.2)	5 (21.7)	0.976
Total cholesterol ≥ 200 (mg/dl)		6 (42.9)	16 (35.6)	0.622	3 (33.3)	9 (39.1)	0.761
SBP ≥ 130 or DBP ≥ 85 (mmHg)		14 (100)	31 (68.9)	0.017*	9 (100)	16 (69.6)	0.073
Waist circumference [men ≥ 102 (cm) or women ≥ 88 (cm)]		8 (57.1)	34 (75.6)	0.184	9 (100)	16 (69.6)	0.061

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, ABI: Ankle-brachial index; BMI: Body mass index; FBS: Fasting blood sugar; LDL: Low-density lipoprotein; HDL: High-density lipoproteins

* P < 0.050

Table 3. The association of ankle-brachial index level and vitamin D serum levels

Logistic regression model	Odds ratio (95% CI)		P
	Vitamin D > 75 (nmol/dl)	Vitamin D ≤ 75 (nmol/dl)	
Crude	1	0.795 (0.299-2.110)	0.645
Model 1	1	1.180 (0.386-3.604)	0.722
Model 2	1	1.096 (0.350-3.432)	0.875

Model 1: adjusted based on sex and age; Model 2: adjusted based on sex and age; CI: Confidence interval

Discussion

In the present study, it was found that MetS is not significantly related to low ABI. Moreover, in the Edinburgh Artery Study (EAS), no association was reported between MetS and peripheral artery disease (PAD) incidence.[18] The present study findings were not in agreement with that of the Women's Health Study, a cohort clinical trial on women free of baseline cardiovascular disease, which showed that MetS was associated with an increased risk of PAD.[19]

The present study showed high level of LDL was more prevalent in patients with desirable levels of vitamin D. However, many studies, such as that by Saedisomeolia et al., have reported a negative relationship between vitamin D deficiency and LDL level.[20]

Scragg et al. in their epidemiological study, reported that blood pressure has an inverse association with vitamin D levels.[21] Rostand also conducted an epidemiological study in this regard and found a direct association between increasing latitude, as a surrogate of low vitamin D levels, and blood pressure.[22] Pfeifer et al. performed a small clinical trial the results of which suggested that systolic blood pressure was reduced as a result of oral vitamin D supplementation.[23] However, in the present study, a significant difference was not observed between group A and B in terms of abnormal blood pressure, but there was a higher rate of abnormal blood pressure in normal ABI patients of group B. In addition, Scragg et al. did not find any relationship between vitamin D and blood pressure, which was in agreement with the present study findings.[24]

A potential mechanism for increased risk of CVD is the association of 25(OH)D deficiency with glucose intolerance[25] and MetS.[26] However, this relationship was not observed in the current study.

Some studies have reported a relationship between low 25(OH)D levels and increased prevalence of coronary heart disease (CHD), stroke,[27] and congestive heart failure.[28] However, some other studies have found inverse relationships between these factors and higher than normal levels of 25(OH)D. Rajasree et al. conducted a case-control study on 143 patients with CHD and 25(OH)D levels of higher than 89 ng/ml.[29] They obtained a multivariable-adjusted odds ratio of 3.18 (95% CI: 1.31, 7.73) for CHD.[29] The case-control study by Scragg et al. on patients with acute myocardial infarction (AMI) showed the protective effect of higher than the median levels of 25(OH)D (≥ 12.8 ng/ml) against CHD (multivariable adjusted odds ratio = 0.43, 95% CI: 0.27, 0.69).[30]

The Framingham Offspring Study was performed on 1739 participants free of CVD at baseline; an association was observed between 25(OH)D level of lower than 15 ng/ml and a multivariable-adjusted 62% higher hazard of first cardiovascular event.[31]

There is evidence of the possibility of the role of vitamin D in the pathogenesis of CVD. Cardiac myocytes possess vitamin D receptors.[32] Xiang et al., in their in-vitro study, found that cardiac myocyte hypertrophy can be inhibited by active vitamin D.[33] Bodyak et al. evaluated the development of left ventricular hypertrophy in Dahl salt-sensitive rats and found that paricalcitol, an active vitamin D compound, weakened its development.[34] Li .et al reported that vitamin D is an inhibitor of the renin-angiotensin system.[35] In addition, Timms et al.[36] and Schleithoff et al.[37] reported improvement in the cytokine profile [C-reactive protein (CRP) and tumor necrotizing factor-alpha (TNF-α) levels] of patients with vitamin D deficiency[36] and congestive heart failure,[37] respectively, as a result of supplementation with various forms of vitamin D. The anticoagulant activity of active vitamin D and its analogs have been shown in cellular experiments. Furthermore, Kasuga et al. found that aortic atherosclerosis is developed in transgenic rats which expressed the vitamin D-25-hydroxylase gene, a model of vitamin D deficiency attributable to continuous degradation of active vitamin D.[38]

The present clinical study was conducted on a small sample by evaluating a limited number of variables; therefore, this might be a preliminary conclusion. It is suggested that future population-based studies be performed with a larger sample size and by measuring a higher number of related

factors to confirm the role of vitamin D in the development of MetS.

Conclusion

In summary, it can be concluded that low 25(OH) D levels in Iranian adults with MetS had no significant relationship with cardiovascular risk factors and ABI. No association was found between the studied variables even after adjustment for age, sex, physical activity, and smoking. To confirm these conclusions, further prospective and mechanistic studies are necessary.

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Conflict of Interests

Authors have no conflict of interests.

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Prediction of the ischemic origin of functional mitral regurgitation in patients with systolic heart failure through posterior mitral leaflet angle

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Original Article

Abstract

BACKGROUND: Differentiating ischemic from non-ischemic functional mitral regurgitation (FMR) in patients with cardiomyopathy is important in terms of the therapeutic decision-making and prognosis, but might be clinically challenging. In this study, the deformation of mitral valve (MV) indices in the prediction of the etiology of FMR was assessed using 2D transthoracic and tissue Doppler echocardiography.

METHODS: This case-control study was conducted from April 2015 to January 2016 in Imam Reza Hospital in Mashhad, Iran. The participants consisted of 40 patients with ischemic cardiomyopathy (ICM) and 22 with non-ischemic dilated cardiomyopathy (DCM) who referred to the heart failure clinic. Transthoracic echocardiography was performed using the conventional 2D and tissue Doppler imaging (TDI). MV tenting area (TA), coaptation distance (CD), anterior and posterior mitral leaflet angles (AMLA and PMLA), and regional systolic myocardial velocity (Sm) were measured.

RESULTS: There were no significant differences in echocardiographic indices between the two groups, besides Sm and PMLA which were significantly lower and higher, respectively, in ICM subjects in comparison with DCM patients ($P = 0.002$). PMLA ≥ 40 degrees and Sm ≤ 4 cm/second have a relatively high value for discriminating the ischemic from non-ischemic origin of functional MR in subjects with systolic heart failure (sensitivity: 80.0% and 70.0%, specificity: 73.0% and 77.3%; $P = 0.001$ and $P < 0.001$; respectively). Multivariable logistic regression identified PMLA and anterior Sm as major determinants for ischemic MR {Odds ratio (OR) [95% confidence interval (CI)] = 0.89 (0.82-0.96), $P = 0.003$, OR (95% CI) = 0.29 (0.14-0.60), $P = 0.001$, respectively}.

CONCLUSION: The present study showed that PMLA and Sm had an independent significant association with the mechanism of FMR. These findings are suggestive of the predictive role of mitral deformation echocardiographic indices in the determination of the etiology of FMR in systolic heart failure.

Keywords: Cardiomyopathies, Systolic Heart Failure, Mitral Regurgitation, Transthoracic Echocardiography

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Introduction

The mitral valve (MV) apparatus includes the mitral leaflets, chordae tendineae, papillary muscles, and mitral annulus. Abnormalities of any of these structures may cause mitral regurgitation (MR).

For clinical purposes, MR is classified as primary organic MR caused by intrinsic disease of the mitral leaflets, and secondary functional MR caused by diseases of the left ventricle (LV) and/or dilatation of MV annulus. Functional MR is further classified

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as ischemic or non-ischemic MR, depending on the contribution of coronary artery disease (CAD) to left ventricular myocardial dysfunction. These are two distinctly different disease conditions, with different pathophysiologies, outcomes, and management considerations.[1,2]

Patients with non-ischemic dilated cardiomyopathy (DCM) have a greater and more significant improvement in functional status during follow-up than those with ischemic cardiomyopathy (ICM), despite the use of similar cardiovascular medical treatments. The current optimal medical therapy (OMT) produces more favorable ventricular remodeling in DCM than ICM.[3]

LV systolic dysfunction and LV remodeling can result in the occurrence of functional mitral regurgitation (FMR). Moreover, the degree of severity of FMR is believed to be associated with morbidity and mortality outcomes.[2-4]

Several echocardiographic parameters including tenting area (TA), inter-papillary muscle distance (IPMD), coaptation distance (CD), and mitral leaflet angle (MLA) have been studied in patients with FMR.[3-7] Some studies have evaluated the correlation between MLA and other deformation indices for the evaluation of MR severity.[6,8,9]

Different complicated methods have been introduced to calculate the degree of severity of MR; however, the use of two-dimensional echocardiographic indices is relatively simple and has been proven to be a reliable method to accurately determine the degree of severity of FMR. Moreover, there is only limited evidence to suggest the predictive role of mitral deformation echocardiographic indices in determining the etiology of FMR in systolic heart failure.

The aim of this study was to elucidate differences in MV deformation indices between patients with ischemic and non-ischemic moderate FMR using transthoracic echocardiography.

Materials and Methods

This case-control study was conducted from April 2015 to January 2016 at Imam Reza Hospital in Mashhad, Iran. All subjects with clinical features of heart failure who had undergone Doppler echocardiography were assessed for eligibility.

Only patients who had been previously diagnosed with chronic heart failure (CHF), a severely reduced LV ejection fraction (LVEF) (EF ≤ 30%), and a moderate functional MR were included in the study. Patients with acute coronary syndrome (ACS), acute myocarditis, organic MV disease, and significant aortic valve disease, and patients undergoing cardiac resynchronization therapy (CRT) or with implantable cardioverter defibrillator (ICD) devices were excluded. All subjects were in sinus rhythm at the time of the study. The local Ethics Committee approved this study and all patients consented to participate in the study (code number: 93516).

All included subjects underwent selective coronary angiography (SCA) by the same experienced cardiologist at the Cath lab of Imam Reza Hospital, to differentiate the etiology of heart failure, and hence, determine the cause of FMR as being either ICM or DCM. Patients with a history of old myocardial infarction as well as subjects presenting with arterial narrowing of ≥ 50% on the proximal of any of the main three coronary arteries on SCA studies were defined as having CAD and categorized as subjects with ICM. Moreover, patients with DCM were defined as those with systolic heart failure in the presence of normal coronary arteries in SCA and no clinical history of myocardial ischemia. Accordingly, the study population was divided into two groups of ICM and DCM.

In addition to SCA, all included patients also underwent conventional as well as tissue Doppler imaging (TDI) as a second modality to assess different echocardiographic parameters, including systolic myocardial velocity (Sm), TA, CD, and MLA, in order to determine which parameter has the best predictive role by analysis considering SCA findings.

Conventional DI and TDI were performed using a Vivid Seven (GE Healthcare, Milwaukee, USA) with a 4S probe by an expert echocardiologist who was blinded to the angiographic data. Three loops of 2D and TDI images were stored for offline analysis. The severity of MR was determined using the proximal isovelocity surface area (PISA) method.

The modified biplane Simpson method determined LV end-diastolic volume (LVEDV) and LVEF.

Anterior and inferior Sm were measured using TDI at mid-anterior and inferior walls in the apical two-chamber view (Figure 1).

MV deformation indices were assessed in mid-systole using the parasternal long-axis and 4-chamber views (Figure 2). The TA of the MV was measured as the area enclosed by the annular line and MV leaflets. The CD was defined as the distance between the annular line and the leaflet's coaptation point. The posterior and anterior MLA (PMLA and AMLA, respectively) were measured

directly between the mitral leaflets and mitral annulus plane in the 4-chamber view as shown in figure 2.

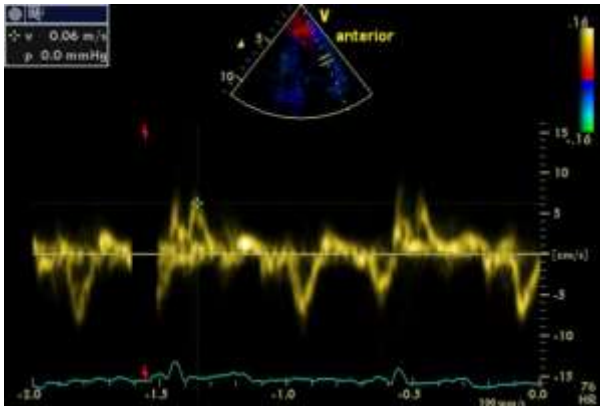

Figure 1. Systolic myocardial velocity measured by PW-based tissue Doppler imaging at the mid-anterior wall in two-chamber view

Standard parasternal and apical views were recorded as three consecutive beats for offline analysis according to the American Society of Echocardiography guidelines.[10]

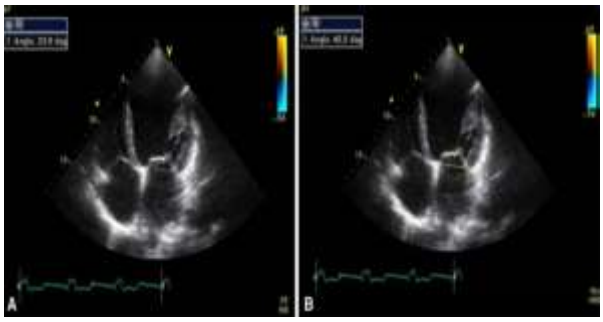

Figure 2. Anterior mitral leaflet angle (a) and posterior mitral leaflet angle (b) at mid-systole in apical four-chamber view

In the present text, continuous data are expressed as mean ± standard deviation (SD) and qualitative parameters are expressed as number (percentage). One-sample Kolmogorov-Smirnov test was used in order to evaluate the normality of parameters. Differences between groups were assessed by independent student's t-test for the normally distributed parameters and Mann-Whitney test for non-normally distributed variables. Categorical variables were compared using the chi-square test. Multivariable stepwise logistic regression analysis was performed with adjustments for age, gender, hyperlipidemia, hypertension, smoking status, anterior and inferior Sm, posterior MLA, and other variables with marginally significant differences ($P < 0.100$) in univariate analyses in order to investigate the independent determinant of functional ischemic MR. A receiver operating characteristic (ROC) curve was constructed to determine the best cut-off values for tissue Doppler echocardiographic parameters. ROC curve shows the relation between true positive (sensitivity) and false positive (1-specificity) for each echocardiographic parameter. The area under the ROC curve was measured. A large area under the ROC curve represents more reliability and good discrimination of the echocardiographic parameter. Youden's index specifies the best cutoff point. Its value ranges from 0 to 1, and has a 0 value when a diagnostic test gives the same proportion of positive results for groups with and without the disease. A value of 1 indicates that there are no false positives or false negatives. The index gives equal weight to false positive and false negative values, so all tests with the same value of the index give the same proportion of total misclassified results.

A two-tailed P-value < 0.05 was considered significant. SPSS statistical software (version 12.0, SPSS Inc., Chicago, IL, USA) and MedCalc Software (version 12.1.4.0, MedCalc Software, Mariakerke, Belgium) were used.

Results

The study population consisted of 62 patients (mean age: 57.21 ± 16.41 years, 37.1% women) with chronic CHF (including 40 suffering from ICM and 22 from DCM). The baseline demographic and clinical characteristics of the two groups are shown in table 1. There were no statistically significant differences between the two groups in terms of NYHA functional class, body surface area (BSA), hypertension (HTN), and bundle branch block (BBB), even though patients with ICM were significantly older and more often had a history of diabetes mellitus (DM), hyperlipidemia (HLP), and smoking compared to patients with DCM ($P < 0.050$).

The severity of LV systolic dysfunction, LVEDV, LVESV, and LVEF were similar in the two groups ($P > 0.050$). In addition, some other echocardiographic parameters such as TA, CD, and AMLA were not significantly different between patients with ICM and DCM ($P = 0.690$, 0.420, and 0.670, respectively). It is noteworthy that patients with ischemic FMR had significantly lower anterior Sm, lower inferior Sm, and a higher degree of PMLA in comparison with patients with DCM ($P < 0.050$). All echocardiographic measurements are shown in table 2.

Table 1. Demographic and clinical characteristics of patients with ischemic cardiomyopathy and non-ischemic cardiomyopathy

Parameter		ICM (n = 40)	NICM (n = 22)	P
Demographic/Clinical	Age (years)	59.28 ± 17.21	47.41 ± 19.24	0.017
	Gender (female %)	10 (25.0)	13 (59.0)	0.008
	NYHA Class- II/III	22 (55.0)	13 (59.0)	0.780
	BSA (m^2)	1.74	1.71	0.190
	Diabetes Mellitus	14 (35.0)	2 (9.1)	0.026
	Hyperlipidemia	6 (15.0)	0 (0.0)	0.001
	HTN	18 (45.0)	5 (22.7)	0.080
	Smoking	23 (57.5)	3 (13.6)	0.001
ECG	Sinus Rhythm	38 (95.0)	20 (90.9)	0.600
	LBBB	15 (37.5)	6 (27.2)	0.780
	RBBB	4 (10.0)	3 (13.6)	0.690

Data are presented as mean ± standard deviation for quantitative variables and n (%) for qualitative variables.
Diabetes: Fasting blood sugar ≥ 126 mg/dl or use of diabetes medications; Hyperlipidemia: LDL > 160 mg/dl or total cholesterol > 240 mg/dl
ICM: Ischemic cardiomyopathy; NICM: Non-ischemic cardiomyopathy; NYHA: New York Heart Association; BSA: Body surface area; HTN: Hypertension; LBBB: Left bundle branch block; RBBB: Right bundle branch block; ECG: Electrocardiography

Among clinical and echocardiographic parameters, age, gender, DM, HLP, smoking, PMLA, and anterior and inferior Sm were shown to have significant associations with ischemic MR by univariate analysis ($P < 0.050$). Table 3 reveals the results of multiple logistic regression analysis regarding the significant predictors of ischemic MR. Smoking was identified as a strong determinant of ischemic MR (OR = 3.16, 95% confidence interval (CI): 2.77-198.52, P = 0.004). Moreover, echocardiographic parameters of PMLA and anterior Sm were identified as determinants with significant prediction value for ischemic MR (OR = 0.89, 95% CI: 0.82-0.96, P = 0.003; OR = 0.29, 95% CI: 0.14-0.60, P = 0.001, respectively).

According to the ROC curve analysis for PMLA and anterior Sm, the optimal cut-off point for discriminating ischemic MR from non-ischemic MR was ≤ 4 cm/second with the sensitivity and specificity of 80.0% (95% CI: 64.5-91.0), and 73% (95% CI: 50.0-91.0), respectively, and area under the curve (AUC) of 0.77 (95% CI: 0.63-0.91, $P < 0.001$). On the other hand, PMLA ≥ 40 degrees had the sensitivity of 70.0% (95% CI: 53.5-83.4), specificity of 77.3% (95% CI: 54.6-92.2), and AUC of 0.73 (95% CI: 0.59-0.86, P = 0.001) for predicting the ischemic etiology of FMR (Table 4) (Figure 3). Although PMLA had a higher positive predictive value (95% CI) than anterior Sm [0.75 (0.67-0.81) vs. 0.74 (0.67-0.80), respectively], this difference was not significant.

Table 2. Echocardiographic indices of patients with ischemic cardiomyopathy and non-ischemic cardiomyopathy

Parameter	ICM (n = 40)	NICM (n = 22)	P
LVEDV/BSA (ml/m^2)	119.32 ± 48.79	120.81 ± 42.72	0.790
LVESV/BSA(ml/m^2)	91.95 ± 38.64	96.49 ± 31.20	0.950
LVEF (%)	23.10 ± 6.60	19.80 ± 9.00	0.105
Tenting area	2.30 ± 0.90	2.30 ± 0.80	0.690
Coaptation depth	10.00 ± 0.40	9.30 ± 0.20	0.420
Anterior Sm (cm/second)	3.75 ± 1.14	5.00 ± 1.53	0.002
Inferior Sm (cm/second)	3.72 ± 1.11	4.80 ± 1.20	0.002
Anterior MLA (degree)	35.21 ± 9.80	34.32 ± 11.76	0.670
Posterior MLA (degree)	50.70 ± 12.21	40.10 ± 11.37	0.002

Data are presented as mean ± standard deviation.
ICM: Ischemic cardiomyopathy; NICM: Non-ischemic cardiomyopathy; LVEDV: Left ventricle end-diastolic volume; LVESV: Left ventricle end-systolic volume; LVEF: Left ventricle ejection fraction; BSA: Body surface area; Sm: Systolic myocardial velocity; MTA: Mitral leaflet angle; AMLA: Anterior mitral leaflet angle; PMLA: Posterior mitral leaflet angle; NS: Non-significant (P > 0.050)

Table 3. Logistic regression analysis for the prediction of ischemic functional mitral regurgitation*

Parameter	OR (95% CI)	P
Univariate		
Age (year)	1.07 (1.02-1.21)	0.010
Gender (male)	1.15 (0.76-1.74)	0.490
Hyperlipidemia	1.73 (1.10-2.72)	0.020
Hypertension	0.66 (0.28-1.50)	0.320
Smoking	3.13 (1.04-9.42)	0.040
Anterior Sm (cm/second)	0.22 (0.11-0.45)	< 0.001
Inferior Sm (degree)	0.99 (0.98-1.00)	0.340
PMLA (degree)	1.16 (1.13-1.19)	0.001
Multivariable		
Age(y)	0.99 (0.94-1.04)	0.740
Hyperlipidemia	1.26 (0.58-2.74)	0.550
Anterior Sm (cm/second)	0.29 (0.14-0.60)	0.001
PMLA (degree)	0.89 (0.82-0.96)	0.003
Smoking	23.46 (2.77-198.52)	0.004

CI: Confidence interval; OR: Odds Ratio; Sm: Systolic myocardial velocity; PMLA: Posterior mitral leaflet angle;
* Stepwise logistic regression was done.

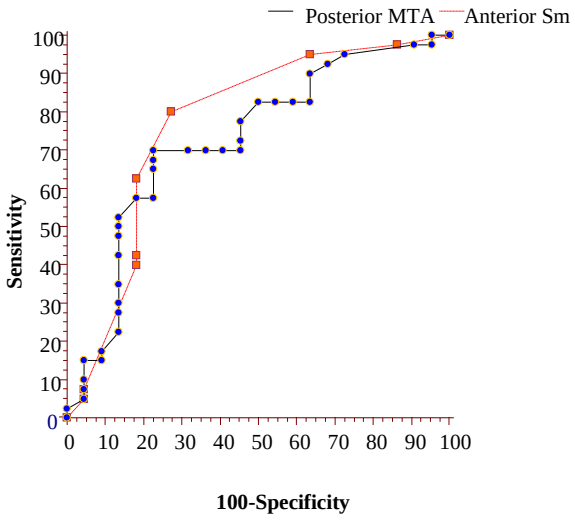

Figure 3. Receiver–Operating Characteristics curve illustrating accuracy of anterior systolic myocardial velocity and posterior mitral leaflet angle for the prediction of the ischemic etiology of functional mitral regurgitation

Discussion

The aim of this study was to evaluate echocardiographic parameters including 2D deformation MV indices and TDI in patients diagnosed with ICM and DCM. Echocardiographic differences were assessed with respect to FMR indices between these two groups. There were no significant differences in MR severity, and LV size and function (as measured by LVEF) between the two groups. The present study showed significant differences in PMLA and Sm between individuals with ICM and DCM. There were no significant differences in AMVL, TA, and CD between ICM and DCM groups. Multivariable logistic analysis demonstrated that PMLA and Sm could predict the etiology of FMR in patients with systolic heart failure. Many studies which have assessed echocardiographic characteristics in subjects with ICM and DCM have mainly focused on the relation of MV indices to MR severity. Konstantinou et al. studied the relation of MV echocardiographic deformation parameters with the severity of MR in ICM and DCM.[10] They found that FMR severity was chiefly determined by the extent of mitral apparatus deformity, and CD and regional myocardial systolic velocity had a significant association with FMR severity.

Table 4. Sensitivity and specificity of the anterior systolic myocardial velocity and posterior mitral leaflet angle for the identification of ischemic etiology in patients with functional mitral regurgitation

Parameter	Cut-off point	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	Positive Predictive value (95% CI)	Negative Predictive Value (95% CI)	P
Anterior Sm	4 (cm/second)	0.80 (0.64-0.91)	0.73 (0.50-0.89)	0.77 (0.63-0.91)	0.74 (0.67-0.80)	0.78 (0.70-0.84)	< 0.001
PMLA	40 (degree)	0.70 (0.53-0.83)	0.77 (0.54-0.92)	0.73 (0.59-0.86)	0.75 (0.67-0.81)	0.71 (0.65-0.77)	0.001

CI: Confidence interval; AUC: Area under the curve; Sm: Systolic myocardial velocity; PMLA: Posterior mitral leaflet angle

In the study by Papadopoulou et al., there were significant differences in LV and MV indices between ICM and DCM with functional MR.[11] In the logistic regression analysis, CD and TA were significantly larger in patients with ICM than those with DCM. TA > 1.27 cm^2 exhibited the highest sensitivity for predicting the ischemic etiology of LV dysfunction. However, this study evaluated patients with varying degrees of MR severity, which can have a confounding effect on the results reported. Therefore, the relatively similar CD and TA between ICM and DCM in our study could be due to the inclusion of subjects with no significant differences in MR severity, LV size, and function.

Upon evaluating the myocardial systolic velocity by TDI at mid-segments of anterior and inferior walls in ischemic and non-ischemic LV dysfunction, significant differences were observed in anterior and inferior Sm between ICM and DCM. Although the two groups had similar LVEF, patients with ICM exhibited lower systolic myocardial velocities, which could be explained by the regional wall motion abnormality detected in TDI. Significantly lower values of Sm were observed in patients with ICM compared to DCM, which was in agreement with previous studies.[8,12,13] MR severity affects Sm,[1] even though all patients in the present study had MR with similar moderate severity. In fact, longitudinal LV contraction could be attenuated in myocardial ischemia. Obstructive CAD leads to regional hypoperfusion of the myocardium which is detected by TDI earlier than visual assessment, presenting with attenuated Sm. In the present study, it was found that Sm values of less than 4 cm/second were independently associated with the probability of diagnosis of ICM rather than DCM.

In the current study, patients with ICM had a higher degree of PMLA angle in comparison with subjects with DCM, which could be explained by the predominant role of posteromedial dysfunction in ischemic MR. Therefore, in addition to the predictive role of Sm in identifying ICM and DCM, PMLA of more than 40 degrees with a high sensitivity and specificity in predicting the ischemic origin of functional MR was observed. This independent predictive role for PMLA in the present survey can be explained by the mechanism of FMR in patients with ICM. Mitral valve tenting is a major determinant of FMR and is directly determined by local LV remodeling, particularly by the displacement of the apical and posterior papillary muscles (PM). The pattern of mitral apparatus deformation is asymmetrical in ICM-related FMR. This could result in augmented tethering of the posterior MV leaflet rather than anterior MV leaflet in patients with ICM because of regional change in LV dysfunction. In contrast, in patients with DCM, global LV dysfunction results in bilateral symmetrical PM displacement.[14-16]

Gorman et al. suggested that, in a sheep model, LV dilatation without prominent geometric changes in the MV apparatus does not cause significant ischemic MR, while with MV annular and posteromedial PM geometric changes, especially in subjects with posterior MI, MR develops.[17] Therefore, ischemic MR is proportional to the degree of deformity of the MV complex, especially the outward displacement of the posteromedial PM, rather than to global LV dilatation. Magne et al. conducted a study on patients with ischemic MR who underwent surgical MV repair.[18] Patients with PMLA of more than 45 degrees had unfavorable results and recurrence of MR was seen frequently in these patients. Their study suggested that a higher degree of PMLA indicates the greater tethering of MV leaflets, resulting in unsuccessful MV repair.

Limitation: The main limitation of this study was that the patient population was relatively small; thus, further studies with larger samples are needed. Moreover, the present study findings were limited to patients with moderate severity of functional MR.

Conclusion

PMLA ≥ 40 degrees in echocardiography could be used with reasonable accuracy to predict the ischemic entity of MR in patients with systolic heart failure. In addition, Sm ≤ 4 cm/second measured by TDI can predict MR of ischemic origin in patients with systolic heart failure.

Acknowledgments

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Conflict of Interests

Authors have no conflict of interests.

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Comparison of the effect of the Dietary Approaches to Stop Hypertension diet with usual dietary advice on expression of peroxisome proliferators-activated receptor gamma gene in women: A randomized controlled clinical trial

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Original Article

Abstract

BACKGROUND: Peroxisome proliferator-activated receptor gamma (PPAR-γ) which controls body weight, glucose homeostasis, and adipocyte differentiation is a valuable candidate gene for insulin resistance (IR). The present study aimed to compare the effects of the Dietary Approaches to Stop Hypertension (DASH) diet and usual dietary advice (UDA) on PPAR-γ gene expression in women at risk for cardiovascular disease (CVD).

METHODS: This randomized controlled trial was performed on 44 women aged 20-50 years at risk for CVD (BMI > 25 kg/m² and low physical activity). Participants were randomly assigned to the UDA (n = 22) or DASH (n = 22) diets for 12 weeks. The DASH diet was rich in fruits, vegetables, whole grains and low-fat dairy products and low in saturated fat, total fat, cholesterol, refined grains and sweets, with a total of 2400 mg/day sodium. The UDA diet was a regular diet with healthy dietary advice. Anthropometric indices and PPAR-γ gene expression were measured and compared between the two groups at the end of the study.

RESULTS: After the intervention, body mass index (BMI) and waist circumference (WC) significantly decreased in the DASH group (P < 0.050) but the results showed no significant differences between the two groups. At the end of the trial, PPAR-γ gene expression was significantly different between the UDA and the DASH diet groups (P = 0.040) and this difference remained significant after adjustment for BMI, and physical activity (P = 0.030).

CONCLUSION: The result of the study showed that the DASH diet significantly decreased the expression of PPAR-γ. This finding was unexpected and future studies on the current topic are therefore recommended.

Keywords: Peroxisome Proliferator-Activated Receptor Gamma, DASH Diet, Gene Expression

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Introduction

Insulin resistance (IR), which is described as decreased physiological response of the peripheral tissues to the action of the normal levels of insulin, is the main sign in some metabolic illnesses, such as metabolic syndrome and type two diabetes mellitus (DM).[1] IR aggregates in families and up to 30–70% of type two DM risks can be associated with genetics.[2] Nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ) which is mostly expressed in adipose tissue and controls body weight, glucose homeostasis, and adipocyte differentiation is a valuable candidate gene for IR. Thus, mutations in this gene might affect IR and lipid metabolism.[3,4] Moreover, dietary factors are the most important environmental components in the pathogenesis and development of the general polygenic, food-related diseases; therefore, diet controlling is a crucial factor in the long-term wellbeing and quality of life (QOL) of individuals

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with IR.[5,6] One of these regimes is the Dietary Approaches to Stop Hypertension (DASH) the impact of which on IR is a controversial topic. As regarding the composition of the DASH dietary pattern that contains high levels of calcium, potassium, magnesium, fiber and antioxidants, it is expected to improve insulin action in humans.[7]

The effect of dietary interventions on insulin action might be modified by genetic factors, so identifying the nutrient-sensitive genotypes seems necessary for optimizing nutrition recommendations based on an individual's genetic profile to reduce disorder.[8] To our knowledge, there were no interventional investigations in humans in the field of the effects of special dietary patterns on gene expression. Therefore, the objective of this study was to evaluate the impacts of the DASH diet versus usual dietary advices (UDA) on PPAR-γ gene expression.

Materials and Methods

This randomized controlled clinical trial (RCT) was performed on healthy women volunteers (20-50 years of age) who were at risk of cardiovascular disease (CVD) and referred to Isfahan Endocrine & Metabolism Research Center, Isfahan, Iran, in January 2015. At risk of CVD was defined as BMI > 25 kg/m^2 and low physical activity. Health was evaluated using a questionnaire on personal health, medical history and biochemical experiments. The inclusion criteria consisted of lack of pregnancy or lactation, lack of history of occurrence of hepatic, cardiovascular, gastrointestinal, renal, and thyroid diseases, and rheumatoid arthritis, DM, lupus, trauma, severe infection, and allergy. Moreover, they could not utilize omega-3 fatty acids supplements, multi-vitamins and minerals, antacid comprising magnesium and calcium, aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs) and anti-inflammatory and anti-depressant drugs. Participants were excluded if they had increased physical activity or weight changes during the study or poor adherence to the study protocol.

The research was conducted based on the standards of the Declaration of Helsinki; the design and purpose of the study were described for the participants, and written informed consent was obtained from all subjects. The study was approved by the ethical committee of Isfahan University of Medical Sciences, Iran. This trial was recorded at the Iranian Registry of Clinical Trials (IRCT) with the registered number of IRCT2014090719072N1.

The participants were randomly allocated to a UDA diet or the DASH diet for 12 weeks after a 2-week run-in period. The run-in period was performed to homogenize the groups in terms of the intake of macronutrients and basis of diets. The UDA diet was prescribed for patients in this stage. Group allocations were performed using random sequencing. The individual who prescribed the diets was aware of the group allocation, but laboratory members were blinded. The participants' socio-economic status was assessed using a validated questionnaire for Iranians.[9] The participants were evaluated every 2 weeks, and anthropometric and physical activity measurements were recorded. The diet was prescribed for patients. They were asked to record their physical activity 3 days every month and not change their activity level record, then, reported activities were scored and coded based on the Compendium of Physical Activities and expressed as the metabolic equivalent (MET). MET is the ratio of work metabolic rate to a standard resting metabolic rate and scored from 0.9 (sleeping) to 18 METs (running at 10.9 mph).[10]

Individuals were randomly allocated to one of two diets; UDA diet and the DASH diet. The UDA group was only recommended to "eat as regular" and received healthy dietary advices. The DASH diet was prescribed for the intervention group. The DASH diet is rich in fruits, whole grains, vegetables, low-fat dairy products, and low in saturated fat, cholesterol, total fat, sweets, refined grains and red meat. Moreover, it comprises 2,400 mg sodium per day, which was based on the Iranian Food Composition (Table 1).[11]

Table 1. Dietary goals of the Dietary Approaches to Stop Hypertension intervention vs. usual dietary advice

DASH	Usual dietary advice
at least eight servings/day of fruits and vegetables	Try to have a variety of foods in your daily diet.
two to three servings/day of low-fat dairy products	Do not skip any meals.
1/2 to 1 serving of nuts, seeds, and legumes daily	Minimize the intake of sugar, sweets, and sweetened drinks.
< 2400 mg/d of Na	Before cooking, remove fats and skin of the chicken and meat.
	Try to use whole-wheat and barley breads instead of rice.

DASH: Dietary Approaches to Stop Hypertension

A Mifflin-St Jeor equation was used to estimate the energy requirement for each person.[12] The participants were met every 2 weeks; each session lasted 45–60 minutes. They were contacted by the nutritionist every week by phone. The calorie count system was used to prescribe the diets, and the participants were taught to use the exchange list for modifying food items and calculating calories. Participants had to deliver their 3-day diet records (2 work days and 1 holiday) every month and their diet was evaluated by analyzing the food record diaries using the Nutritionist IV software (version 7.0; N-Squared Computing, Salem, OR, USA) that was adapted for Iranian food items.

Participants were weighed with minimal clothing and without shoes using digital scales (SECA, Hamburg, Germany) with an accuracy of approximately 0.1 kg. Height was measured in a standing position using a tape measure without shoes. Waist circumference was measured where the waist was narrowest over light clothing, using an unstretched tape measure, without pressure on the surface of the body and amounts were recorded with an accuracy of approximately 0.1 cm.

Peripheral blood mononuclear cells isolation, RNA isolation, and real-time polymerase chain reaction: Human peripheral blood mononuclear cells isolation (PBMC) was conducted by centrifugation on a Ficoll-Paque Plus (Amersham Biosciences Corp., Little Chalfont, UK) density gradient. Total RNA was isolated from the PBMC using Trizol® reagent (Invitrogen) according to the manufacturer's instructions. Isolated RNA was dissolved in RNase-free water, and the amount of RNA was determined by measuring absorbance at 260 nm with a spectrophotometer. The RNA samples were treated with DNase I (Thermo Scientific, Waltham, MA, USA) in order to avoid potential contamination with genomic DNA. To synthesize double-stranded cDNA, 2 µg of total RNA was consumed using RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Waltham, MA, USA) and oligodT primers. The primers for all assayed genes were designed using the Allele ID software (version 7.6; Primer Biosoft, Palo Alto, CA, USA) (Table 2). The real-time polymerase chain reaction (PCR) was performed using SYBR Green PCR Master Mix (Thermo Scientific, Waltham, MA, USA) and the StepOnePlus™ Real-Time PCR Detection System (Applied Biosystems, Foster City, CA, USA). Glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The expression level of each target gene was calculated as $2^{-\Delta\Delta Ct}$, as previously described.[13]

Table 2. Primers used in real-time polymerase chain reaction

Gene	Primer sequences	Size (Base pair)
PPARG-F	GCCTTTTGGTGACTTTATGGA	21
PPARG-R	GTAGCAGGTTGTCTTGAATG	20
GAPDH-F	AAGCTCATTTCCTGGTATG	19
GAPDH-R	CTTCCTCTTGTGCTCTTG	18

PPARG: Peroxisome proliferator-activated receptor gamma; GAPDH: Glyceraldehydes-3- phosphate dehydrogenase

The normality of continuous variables, such as age, weight, BMI, waist circumference and physical activity, was evaluated by normal plots and one-sample Kolmogorov-Smirnov test. ANCOVA was used for assessing gene expression differences between the UDA and DASH diet after 12 weeks with changes in weight and waist circumference (WC) and physical activity as covariate. For each dependent variable, the changes from baseline were computed by subtracting the end-of-trial value from the baseline value. Within-group and between-group changes in anthropometric measures as well as biochemical indicators were compared using paired samples t-test and independent t-test, respectively. In addition, chi-square test was used to compare qualitative variables. The SPSS (version 16.0, SPSS Inc., Chicago, IL, USA) was used for statistical analyses and P values of less than 0.05 were considered as significant.

Results

Characteristics: Of the 51 participants, 44 individuals completed the study. During the study, 1 patient was diagnosed with polycystic syndrome and another with high weight change, so these 2 patients had to be excluded from the analyses. Moreover, 5 patients deviated from the study protocol, and therefore, their data were not available. The consort diagram is shown in figure 1. Differences in distribution of several characteristics among 22 individuals in the DASH group and 22 subjects in the UDA group are shown in table 3. The mean age of patients was 38 ± 8 years in the UDA group and 37 ± 9 years in the intervention group. There was no difference between the groups regarding age, socioeconomic status, weight, physical activity (PA) and gene expression at the baseline.

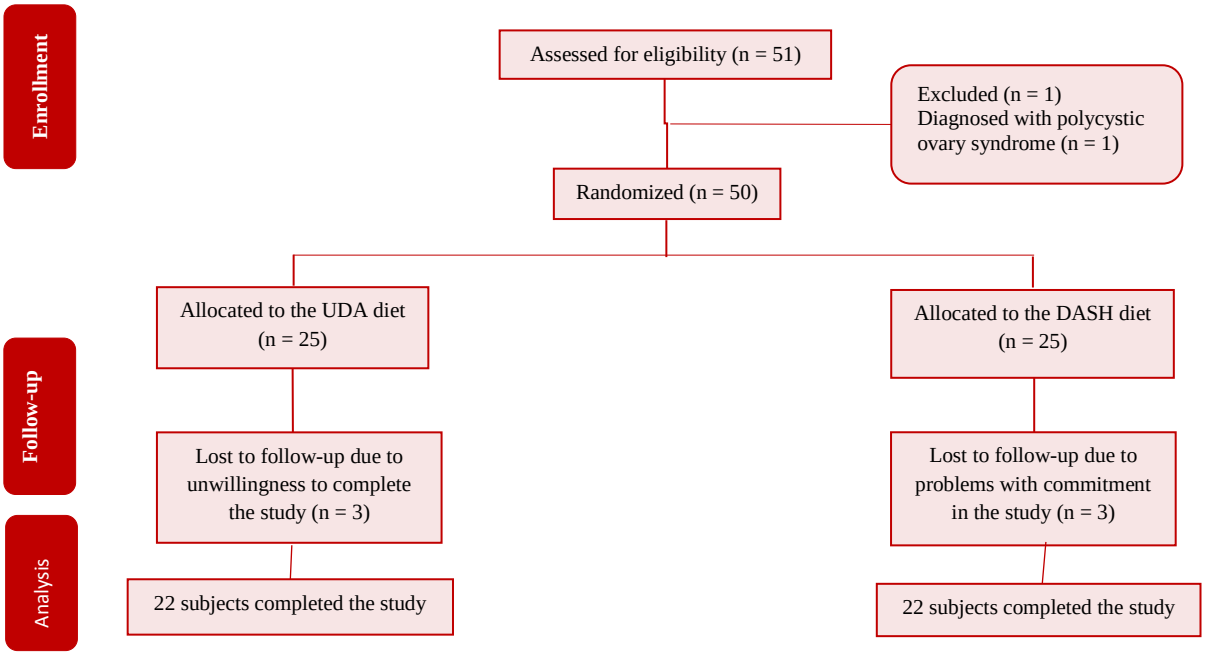

Figure 1. Flowchart of participants' recruitment and enrollment in the study
DASH: Dietary Approaches to Stop Hypertension; UDA: Usual dietary advice

Analysis of diet showed that calorie and protein intake of the two groups did not significantly differ, but these two diets were different in terms of total fat and fat composition intake, as well as the percentage of carbohydrate intake. These two diets were different in terms of sodium content. Although these differences were not statistically significant, they were nutritionally important. The DASH diet had a higher amount of calcium, potassium and fiber (Table 4).

Table 3. Baseline characteristics and effects of the Dietary Approaches to Stop Hypertension diet vs. usual dietary advice on anthropometric measures (mean values with their standard deviation)

Variable	UDA† (n = 22)	DASH* (n = 22)	P
Age (year)	38.9 (7.7)	37.3 (9)	0.530
Socio-economic status			
Low [n (%)]	6.0 (26.1)	9.0 (37.5)	
Medium [n (%)]	11.0 (47.8)	6.0 (25.0)	0.270
High [n (%)]	6.0 (26.1)	9.0 (37.5)	
Physical activity (MET-h/d)			
Baseline [n (%)]	42.0 (5.9)	40.0 (4.2)	0.200
End-of-trial [n (%)]	41.9 (6.3)	38.9 (3.5)	0.070
Difference (95%CI)	0.17 (-0.8,1.14)	1.04 (-0.62,2.70)	
BMI (kg.m^2)			
Baseline [n (%)]	32.8 (2.7)	33.46 (3.6)	0.300
End-of-trial [n (%)]	32.64 (2.6)	33.01 (3.8)	0.700
Difference (95%CI)	-0.28 (-0.98,0.42)	-0.39 (-0.69,-0.09)	
WC (cm)			
Baseline [n (%)]	99.8 (6.7)	102.3 (10.9)	0.020
End-of-trial [n (%)]	100 (6.7)	99.9 (8.7)	0.900
Difference (95%CI)	0.11 (-0.64,1.43)	-2.4 (0.09,4.60)	

* The DASH diet was high in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fats, total fats, cholesterol, refined grains, and sweets. † The usual dietary advice group received general oral and written information about healthy food choices. DASH: Dietary Approaches to Stop Hypertension; UDA: Usual dietary advice; MET: Metabolic equivalent; BMI: Body mass index; WC: Waist circumference; CI: Confidence interval
P < 0.050 is significant, Obtained from independent t-test.

Table 4. Daily energy and nutrient intakes in the Dietary approaches to stop hypertension and Usual Dietary Advice groups at baseline at the end of the study (Mean values with their standard deviation)

Intake	UDA group (n = 22)	DASH group (n = 22)	P
Energy (kcal)	1688.3 (799.7)	1633.4 (391.8)	0.770
Protein (g/day)	63.0 (34.5)	66.9 (24.0)	0.270
Total fat (g/day)	69.0 (35.0)	48.0 (21.0)	< 0.001
Carbohydrate (g/day)	211.0 (108.0)	239.0 (50.0)	< 0.001
Saturated fat (g/day)			
Crude†	15.2 (3.3)	13.4 (3.7)	0.200
Model I1‡	15.2 (4.9)	13.3 (5.0)	0.060
PUFA (g/day)			
Crude	26.5 (12.0)	16.3 (9.0)	< 0.001
Model I	26.0 (6.8)	16.7 (6.8)	< 0.001
MUFA (g/day)			
Crude	13.0 (6.0)	15.8 (6.0)	0.140
Model I	13.3 (4.4)	15.6 (2.9)	0.040
PUFA/SFA Ratio			
Crude	1.75 (0.6)	1.2 (0.8)	< 0.001
Model I	2.4 (2.1)	0.9 (3.5)	0.090
Fiber (g)			
Crude	14.6 (6.7)	14.8 (5.2)	0.940
Model I	11.2 (4.5)	14.3 (5.8)	0.050
Potassium (mg)			
Crude	2362.2 (1039.7)	2796.5 (1086.6)	0.190
Model I	2325.0 (542.0)	2831.0 (769.0)	0.010
Calcium (mg)			
Crude	674.1 (318.9)	875.1 (378.9)	0.060
Model 1	664.5 (260.0)	884.0 (287.0)	0.010
Magnesium (mg)			
Crude	249.3 (207.0)	255.3 (15.1)	0.910
Model I	246.0 (185.0)	259.0 (93.0)	0.800
Sodium (mg)			
Crude	1544.3 (151.2)	1613.7 (1625.4)	0.870
Model I	1682.0 (1242.0)	1645.0 (849.0)	0.700
Vitamin C (mg)			
Crude	104.6 (73.9)	138.2 (94.7)	0.200
Model I	102.9 (64.0)	140.0 (87.0)	0.120

Obtained from independent t-test; †crude model did not adjusted; ‡ Model 1 adjusted for energy intake (data are means ± SD); Data are means ± SD; P < 0.050 is significant.

DASH: Dietary Approaches to Stop Hypertension; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acid; SFA: saturated fatty acid; SD: Standard deviation; UDA: Usual dietary advice

Nutrient intake and anthropometric measurements: The reported dietary intakes confirmed that participants modified their intake of nutrients in the direction of the intervention; however, the targets were not fully achieved. The estimated nutrient content of the 3-day food records consistent with the patients' reports is shown in table 4.

As shown in table 3, no significant differences were observed in body composition between the two groups. Nevertheless, after the trial, BMI significantly decreased (P < 0.050) and WC marginally decreased in the DASH group (P = 0.055).

Gene expression changes: The outcome of reverse transcription-PCR indicated that the DASH diet significantly decreased the expression of PPAR-γ compared to the UDA diet (P = 0.040), and after weight change and physical activity adjustment, the results did not noticeably alter (P = 0.030) (Table 5).

Discussion

The results of this investigation indicated that the expression of PPAR-γ in the UDA group was higher than the DASH group. To the best of our knowledge, the effect of the DASH diet on PPAR-γ gene expression in humans has not been reported previously, but some studies have been performed on different polymorphisms of this gene.[14,15]

Table 5. The effects of the Dietary Approaches to Stop Hypertension diet vs. usual dietary advice on gene expression (Mean values with their standard deviation)

Gene expression	UDA			DASH			P	P‡
	Baseline	12th week	P*	Baseline	12th week	P		
	11.07 ± 3.90	12.34 ± 4.75	0.180	10.80 ± 0.70	9.28 ± 3.90	0.230	0.040	0.030

All values are mean ± SD
The UDA group had the usual diet.
The DASH diet was high in fruits, vegetables, whole grains, and low-fat dairy products and low in saturated fats, total fats, cholesterol, refined grains, and sweets.
The amount of sodium intake was 2400 mg/day
* Obtained from paired t-test through the comparison of between-group differences by ANCOVA; ‡ Adjusted for a change in weight, WC, and PA; P < 0.050 is significant. UDA: Usual dietary advice; DASH: Dietary Approaches to Stop Hypertension; SD: Standard deviation; WC: Waist circumference; PA: Physical activity

PPAR-γ activation improves insulin signaling, glucose transportation, glycogen synthesis, mitochondrial function and fat mobilization.[16-18] Some mechanisms have been suggested for these effects including activation of fatty acid transporters such as fatty acid transport protein 1 (FATP1), a cluster of differentiation 36 (CD36), glycerol kinase (GK) and phosphoenolpyruvate carboxykinase (PEPCK), and thus, the retaining of fatty acids in adipose tissue.[19,20] PPAR-γ modulates the endocrine activity of adipose tissue by regulating the synthesis of secreted adipocyte proteins (adipokines) that affect insulin signaling in hepatic and peripheral tissues.[21] Thus, adiponectin expression increases, whereas the production of plasminogen activator inhibitor-1 (PAI-1), leptin, tumor necrosis factor-a (TNF-a), resistin, and interleukin 6 (IL-6) reduces.[22] Furthermore, it directly increases adipocyte glucose disposal by induction of the glucose transporter type 4 (GLUT4).[23]

The characteristics of physiologically related activators of PPAR-γ are not clear, although PPAR-γ is activated by fatty acids.[16,24-26] As previously mentioned, fat intake was significantly higher in the UDA group, and its composition differed in the two groups. For example, the polyunsaturated fatty acid (PUFA): saturated fatty acid (SFA) ratio was significantly higher in the UDA group (P = 0.009). These results are in agreement with the findings of previous studies which have shown that PUFAs could act as ligands of PPAR-γ or could modify its expression.[27-30] It is also consistent with the findings of studies that have revealed that some n–3 and n–6 PUFAs activate PPAR-γ.[31] Moreover, they are in agreement with findings of studies which have reported the main interaction between usual dietary fat composition and the PPAR-γ Pro12Ala polymorphism.[32,33] Furthermore, after the intervention, weight and WC significantly decreased in the DASH group compared with the UDA group, which is acceptable regarding the DASH composition. This result is in agreement with one study which showed a 25% reduction in PPAR-γ mRNA expression after a 10% decrease in body weight.[27] These findings suggest that PPAR-γ is required in the maintenance of normal insulin sensitivity in mice, but also creates the fascinating idea that it may be required for the adversative effects of a high-fat diet on carbohydrate metabolism.[34]

Conclusion

The present study was proposed to determine the impact of the DASH diet on the PPAR-γ gene expression. This study indicated that BMI and WC decreased significantly in the DASH group in comparison with the UDA group. The second major finding was that the DASH diet significantly decreased the expression of PPAR-γ. This finding was unexpected, and future studies on the current topic are therefore recommended.

Limitations: A number of important limitations need to be considered. First, dietetic intake in the present investigation was self-reported, and participants were advised to follow a specific diet rather than delivering prepared foods, thus resulting in possible imperfect adherence to the recommended diets. The second limitation in this study was that the sample volume was relatively small, so further investigations and experimentations in different population are strongly recommended. Third, the findings cannot be generalized because the study participants were restricted to women.

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Conflict of Interests

Authors have no conflict of interests.

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Epicardial fat thickness and severity of coronary heart disease in patients with diabetes mellitus type II

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Original Article

Abstract

BACKGROUND: Clinical imaging studies have demonstrated a strong direct correlation between epicardial fat and abdominal visceral adiposity. There are several studies about positive correlation of epicardial fat and atherosclerotic coronary disease in general population. This study aimed to evaluate the association of epicardial fat thickness with atherosclerotic coronary disease in patients with diabetes mellitus type II.

METHODS: This cross-sectional observational study involved 80 patients with diabetes mellitus type II. The patients were chosen using simple sampling method from patients with diabetes mellitus who were referred for angiography because of suspected coronary artery disease. The severity of coronary atherosclerotic lesions was evaluated using modified Gensini scoring system. Epicardial fat thickness was measured by transthoracic echocardiography within 90 days after coronary angiography. Multiple linear regression method was used to evaluate the association between mean epicardial fat thickness and Gensini score.

RESULTS: After adjustment for the effects of body mass index (BMI), age, angina, and sex, there was a significant association between Gensini score and epicardial fat thickness ($\beta = 0.825$; $P < 0.001$). Patients with higher blood pressure and higher body mass index also had a higher Gensini score ($P < 0.010$).

CONCLUSION: In patients with diabetes mellitus type II, there is a positive association between epicardial fat thickness and severity of coronary artery disease. So, by echocardiography evaluation of epicardial fat thickness, we could have an estimation of the severity of coronary arteries diseases before using more invasive techniques.

Keywords: Body Fat, Coronary Artery Disease, Stenosis, Diabetes Mellitus

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Introduction

Obesity is an important risk factor for the development of all features of metabolic syndrome and atherosclerotic cardiovascular disease.[1-6] Clinical imaging studies have demonstrated a strong direct correlation between epicardial fat and abdominal visceral adiposity. Epicardial fat covers 80% of the heart's surface and constitutes 20% of total heart weight.[7] Epicardial fat is three to four folds more associated with the right than the left ventricle.[7]

There is a lot of publications about the physiological and metabolic importance of epicardial adipose tissue. Both the epicardial fat thickness and volume have strong association with obesity, impaired fasting glucose, insulin resistance, metabolic syndrome, hypertension, diabetes mellitus, and atherosclerosis.[8] An association between insulin resistance and central adiposity, and clinical parameters of cardiovascular risk including low-density lipoprotein (LDL) cholesterol and blood pressure had been shown in previous studies.[9]

Epicardial fat is independently associated with coronary artery disease (CAD). This correlation may be explained by systemic inflammation induced by visceral fat including epicardial fat.[10]

Epicardial adipose tissue (EAT) mediates inflammatory process within the atherosclerotic plaque.[11] The paracrine or vasocrine secretion of

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epicardial inflammatory adipokines, such as tumor necrosis factor alpha, plasminogen activator inhibitor-1, interleukin-6, interleukin-1b, monocyte chemo-attractant protein-1, and resistin contribute to the metabolic and inflammatory milieu that promotes atherogenesis and insulin resistance.[12] This mechanism may explain the positive relationship between the amount of fat surrounding the heart and vessels and several components of the metabolic syndrome and diabetes mellitus type II.[13]

There are few studies about the association of epicardial fat thickness and severity of CAD in subgroup of patients with diabetes mellitus. In this study, we aimed to determine the relationship between the epicardial fat thickness and severity of CAD in patients with diabetes mellitus type II.

Materials and Methods

This cross-sectional observational study was performed in Chamran hospital, Isfahan University of Medical Sciences, Iran. Eighty five patients with diabetes mellitus type II aged 40 to 80 years took part in this study. The cases were chosen via simple sampling method, from the patients with diabetes mellitus type II, who were referred for coronary angiography because of suspected CAD during August 2015 to May2016.

All the patients underwent detailed history, clinical examination, anthropometric measurement, routine biochemistry, electrocardiography (ECG), and transthoracic echocardiography.

Patients who had chest deformities, chronic lung disease, poor echo window, pericardial and/or pleural effusion on transthoracic echocardiography, previous coronary artery bypass graft (CABG) surgery, and percutaneous coronary intervention (PTCA) were excluded from study. Patients with chronic kidney disease defined by rise in creatinine or albuminuria, patients with any kind of metastatic on non-metastatic cancer, and patients with increased liver enzyme were also excluded.

Blood pressure was measured from right hand after 10 minutes of rest. Hypertension was defined as systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or requirement of antihypertensive medication.[10] Body mass index (BMI) was calculated as body weight in kilograms divided by height squared. Obesity was defined as having a BMI ≥ 30 kg/m^2. Diabetes mellitus type II was defined according to the criteria of the American Diabetes Association.[14] These criteria included:

1- Symptoms of diabetes plus random blood glucose concentration over 200 mg/dl

2- Fasting blood glucose over 126 mg/dl

3- Glucose over 200 mg/dl during glucose tolerance test

4- Glycated hemoglobin (HbA1c) over 6.5%

Hyperlipidemia was defined as total cholesterol higher than 220 mg/dl or triglycerides ≥ 150 mg/dl.[15]

In a fasting state, coronary angiography was performed using the Judkins' technique,[16] by the femoral or radial artery approach. The severity of coronary atherosclerotic lesions was evaluated from at least 3 projections in all the patients by modified Gensini scoring system.[17] According to this scoring system, coronary arterial system was divided into 8 segments and the most severe luminal narrowing in each coronary segment was graded with 1 to 4 points (between 1% and 49%, 1 point; 50% and 74%, 2 points; 75% and 99%, 3 points; 100%, 4 points). Each patient was evaluated with a total score between 0 and 32 points. Each point was multiplied with separate coefficients based on vessel and its segments; these coefficients were 5 for left main coronary artery, 2.5 for proximal left anterior descending (LAD), 1.5 for middle LAD, 1.5 for distal LAD, 1 for diagonal LAD, 2.5 for proximal circumflex artery, 1 for marginal obtuse and posterolateral branch, 1.5 for right proximal coronary, 1 for posterior descending artery, and 0.5 for others. The points were added and total Gensini points were calculated for each patient.[18]

Epicardial fat thickness was measured using transthoracic echocardiography within 90 days of coronary angiography. Echocardiographies were performed by a single cardiologist with a GE Vivid 3 instrument (Providian Medical, LLC, USA) according to standard techniques, with subjects in the left lateral decubitus position. Cardiologist that performed echocardiography was not aware of angiography results.

The epicardial fat thickness was measured perpendicularly on the free wall of the right ventricle at end-systole for 3 cardiac cycles. The measurement was performed at a point on the free wall of the right ventricle where the fat thickness was highest. All data were analyzed via SPSS software (version 15, SPSS Inc., Chicago, IL, USA).

Continuous data were demonstrated as mean and standard deviation. Categorical data were shown as absolute number and percent. The normality of data was evaluated via Kolmogorov-Smirnov test. Independent t test was used for continuous data and chi-square test for categorical data analysis. Multiple linear regression was used to evaluate the relationship between means of epicardial thickness and modified Gensini score.

Table 1. Demographic characteristic of patients and comparing between the two groups based on the median of epicardial fat thickness

Variables	Equal or less than 0.7 mm (n = 45)	More than 0.7 mm (n = 40)	P	Total (n = 85)
Sex (Men)	25 (55.6)	20 (55.0)	0.610	45 (52.9)
History of Heart failure	6 (13.3)	11 (27.5)	0.100	17 (20.0)
Smoking	13 (28.9)	18 (45.0)	0.120	31 (36.5)
Hypertension	33 (73.3)	33 (82.5)	0.310	66 (77.6)
Dyslipidemia	15 (33.3)	8 (20.0)	0.160	23 (27.1)
History of MI	9 (20.0)	21 (52.5)	0.002	30 (35.0)
History of angina	39 (86.7)	35 (87.5)	0.910	74 (87.0)
Age (year)	58.20 ± 8.34	62.60 ± 7.83	0.014	60.31 ± 8.36
Weight (kg)	73.30 ± 11.40	79.20 ± 8.85	0.010	76.09 ± 10.62
Height (cm)	168.90 ± 7.69	167.20 ± 6.57	0.280	168.14 ± 7.20
Body mass index (BMI)	25.60 ± 3.24	28.40 ± 3.44	< 0.001	26.93 ± 3.60
Duration of diabetes (year)	7.44 ± 2.88	8.57 ± 3.42	0.100	7.97 ± 3.18
Modified Gensini score	12.10 ± 4.98	24.40 ± 5.45	< 0.001	17.89 ± 8.04

All continuous variables reported as mean ± standard deviation (SD) and categorical variables reported as absolute number (percent).
MI: Myocardial infarction

Patients divided into two groups of below and above the median of Gensini score and mean z. The difference between the 2 groups was evaluated using t-test. P-value lower than 0.050 was considered significant.

Results

Eighty five patients took part in this study, 45 men (53%), and 40 women (47%). Participants were divided into two group according to median (7 mm) of epicardial fat thickness (EFT). The patients with EPT of over 7 mm were significantly older (62.6 ± 7.83 vs. 58.2 ± 8.34; P = 0.014) and fatter (28.4 ± 3.44 vs. 25.6 ± 3.24; P < 0.001), and had more severe CAD (mean Gensini score of 24.4 ± 5.45 vs. 12.1 ± 4.98; P < 0.001) (Table 1).

After adjustment the roles of BMI, age, angina history, and sex, multiple linear regression analysis revealed a significant association between modified Gensini score and epicardial fat thickness (Crude Model: 0.83, Adjusted Model: 0.72; P < 0.001 for both). One millimeter increase of mean epicardial fat thickness was associated with 0.82 unit of modified Gensini score (Figure 1).

Discussion

In this study, we found a correlation between the severity of CAD calculated by modified Gensini method and echocardiographic epicedial fat thickness in patients with diabetes mellitus type II. We also found that there was a relationship between obesity and hypertension with the severity of CAD; but this relationship was not linear.

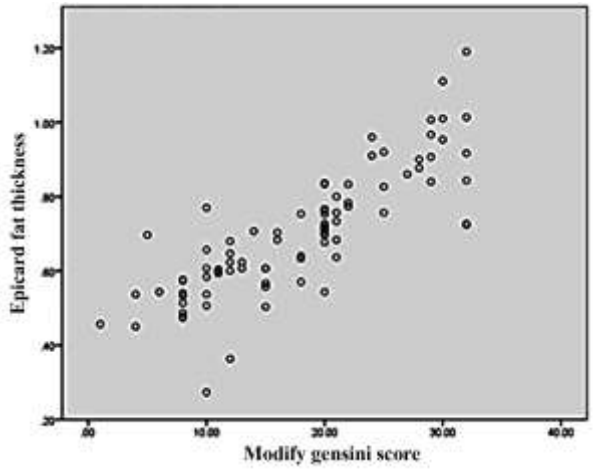

Figure 1. Association of modified Gensini score and epicardial fat thickness

Magnetic resonance imaging (MRI) and computed tomography (CT) scan are currently gold standards for measuring epicardial fat; but these are expensive, and are not routinely performed in a typical cardiac patient.[19] So, we used echocardiography for measurement of epicardial fat thickness, which is an estimation of epicardial fat content.

There are a lot of studies about the epicardial fat thickness and atherosclerotic diseases of CAD; but, there are few studied in the subgroup of patients with diabetes mellitus. In a recent study on 123 patients with CAD, echocardiographic epicardial fat thickness was significantly correlated with the presence and severity of angiographically detected CAD. They used Gensini scoring system for measurement of the severity of CAD.[17] In another study on 110 patients, epicardial fat thickness in men and women was not statistically different and coronary artery lesions

measured by Gensini score showed linear association with severity of CAD, and epicardial fat thicknes.[20]

Nakazato et al. measured epicardial fat volume by CT scan instead of echocardiography, and found that epicardial fat valium was independently and linearly associated with existence of CAD and its severity.[21]

The location of lipid accumulation around the heart may be important in increasing the probability of coronary stenosis. In a study on 157 patients with diabetes mellitus type II and without CAD history, left atrioventricular groove epicardial adipose volume was an independent predictor of CAD.[22]

Cystatin C, a 13-kD endogenous cysteine proteinase inhibitor, is ubiquitously expressed, mainly in the brain, testis, lung, spleen, and adipose tissue.[23] Recently, a strong association between epicardial fat and cystatin C in patients with diabetes type II is founded. This means that epicardial fat accumulation play an essential role in cystatin C secretion, that contributing to atherosclerosis risk in these patients.[24]

There is a decreased expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α) and uncoupling protein 1 (UCP1) mRNA in epicardial fat tissue of patients with CAD and diabetes mellitus type II that may be caused by a loss of brown-like fat features. There is a higher prevalence of CAD in patients with decreased expression of PGC1α in epicardial adipose tissue.[25]

Besides, its effect on coronary artery pericardial fat is related to other cardiac conditions such as heart failure calcification of coronary arteries, coronary artery spasm, etc.

Ng et al. found that in with patients mellitus, epicardial fat is independently associated with impaired myocardial systolic function despite preserved 3 dimensional (3D) left ventricular ejection fraction and absence of obstructive CADs. They measured epicardial fat using 3D echocardiography.[26]

Coronary artery calcium score is associated with epicardial fat thickness, too. A cohort study showed that progression of coronary artery calcification was correlated with epicardial fat thickness, and this score also had significant correlation with systemic inflammation markers.[27]

A 5-year CT scan follow-up study by Hwang et al. showed that greater amount of epicardial fat at baseline CT scan independently predicted the development of non-calcium coronary plaque in asymptomatic individuals.[28] The development of coronary artery calcification may be mediated by epicardial fat volume via the activation of local inflammatory cytokines.

Epicardial fat is related to the presence of coronary artery calcification but not to aortic valve or ascending aorta calcification. These findings support a local paracrine effect of epicardial fat in mediating coronary atherosclerosis.[29]

Epicardial fat volume also correlated with atherosclerotic plaque vulnerability. There was an association between epicardial fat volume and development of coronary atherosclerosis and the most dangerous types of plaques in Ito et al. study.[30]

Ergonovine-induced epicardial coronary artery spasms is also related to epicardial fat valum.[31] So, increased epicardial fat thickness may predict the probability of angina attack in patients with non-significant coronary stenosis.

Conclusion

In conclusion, this study showed significant correlation between epicedial fat and the severity of CAD; epicardial fat also related to other cardiac conditions including left ventricular dysfunction, myocardial fibrosis, coronary artery calcification, and coronary spasm. It needs new studies finding best solution to prevent and treat this condition.

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Conflict of Interests

Authors have no conflict of interests.

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Unusual management of parturient patient with severe bicuspid aortic valve stenosis and congestive heart failure

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Case Report

Abstract

BACKGROUND: Critical aortic stenosis (AS) is an unusual cardiac pathology in pregnancy, but has significant impact on the fetal and maternal outcomes of pregnancy. Pregnant patients with aortic stenosis and heart failure represent a major challenge for the heart team and anesthesiologist who should balance the risks and benefits of different treatment strategies and their effects on the mother and fetus.

CASE REPORT: We present a 26-year-old parturient who underwent cesarean section at 30 weeks of gestation under general anesthesia in the presence of cardiac surgical team followed by deferred aortic valve replacement after two weeks.

CONCLUSION: This report describes the importance of multidisciplinary preoperative evaluation, and careful surgical and anesthetic planning to avoid the deterioration of perioperative cardiac condition in such patients.

Keywords: Pregnancy, Aortic Stenosis (AS), Bicuspid Aortic Valve (BAV), Aortic Valve Replacement (AVR), Congestive Heart Failure (CHF)

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Introduction

Significant aortic stenosis (AS) in pregnancy has been described infrequently and there is no reported case series large enough to reach consensus about the its optimal management.[1,2] Altered hemodynamic function during pregnancy in addition to the relatively fixed cardiac output caused by severely stenotic valve precipitates congestive heart failure (CHF) with associated maternal and fetal mortality.[3]

Pregnant patients with CHF represent a major challenge for the anesthesiologist and heart team who should balance the risks and benefits of different anesthesiological strategies and their effects on the mother and fetus.

We describe successful unusual management of a parturient patient with severe AS and CHF in whom cesarean section and subsequent aortic valve replacement (AVR) wer deferred under strict surveillance to optimize the fetus viability and minimize the maternal mortality.

Case Report

A 26-year-old, 73-kg primigravida woman presented at 28th week of gestation being referred from a rural clinic to our university referral hospital, complaining of worsening respiratory distress with primary suspicion of pulmonary embolism.

Significant lower extremity edema and severe orthopnea with pulmonary rales were appreciated at the physical examination.

The echocardiographic investigations showed bicuspid aortic valve (BAV), thickened and calcified leaflets, pressure gradient of aortic valve of 88/53 mm Hg (peak/mean), severe AS with aortic valve area (AVA) of 0.75 cm^2, ejection fraction (EF) of 30%, concentric left ventricular hypertrophy (LVH), and up to moderate functional mitral regurgitation (MR).

After discussion and consultation with the cardiologists, cardiovascular surgeons, and obstetricians, medical treatment for patient's CHF and watchful waiting till maturation of the fetus was planned. The patient was admitted at intensive care

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unit (ICU), and anti-CHF therapy was started with inotrope and furosemide, with meticulous monitoring of cardiovascular status, and fetus surveillance.

During ICU admission, patient's symptoms were relieved dramatically and elective cesarean section (CS) was planned for the delivery of the fetus at 30th week of gestation. As the patient did not offer consent for simultaneous CS and valve replacement, decision was taken to proceed to cesarean section under general anesthesia with attendance of the cardiac surgical team to intervene if required.

During general anesthesia, hemodynamic parameters were planned to be sustained close to the baseline with balance of vasoactive and anesthetic agents; and emergency institution of cardiopulmonary bypass (CPB) was possible if hemodynamic deteriorates or cardiac arrest happened.

Immediately after general anesthesia, the consultant obstetrician proceeded to deliver the baby by lower segment CS (LSCS). Delivery of a healthy female infant with the weight of 2750 g was achieved in 3 minutes. APGAR score at 1st and 5th minutes was 8 and 9, respectively. To facilitate uterine muscle contraction, uterine massage, and infusion of oxytocin (10 units/hour) along with intramuscular injection of prostaglandin (250 µg) were applied.

The patient's recovery was uneventful and she was successfully extubated at operating room, then transferred to ICU for hemodynamic monitoring. Two weeks later, along with worsening of patient's symptoms of CHF, she finally consented to aortic valve replacement (AVR).

After establishment of cardiopulmonary bypass, AVR with mechanical St Jude No. 21 combined with septal myectomy was performed. Postoperative transthoracic echocardiography (TTE) revealed peak and mean gradients of 25 and 16 mmHg, respectively, trace MR, and left ventricular ejection fraction (LVEF) of 45%. The patient was discharged to home on her 5th day of valve replacement with acceptable condition. Clinical and echocardiographic assessment during one-year follow-up period was satisfactory.

Discussion

Pregnancy in patients with severe AS is associated with high risk of mortality reaching as high as 17%. Pregnancy with severe AS is characterized by an increased incidence of CHF (16.7%), poor class of New York Heart Association (NYHA) classification, shorter duration of pregnancy, and premature labor (25%).[4]

As bicuspid AS is more common in men, and in childbearing women's severe AS is uncommon, experience of pregnancy in these patients is scarce.[5]

Physiological changes during pregnancy including increased intravascular blood volume, cardiac output, and diminished systemic vascular resistance (SVR) may lead to deterioration in the cardiac status of patients with AS during pregnancy.

Different strategies have been described in literature for patients with severe AS and pregnancy based on the severity of symptoms, presence of CHF, and gestational age. Severe AS has been reported in 15 pregnant patients of whom four patients manifested CHF. Six patients required cesarean section at a mean gestational age of 33.8 ± 4.5 weeks (median, 33.5 weeks). Intervention for AS was required in nine patients of whom four had balloon aortic valvuloplasty during pregnancy, two patients underwent AVR after delivery and three patients underwent concomitant AVR and cesarean section.[6]

The induction of anesthesia is a critical step in patients with significant AS. Avoidance of hypovolemia, myocardial depression, vasodilation, tachycardia, or dysrhythmias is important, as all of these can lower the cardiac output precipitously ending in sudden cardiac arrest. Moreover, intravenous bolus administration of oxytocin after delivery can induce significant hypotension and must be avoided.[7]

In our presented case, balloon dilatation of the stenotic aortic valve was not possible due to severe calcific BAV. In parturient patients with CHF symptoms and viable fetus, concomitant cesarean and aortic valve replacement can be done to decrease the cardiac events and mortality. Nevertheless, this was not an option as the patient refused cardiac surgery. As CHF symptoms decreased after medical treatment, a multidisciplinary team decided for LSCS and deferred AVR in another setting. To the best of our knowledge, there is no such report of CS and deferred AVR in pregnant patient with severe AS and CHF symptoms in the literature. Of note, anestethical considerations are of great importance in the management of such patients.[8,9] Although deferring AVR can diminish the complications of concomitant CS, but it carries the potential risks of severe AS itself. In our experience, strict hemodynamic monitoring of such patients before, during, and after any intervention is a great tool for minimizing the possible adverse events of patients with severe AS.

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Conflict of Interests

Authors have no conflict of interests.

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A rare case of spontaneous and simultaneous multivessel coronary artery spasm leading to multisite myocardial infarction and ventricular fibrillation

Leili Iranirad[(1)], Mohammad Saleh Sadeghi[(2)]

Case Report

Abstract

BACKGROUND: Coronary artery spasm (CAS) can result in life-threatening arrhythmia and sudden cardiac death. Although this disorder has been known for a long time, little is known about it, and its mechanisms have been not identified yet.

CASE REPORT: We describe a 52-year-old woman with no significant cardiovascular risk factors who experienced several episodes of spontaneous and coincident multivessel coronary artery spasm, which led to myocardial infarction as well as malignant arrhythmias. Coronary angiography revealed severe migratory narrowing in the left anterior descending artery and right coronary artery.

CONCLUSION: Simultaneous multivessel coronary artery spasm develop multisite myocardial infarction (MI), and malignant arrhythmias could occur even in the absence of significant stenosis and triggering factors, which would lead to an increased risk of life-threatening cardiac events.

Keywords: Variant Angina Pectoris, Myocardial Infarction, Coronary Angiography

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Introduction

Variant angina is a discrete form of angina pectoris, which typically occurs during normal activity or at rest, without evident classical triggers such as exercise. In this syndrome, episodes usually occur at night or in the early hours of the morning.[1-3]

The occurrence of coronary artery spasm (CAS) shows extensive variances in different countries. For instance, the incidence of CAS seems to be three-times greater in the Japanese population compared to Caucasians, suggesting the probable role of genetic factors in the pathogenesis.[2,4]

CAS is rare in young persons. Most patients suffering from CAS are between 40-70 years old. Death rates reported in patients with Prinzmetal angina are relatively low.[4,5] Although this disorder has been described since long time ago, little is known about it and its mechanisms remain unclear. In this report, we describe a rare occurrence of spontaneous and simultaneous multivessel CAS, which led to myocardial infarction and malignant arrhythmias.

Case Report

A 52-year-old woman was presented to the emergency room with acute epigastric pain and cold sweating. Admission electrocardiography (ECG) indicated ST-segment raising in the inferior leads (Figure 1); and the patient was managed with trinitroglycerin (TNG) and fibrinolytic therapy.

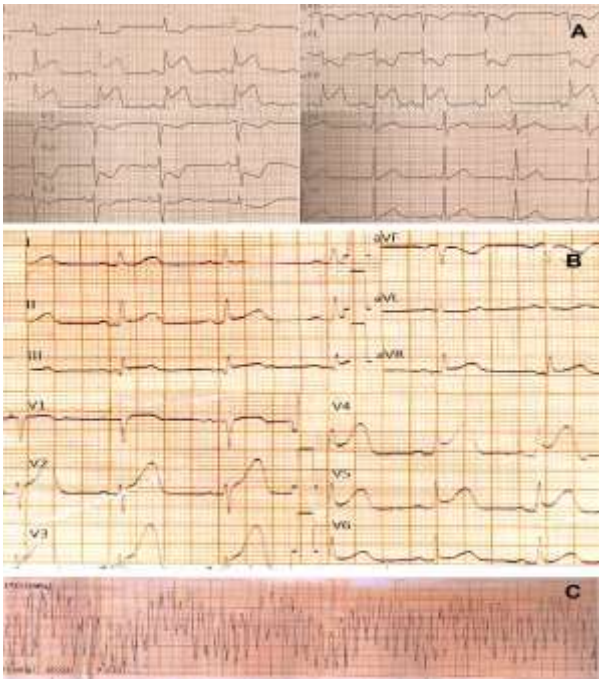

Figure 1. A: ST elevation in inferior leads; B: ST elevation in anterior leads; **C:** Polymorphic ventricular tachycardia

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Coronary angiography (CAG) was performed and showed severe stenosis in the left anterior descending artery (LAD) and right coronary artery (RCA) (Figures 2 and 3). Biochemical tests showed troponin level of 7.1 ng/ml (normal < 0.01 ng/ml); and other important factors such as complete blood count (CBC), fasting blood sugar (FBS), blood sugar (BS), triglyceride (TG), total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), Na, K, Mg, Ca, Cr, prothrombin time (PT), partial thromboplastin time (PTT), and international normalized ratio (INR) were at normal levels. The patient did not any have risk factors such as diabetes, smoking, alcohol consumption, use of ergonovine or other drugs, family history of cardiovascular disease, hypercholesterolemia, or history of angina.

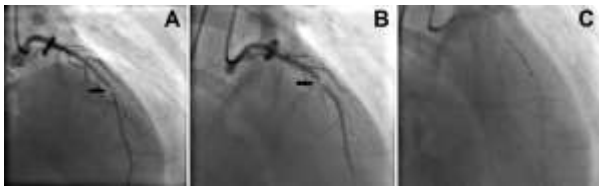

Figure 2. A: Severe stenosis at mid-portion of left anterior descending artery (LAD); B: Severe stenosis before last seen lesion; **C:** Stenting of LAD lesion

Since the patient showed stable vital signs and did not experience any chest pain, revascularization was planned for 48 hours later, based on the literature.[6] Suddenly, after a day, the patient developed polymorphic ventricular fibrillation (VF) and was treated with successful defibrillation.

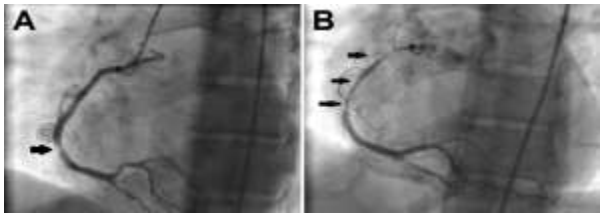

Figure 3. A: Severe stenosis at mid-portion of right coronary artery (RCA); B: Severe long stenosis at proximal to mid-portion of RCA

After defibrillation, ECG showed ST-segment elevation in anterior leads; thus, coronary stenting was urgently performed in the LAD. Surprisingly, there was no stenosis at the prior position when we proceeded with RCA revascularization; this indeed signified a spasm that had shifted to the proximal site. Furthermore, we understood that stenting of the LAD was mistakenly performed on a spasm because stenosis had shifted slightly in relation to the last performed angiography. Interestingly, in spite of spasm, the patient did not have ischemic symptoms during the intervention.

Finally, the patient was placed on oral diltiazem, isosorbide mononitrate, and nicorandil to suppress coronary artery spasm attacks. During a one-year follow-up, the patient was free of symptoms.

Discussion

In 1950, Prinzmetal et al. described a variant form of angina pectoris resulting from temporary occlusion of a large diseased coronary artery with a narrow lumen due to increase in the tonus of the vessel wall.[2,3] Although the exact pathophysiology of Prinzmetal angina remains unclear, the possible mechanisms that have been suggested include endothelial dysfunction, increased vasomotor tone, and increased platelet activation. Other precipitating factors include increased oxidative stress, physical or mental stress, magnesium deficiency, hyperventilation, inflammation, ergot alkaloids, alcohol consumption, and genetic susceptibility.[2-4] Furthermore, cigarette smoking, age, and C-reactive protein with high sensitivity (hs-CRP) are major risk factors for vasospastic angina.[4,5]

Several cases have been reported of coronary spasms in the literature.[5,7-9] In a report, a 57-year-old man with a history of hypertension and diabetes mellitus, and variant angina developed simultaneous anterior and inferior MI, cardiogenic shock and VF.[7] In another report, a 58-year-old woman with a history of hypertension and hypercholesterolemia developed ST-segment elevation and VF. Coronary angiography was initially performed and the second CAG revealed no lesions and she was diagnosed with CAS.[8]

Here, we described a case with simultaneous multiple CAS leading to multisite MI and malignant arrhythmias, a rare occurrence of CAS. Furthermore, the present case did not exhibit any risk factors and triggering factors; however, some cardiac episodes were silent, which is noteworthy.

ECG changes usually develop; however, they may appear ordinary at the start of CAS or in mild CAS.[3,4] ST-segment elevation shows entire or subentire spasm of a main coronary artery. However, CAS is more often related to ST-segment depression, subendocardial myocardial ischemia, which indicates less severe case than ST-segment elevation. In addition, a taller and broader R wave, disappearance of the S wave, a taller T wave, and negative U wave may also appear during ST-segment changes.[2,4]

The only convinced method for diagnosing CAS relies on coronary angiography and provocative tests. However, coronary angiography is normal in about half of the cases.[3,4]

The occurrence of arrhythmias is prevalent during variant angina crises. Bradyarrhythmia, complete atrioventricular block, paroxysmal atrial fibrillation, ventricular tachycardia (VT), VF, and asystole are among the severe arrhythmias. Therefore, continuous ECG monitoring or Holter monitoring is useful for detecting ECG changes in patients suffering from variant angina.[2-4]

Early treatment of variant angina is important to prevent complications such as acute MI, fatal arrhythmias, and sudden death. Intravenous or sublingual nitroglycerine are effective in relieving attacks of variant angina.[2] It is obvious that any factor accelerating CAS probably, specially smoking, or specific drugs (e.g., ergotamine, sumatriptan) must be avoided. Calcium channel blockers have a crucial role in controlling CAS. Long-acting calcium antagonists are recommended to be taken at night when frequent CAS attacks occurrence, in this regard.[1,4] For suppressing CAS attacks in patients with variant angina, nicorandil, a nitrate, and K-channel opener are also useful.[2,4] A combination of different classes of calcium antagonists and nitrates or nicorandil or both is essential for patients suffering from variant angina, which is resistant to standard antianginal medications.[2]

Coronary stenting may express a different and viable option for some patients who are resistant to medical treatment. Chu et al. reported that for severe refractory coronary vasospasm, coronary stenting was effective with no serious complications.[10] Moreover, it is suggested that coronary stenting together with adequate medical treatment can be considered in patients with CAS suffering considerable coronary stenosis.[4] However, adequate information on late clinical consequence followed by stenting is limited, and further controlled clinical studies are necessary to determine coronary stenting for drug-refractory CAS. In this regard, revascularization procedures such as coronary artery bypass surgery (CABG) have resulted in limited success.[1,4] Using an implantable cardioverter defibrillator in CAS cases are associated with life-threatening arrhythmias, VT, or VF.[1,4] Long-term survival in patients with variant angina seems to be generally good.[2,4]

In conclusion, simultaneous multivessel CAS developed multisite MI and malignant arrhythmias could occur even in the absence of significant stenosis, risk factors, and triggering factors, and would lead to an increased risk of life-threatening cardiac events.

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Conflict of Interests

Authors have no conflict of interests.

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Off-center cardiac rehabilitation focused on extended emotional relationship and common health gains

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Letter to Editor

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Dear Editor-in-Chief

In recent years, cardiac rehabilitation (CR) programs have been well advanced.[1,2] However, failure to adhere to these programs by patients and failure to follow a healthy lifestyle during and after CR is still a serious disadvantage.[2,3] In Iran, hospital-based delivery format is still the preferred approach. Although, obstacles such as access have challenged active presence of patients.[4,5] Thus, providing measures to increase adherence by patients and prevent withdrawal of the treatment program is one of the priorities of the management of CR field.[2] In this regard, previous studies have contributed to several factors. But, it seems that the strategies for solving this problem must be the function of social and cultural context of each society.[4] Therefore, proposing practical suggestions tailored to social and cultural situation of each country can be effective in solving this problem.

A brief study of the executive structure of CR centers in Iran shows that these centers are generally active during before midday time (ante meridiem or am). All patients attending hospital-centered CR take part in exercise and lifestyle modification training within 8-12 weeks (three times a week) during the hours of before midday.[2] Despite the awareness of the centers' health team of different levels of patients heart risk (low, moderate, and high risk), the limited number of CR centers throughout the country has led all patients to be managed in a single timetable and delivery format. Obviously, the level of heart risk is effective in choosing exercise schedules, and its duration and severity.[5] Hence, it is better to design the structure of the treatment plans of each group based on heart risk.

Based on these considerations and in order to optimally use the physical space and hardware facilities of the CR centers, it is recommended that patients be divided into two groups of low-medium risk and high risk.[6] Then, low-medium risk and high-risk patients respectively participate in the comprehensive CR programs during the hours of before and after midday (post meridiem or pm). Secondly, it is recommended that several health centers be set up in several different parks in each city. The members of these centers consist of a sports medicine specialist, a nutritionist, and two nurses.

In the next step, the provision of services can be designed based on the cultural context of the country. For example, designing and implementing health promotion side plans with the emphasis on developing emotional relationships of the CR group is likely to be helpful.[7] In the framework of such approaches, patients can participate in off-center group activities. Group exercise and conduct retraining sessions around a health facility are helpful for patients. Given that patients only exercise for three days at a CR center, on other days of the week, group sports can be transferred to out-of-center (adjacent health centers). Previous reports indicate that perceived social support is associated with an increase in the quality and quantity of walking.[8] A group walking with an emphasis on the extended emotional relationship and common health gains makes the low-medium risk patients benefit and enjoy a lot of common interactive and targeted activity. Meanwhile, according to the nutritionist guidelines, patients can use a designated food basket and use healthy food after the rest at the terminal. It seems that the creation of this health program with a positive emotional atmosphere is also effective in managing patient's stress.[9]

In relation to high-risk patients, it is evident that participation in hospital-centered CR is safer. These patients need to be fully supervised by the CR team. Therefore, off-center programs are not very suitable

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for them.[5] In addition; patients away from the center and living in remote areas cannot participate in off-center programs. However, our proposed program can be appealing for a significant proportion of patients and increase their adherence to CR. Implementing our proposed program may also be effective in adopting a healthy lifestyle in the long-term.[3] Therefore, we recommend that this approach is used as a pilot in country's CR centers.

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Conflict of Interests

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